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ORIGINAL ARTICLE

The Effect of Gibberellic Acid and Benzyladenine in Growth and Flowering of Lily (*Lilium longiflorum*)

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H. Emami, M, Saeidnia, A. Hatamzadeh, D. Bakhshi, E. Ghorbani; The effect of gibberellic acid and benzyladenine in growth and flowering of Lily (*Lilium longiflorum*)

ABSTRACT

In order to study growth and flowering characteristics of *Lilium longiflorum* by using tow growth regulators, gibberellic acid and 6-benzyladenine, an experiment was performed in 2008 by using factorial experiment arranged in a completely randomized design with three replication in research greenhouse, college of agricultural science, University of Guilan. In this experiment, unit bulbs soaked for 24 hours by gibberellic acid and 6-benzyladenine, and then they planted in greenhouse. Results showed that the maximum bud number was related to gibberellic acid 150 ppm with benzyladenine 75 ppm. The highest chlorophyll a content was related to gibberellic acid 75 ppm with benzyladenine 75 ppm, gibberellic acid 75 ppm and benzyladenine 75 ppm respectively. In addition, the highest total chlorophyll achieved when we used gibberellic acid 75 ppm with benzyladenine 75 ppm. The highest initial fresh weight was related to gibberellic acid 75 ppm. Gibberellic acid 75 ppm, benzyladenine 150 ppm and benzyladenine 75 ppm showed the maximum anthocyanin content respectively. The maximum vase life was related to gibberellic acid 75 ppm. In order to achieve qualification, longevity of vase life and economic aims, we suggested using gibberellic acid 75 ppm with 6-benzyladenine 75 ppm.

Key words: Antochyanin, chlorophyll, Vase life, Lilium

Introduction

Lily flower (*Lilium longiflorum* Thunb.) is commercially produced as a potted plant in the US. The most important factor determining its market value is the accuracy and uniformity with which the crop flowers in time for sales. Gibberellins and cytokinin are plant growth regulators that affect many phases of plant growth and development from germination, leaf expansion and shoot elongation to flowering, fruit development and mobilization of seed reserves. Potential of using growth regulators specially Gibberellins and cytokinin is not studied in Lilium sufficiently.

Emongor and Tshwenyane [6] reported that cytokinins, increase chlorophyll development and chlorophyll synthase. Chlorophyll degradation in leaves of cut flowers is controlled by gibberellins [27]. Sing *et al* [25] said that gibberellic acid treatment cause to increase in longevity of gladiolus cut flowers. Mutuee *et al* [18], said that gibberellins increase fresh weight of cut flowers.

The aim of this study is to study on some important traits of lily cut flowers treated with gibberellic acid and 6-benzyladenine.

Material and method

In this study we used *Lilium* hybrid *Asiatic*×

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Longiflorum c.v. Menorca. 108 forced bulbs with 16-18 centimeter diameter were supplied from Khadem greenhouse in Pakdasht, Varamin, Iran. I.R. An experiment was performed by using factorial experiment arranged in a completely randomized design with three replications. Also, we used two factors, gibberellic acid and 6-benzyladenine, each in three levels (0, 75 and 150 ppm). Before planting lilium bulbs were disinfected with 1 % (w/v) benomyl fungicide. Bulbs were in treatment for 24 hours before culture. These treatments were contents of control, 75 ppm gibberellic acid, 150 ppm gibberellic acid, 75 ppm 6-benzyladenine, 150 ppm 6-benzyladenine, 75 ppm gibberellic acid with 75 ppm 6-benzyladenine, 150 ppm gibberellic acid with 150 ppm 6-benzyladenine, 75 ppm gibberellic acid with 150 ppm 6-benzyladenine and 150 ppm gibberellic acid with 75 ppm 6-benzyladenine. In order to supply perfect temperature for lily growth, day temperature was regulated at 18-22°C and night temperature was regulated at 16-18°C [4,5]. Photoperiod was 13 hours and light density in greenhouse was 302 micro $mol{\cdot}m^{-2}{\cdot}s^{-1}$ which is measured by illuminati meter (model INS-DX-200).In this study, flowers were harvested when flower bulbs colored completely. Flower harvesting was down at morning. Cut flowers were quickly transferred to horticultural laboratory. Then cut flowers weighed and lodged in equal glasses with distinct distilled water. The cut flowers were stored at room temperature with 70% Relative humidity. Light value was 21 micro mol / m^2 / s, which was measured by illuminati meter.

Data were subjected to ANOVA procedure in SAS (ver. 9.0, SAS Institute, Inc.) and appropriate means were separated using the LS means.

Result:

Number of Yellow Leaf:

Variance analysis showed that effects of gibberellic acid and interaction effect of gibberellic acid and benzyladenine was significantly positive ($p < 0.05$) but effects of 6-benzyladenine was not significant. Comparison of means showed that the maximum number of yellow leaves was related to control treatment and the minimum number was related to gibberellic acid 150 ppm (table 1).

Chlorosis is an ordinary problem in lily production. Leaf yellowing in lily during production occurs as a gradually physiological problem at a greenhouse or occurs suddenly during the storage [6,13]. Flower senescence is along with leaf fading and chlorophyll decrease [13]. In one place Gibberellic acid and benzyladenine prevent from proteolysis and chlorophyll degradation [22,23,6] and on the other hand prevent from leaf senescence and yellowing by increasing in chlorophyll synthesis and chloroplast development [6].

In addition, gibberellic acid in chloroplast membrane causes to regulate photosynthesis process and increases leaf longevity [13].

Our results is similar to results of Nowak and Mynett [19] on lily, Jordi *et al* [14] on Alestromeria, Han [11,12] on lily, Pun *et al* [22] on gladiolus, Ranwala and Miller [24] on lily and Kim and Miller [15] on tulip, confirm the positive and significant role of gibberellic acid and benzyladenine on decreasing number of yellow leaf.

Number of Flower Bud:

Variance analysis showed that effects of gibberellic acid was significantly positive ($p<0.01$) and effects of 6-benzyladenine and interaction effect of gibberellic acid and benzyladenine was significantly positive ($p<0.05$). Gibberellic acid 150 ppm with benzyladenine 75 ppm showed the maximum number of flower bud and the control treatment had the least.

Gibberellic acid may cause to increase in the accessible substances in flowering initiation time. This subject can be related to flowering increase [3]. Our results like the results of Funnel *et al* [8] on *Zantedeschia*, Ali and Elkiey, [3] on *Zantedeschia*, confirm the positive and significant role of gibberellic acid on increasing bud number and likewise the results of Pogroszewska *et al* [21] on *Allium karataviense*, it is showed the positive and significant role of benzyladenine.

Chlorophyll a:

Variance analysis showed that effects of gibberellic acid and benzyladenine was significantly positive ($p<0.01$ and $p<0.05$ respectively). All the treatment caused to increase in chlorophyll a content except control (table 1).

Chlorophyll Content:

Variance analysis showed that effect of gibberellic acid and interaction effect of gibberellic acid and benzyladenine on total chlorophyll content were significant ($p < 0.01$). Comparison of means showed in table 1.

Chlorophyll degradation in leaves of cut flowers is controlled by gibberellins [27], on the other hand, cytokinins prevent leaves senescence and delay proteolysis and chlorophyll degradation [6,28]. In addition, Emongor and Tshwenyane [6] reported that cytokinins, increase chlorophyll development and chlorophyll synthase.

Our results showed the effective role of gibberellic acid and benzyladenine on preventing of chloroplast and chlorophyll degradation. which result to decreasing in leaves senescence and increasing in total chlorophyll content. Our results is similar to results of Van Doorn and Van Lieburg [27] in Alestromeria, Gan and Amasino [9], Wingler *et al* [28], Guo *et al* [10] in chrysanthemum and Emongor and Tshwenyane [6] in Lilium.

Total Anthocyanin Content:

Variance analysis of this trait showed that gibberellic acid and interaction effect of gibberellic acid and benzyladenine on total anthocyanin content were significant ($p < 0.05$) and ($p < 0.01$) respectively. Comparison of means showed that samples, which are treated by gibberellic acid 75 ppm, benzyladenine 150 ppm, and benzyladenine 75 ppm, have the highest content of anthocyanin respectively (table 1).

Anthocyanin pathway activity in petal development duration, need a set of action and reaction between environment and developmental signals. Gibberellins, sugars and light are needed for infusing anthocyanin gene transcription and for aggregating pigments in corrolla. The effects of these signals are not just for pigment biosynthesis. They also control petal cell extension and infusion of gene expression from different ways. These reports suggest a model, which in this model gibberellins, sugar and light increase the activity of transcription regulator that is control different ways for development completion [28]. Weiss [28] reported that gibberellic acid could be a promoter for starting extension and coloration of flower. This plant growth regulator is produced in anthers, which are in developmental step and then transfer in to corolla and at last relate to physiological and biochemical processes such as petal growth and pigment production. Weiss, reported that gibberellic acid could infuse some genes of essential enzymes in anthocyanin production pathway such as chalkon synthetase, chalkon isomerase, di-hydro flavenol redoctase. Kwack *et al* [16] reported that gibberellic acid regulate phenyl alanin amoniliyase activity which is increase anthocyanin content in plant culture. Our results like Weiss [28] and kwack *et al* [16] showed that using gibberellic acid and benzyladenine increased anthocyanin content compare with control treatment.

Longevity:

Variance analysis showed that gibberellic acid and interaction of gibberellic acid with benzyladenine were significantly effective on longevity ($p< 0.05$). Comparison of means showed that using gibberellic acid 75 ppm related to the highest longevity. In addition, after that using gibberllic acid 150 ppm with benzyladenine 75 ppm and gibberellic acid 75 ppm with benzyladenine 150 ppm caused to the highest longevity respectively (table1).

Kwack *et al* [16] reported that flower senescence is a process that is caused by different processes such as oxidation actions which caused by aggregation of hydrogen peroxide radical, destruction of lipids and phospholipids structures and then increasing in ions leakage which is caused by cell membrane damage. Cytocynins delay petal senescence by protecting cells and proteins. Benzyladenine can increase cut flower longevity by improving cell membrane permanency, delaying membrane lipid peroxidation in cell and decreasing in ions leakage. In addition, benzyladenine can increase hegxos sugars (sucrose and fructose) availability in cell by increasing in α- amylase and invertase enzyme activity [17]. Our results also showed the important role of gibberellic acid and benzyladenine in significant increasing in lily longevity like Marousky [17], Guo *et al* [10], Ranwala and Miller [24] and Singh *et al* [25].

Carotenoid Content:

Variance analysis showed that effect of gibberellic acid and benzyladenine on carotenoid content were significant ($p < 0.01$ and $p < 0.05$ respectively).

Our results like the results like the results of Abdel-Wahid and Sweify [2] on Beaucarnea recurvata showed the effective role gibberellic acid in increasing carotenoid content and our results like the results of Abd El-Aziz [1] in croton plant and Penner and Wiely [20] in cucumis and cucurbita showed the effective role of benzyladenine in increasing carotenoid content.

Initial Fresh Weight:

Variance analysis of this trait showed that gibberellic acid and benzyladenine effects on fresh weight was significantly positive ($p< 0.05$). Comparison of means of different gibberellic acid densities showed that gibberellic acid 75 ppm had the highest initial fresh weight and the control treatment had the lowest initial fresh weight (fig 1). Comparison of means for benzyladenine showed that benzyladenine 75 ppm had the highest initial fresh weight and the control treatment had the lowest (fig 2).

Emongor and Tshwenyane (2004, Mutui *et al*, [18] reported that gibberellins increase cut flowers fresh weight by negation of cell water potential. Also benzyladenine increase hegxos sugar (sucrose and fructose) availability by increasing in α- amylase and invertase activity in cell and osmotic potential and at last increase the fresh weight [17].

Table 1: Comparison of means of investigated traits.

	Carotenoid content	Total chlorophyll	Chlorophyll a	Total anthocyanin
Control	0.009 e	1.85 c	0.85 b	0.26 d
Gibberellic acid 75 ppm	0.111 a	3.09 ab	1.72 a	4.45 a
Gibberellic acid 150 ppm	0.62 bc	2.90 ab	1.52 a	2.46 bc
Benzyladenine 75 ppm	0.22 cde	2.88 ab	1.56 a	2.92 bc
Benzyladenine 150 ppm	0.16 de	2.68 ab	1.45 a	3.31 b
Gibberellic acid 75 ppm Benzyladenine 75 ppm	0.35 cde	3.20 a	1.73 a	2.11 c
Gibberellic acid 150 ppm Benzyladenine 150 ppm	0.59 bcd	2.84 ab	1.52 a	2.85 bc
Gibberellic acid 75 ppm Benzyladenine 150 ppm	0.6 bcd	2.85 ab	1.55 a	2.51 bc
Gibberellic acid 150 ppm Benzyladenine 75 ppm	0.83 ab	2.05 bc	1.36 a	2.38 bc

Means followed by similar in each column are not significantly different at 5% level.

Table 1: Comparison of means of investigated traits.

	Longevity	Number of yellow leaf	Number of flower bud
Control	13.61 c	8.3 a	4.08 c
Gibberellic acid 75 ppm	15.77 a	6.75 b	4.75 bc
Gibberellic acid 150 ppm	15.11 ab	5.16 c	4.58 bc
Benzyladenine 75 ppm	15.44 ab	7.50 ab	4.41 bc
Benzyladenine 150 ppm	14.55 bc	7.16 ab	4.50 bc
Gibberellic acid 75 ppm Benzyladenine 75 ppm	14.77 ab	6.97 ab	4.63 bc
Gibberellic acid 150 ppm Benzyladenine 150 ppm	15.22 ab	7.33 ab	4.91 b
Gibberellic acid 75 ppm Benzyladenine 150 ppm	15.52 ab	7.75 ab	5.00 b
Gibberellic acid 150 ppm Benzyladenine 75 ppm	15.66 a	7.16 ab	6.41 a

Means followed by similar in each column are not significantly different at 5% level.

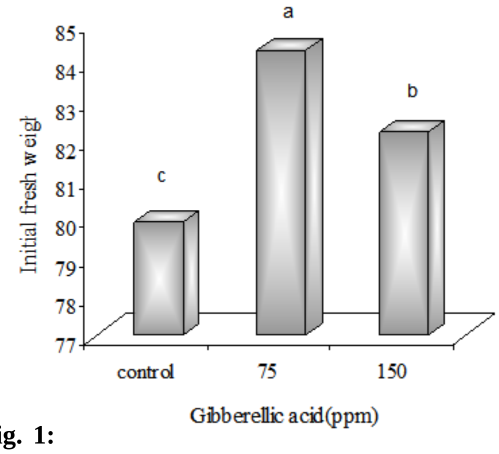

Fig. 1:

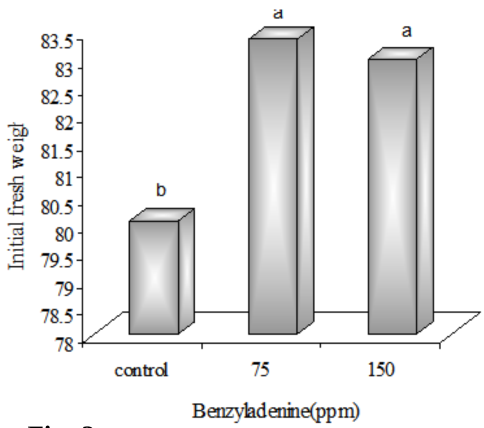

Fig. 2:

Our results like Emongor and Tshwenyane [6] and Mutui *et al* [18] showed that gibberellic acid had significant positive role in increasing fresh weight and in our results benzyladenine had the significant positive role in increasing initial fresh weight too.

Petal Total Soluble Solid:

Variance analysis of this trait showed that the effect gibberellic acid on petal total soluble solid was significantly positive ($p<0.05$). Comparison of means of different gibberellic acid densities showed that gibberellic acid 150 ppm had the highest and the control treatment had the lowest total soluble solid (fig 3).

Gibberellic acid can increase starch hydrolysis to glucose and fructose and sucrose, which is resulted of combination of these two monosaccharide, will cause to boost of cell wall. On the other hand using gibberellic acid can cause to more production and conservation of carbohydrate in stem and petal tissues [26]. Singh et al at 2008 in studying growth regulators and sucrose on post harvest physiology, membrane persistence and gladiolus cut flower vase life reported that gibberellic acid with sucrose increase gladiolus vase life via increasing lipids peroxidation and then decrease activity of lipoxigenase enzyme and improving petal cell wall substance. Our results like Singh *et al* [25] on gladiolus and Taheri Shiva [26] on lily showed the positive and significant effect of gibberellic acid on petal soluble solid.

Conclusion:

As we said, results of this study and some other studies confirm that gibberellic acid and 6-benzyladenine increase some process such as cell wall tension and thus cell water potential decline [7] and more water absorption to cell and at last increase fresh weight and flower succulence [18,6]. On the other hand effect of gibberellic acid and 6-benzyladenine on increasing of carbohydrate hydrolysis induction cause to stability of respiration

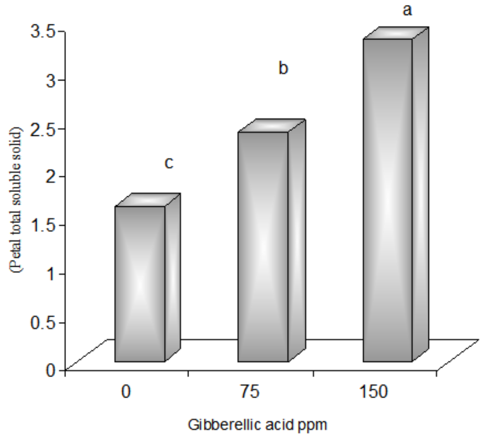

Fig. 3:

and thus increase of cut flowers vase life [5].

Effect of gibberellic acid and 6-benzyladenine on preventing of senescence [22,24,6] and its effect on chlorophyll synthesis and chloroplast development increasing prevent from leaves yellowing [10,6].

Cut flower harvesting and recutting them cause to stress is occurred .This stress decrease photosynthesis by effecting on tylacoeid membrane, tylacoeid osmotic potential and increase production of ROS. Carotenoids have an antioxidant role and prevent or decrease efficacies of ROS stress. On the other hand, anthocyanins prevent cell oxidation process and retard senescence that is occurred because of hydrogen peroxide aggregation and degradation of cell membrane, proteins, lipids and increase of ions leakage [28,26]. Therefore, according to result of this study and some other studies showed that increasing in anthocyanin and carotenoid would increase lily vase life. Thus, gibberellic acid interferes in anthocyanin and carotenoid biosynthesis and retard chlorophyll destroy. Therefore, a section of this effect (increase vase life) is caused to chlorophyll preservation that is attendant with leaf nitrogen level preservation.

According to these points, necessity of using growth regulator to improve quality, marketing and flower vase life is completely justification. Among treatments that we used in this study, gibberellic acid 75 ppm and gibberellic acid 150 ppm with 6-benzyladenine 75 ppm showed the high longevity and their means did not have significant difference with each other, but they showed significant difference with other treatments. Gibberellic acid 150 ppm with 6-benzyladenine 75 ppm had the most flower bud number that is showed significant difference with other treatments. Acid gibbberellic with benziladenine 75 ppm was the most effective treatment on chlorophyll a and total chlorophyll. This treatment was significantly difference with control respecting to chlorophyll a and was significantly different with control and gibberellic acid 150 ppm with 6-benzyladenine 75 ppm respecting to total chlorophyll. Gibberellic acid 75 ppm was the most effective treatment for vase life. This treatment only had significant difference with control and 6-benzyladenine 150 ppm. Gibberellic acid 75 ppm and 6-benzyladenine 75 ppm showed maximum positive effect on initial fresh weight. Gibberellic acid 75 ppm was significantly different with other treatment but 6-benzyladenine was only significantly differed from control. Gibberellic acid 75 ppm was the most effective treatment respect to carotenoid content and anthocyanin. This treatment had no significant difference with other treatment except gibberellic acid 150 ppm with 6-benzyladenine 75 ppm but gibberellic acid 75 ppm was significantly differ from all other treatment respect to anthocyanin.

Therefore according to all results and correlation of traits and in order to achieve economic aims, lily cut flowers with high quality, marketing and vase life, using gibberellic acid 75 ppm with 6-benzyladenine 75 ppm is suggested.

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