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**ORIGINAL ARTICLE**

# EFFECT OF INDOLE-3-ACEDIC ACID AND BENZYL ADENIN ON MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF GLADIOLUS

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**ABSTRACT:** In order to investigate effect of Indol-3-acetic acid (IAA) and benzyladenine (BA) on growth, flowering, corm production and biochemical properties of cut flower Gladiolus (*Gladiolus grandiflorus* L. cv. White Prosperity) during vase life, the experiment was conducted in complete randomized block design (RCBD) with three replicates in Agricultural and Natural Resources Research Center of Markazi Province, Iran, during 2010. Primarily bulbs were treated with four different concentrations of IAA (0,100,150,200 mg/lit) and benzyladenine (0, 100, 150, 200 mg/l) and solely for 6 hours then planted. The results of experiment indicated that IAA and BA increased germination rate of gladiolus, significantly ($P<0.0$). Also, onset stalk flower, diameter of floret and bulb wing affected by IAA and BA, significantly. The results showed that highest content of sugar were in petal and leaves which treated with IAA 100 and 200 mg/litr, respectively. Also, IAA in concentration of 150 mg/litr and BA in concentration of 200mg/lit enhanced protein content of petal and leaves, significantly. These results emphasize the significant roles of IAA and BA in increasing insoluble sugar and retarding degradation protein and chlorophyll longevity of cut flowers.

**KEYWORDS**: Protein, Sugar, chlorophyll, germination, flowering

## INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is one of the major produced flower bulb in all over the word. The marketability of Gladiolus cut flower is limited by their short display life and frequent failure to quality. 90% of flower bulbs (ornamental geophytes) production area comprise with tulip, gladiolus, hyacinthus, iris, lilium and narcissus in all over the world and among these flowers, gladiolus especially paid attention (Gursan, 1998).

Gladiolus is grown as flower bed in gardens, used in floral arrangements for interior decoration, as well as making high quality bouquets. In order to enhance of the yield and quality of any flower crop various cultural management practices are required which including good planting material, spacing, irrigation, plant protection and etc. One of the important factors which affects the growth and development of gladiolus is the kind of plant materials like corms.

Plant growth regulators controlled the physiological functions which occurred inside the corms In fact, plant growth regulators are the organic compounds which modify or regulate physiological processes in an appreciable measure in plants when used in small concentrations. It is well known that application of growth regulators such as Gibberellic acid (GA3), Naphtalin acetic acid. (NAA), Cycocel. (CCC) and Malic Hydrazvl (MH) had positive effects on growth and development of gladiolus plants at different concentrations. The previous studies indicated that the growth and yield of gladiolus was enhanced through application of GA3 (Umrao Vijai et al., 2007), NAA, CCC by (Patel *et al.*, 2010; Ravidas *et al.*, 1992) and MH by (De et al., 2002). All concentrations of IAA, $GA_3$ and 1000 mg $l^{-1}$ cycocel enhanced the number and size of the flowers.

Sugars play an important role in plants as substrates for respiration, materials for cell wall synthesis, and osmolyte. It seems that sugar concentration in petals affects the vase life of cut flowers through ethylene production (Kazoo and Kenichi, 1999). It is found that carnation petal wilting effectively was delayed following application of Indole-3-Acedic Acid (IAA). Maybe an inhibition of ethylene biosynthesis on ACC synthesis level is accounted for that dilation. It has been founded that active degradation of starch occurred more intensively in senescing and stressed tissues in where induction of ∞-amylase was enhanced (Koizuka *et al.*, 1995) Not only the post-harvest life of flowers is strongly depend on the content of carbohydrate but also and the acceptable amount of metabolic sugars affect the rate of senescence (Emami *et al.*,

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2011). So, to extending the vase-life of flower, keeping them in solutions containing sucrose could be an applicable way The post-harvest life of flowers is strongly dependent on the carbohydrate status and the acceptable amounts of metabolic sugars are factors that affect the rate of senescence (Ho and Nichols, 1997). It is the fact that the leaf senescence is prevented by application of plant grow regulators through arresting degradation of protein and chlorophyll (Sacher, 1973). The postharvest life of *Alstroemeria* floral organs is typically long and is terminated by petal abscission. However, in many cultivars, yellowing of the leaves on cut stems occurs within a few days, and proceeds very rapidly (Ferrant *et al.,* 2003). As a consequence, leaf yellowing can reduce the overall display life of selected *Alstroemeria* cultivars. Several treatments have been tested for their ability to delay leaf yellowing and thereby extend the vase life of cut flowers Gibberellins treatment to excised Easter lily leaves resulted in high chlorophyll retention (Han, 1995). It is reported that vase life of many cut flowers can be extended by treatment with cytokinins (Nowak *et al.,* 1988). Pulse treatment with 25 and50 mg L-1 benzyladenine (BA) delayed ethylene production and extended the vase life of cut *Eustoma* flowers (Hassanpour Asil and Karimi, 2010). Application of IAA at 100 mg $l^{-1}$ increased the weight and number of bulblets. The vase life of gladiolus cut flowers was determined. Previous studies showed that PGR (Plant Growth Regulators) increase vase life of cut flower. However these studies well challenged the point of carbohydrates in postharvest life of cut flower but this study aims to further establish the effectiveness of IAA and BA in biochemical properties (Sugar, Protein and chlorophyll content) in Gladiolus postharvest life and conducted to investigate effect of IAA and BA on morphological in Gladiolus (Germination, Onset pedicel, stalk elongation, diameter florets and Bulb yield).

## MATERIALS AND METHODS

*2.1. Plant materials*

The experiment was carried out in Agricultural and Natural Resources Research Center of Markazi Province, Iran, during 2010. The experiment was conducted in a completely randomized design (CRD) with three replications. Bulbs The Bulbs were treated by IAA in concentrations of 0, 100, 150 and 200 mg/L and BA concentrations of 0, 100, 150 and 200 mg/L, then grown under commercial conditions in field. The flowers are borne on an emergent shoot enclosed within 7 leaves. Stage flowering was determined onset stalk along flower Specified stems were harvested when the first flower bud showed full color. Cut stems were transported to the laboratory. The cut flowers were immediately put into 300 $^{cc}$ glasses containing water. During the experiment light intensity was full natural light, temperature was 20±2 and relative humidity was 60% until end vase life. Diameter of floret determine by kulice verny The vase life of gladiolus cut flowers was determined by changes in carbohydrate content in leaves and petals was measured using Paquan and Lechasseur, (1976), Protein content was measured using Bradford, (1976) and also leaves chlorophyll content was measured using Osati, (1980) method. In this experiment, Gladioulus grandifloras bulbs were imported from the Netherlands then treatment by plant growth regulators indole-3-acedic Acid and Benzyl Adenin (Merck of Germany) Primarily bulbs were treated with four different concentrations of IAA (0, 100, 150, 200 mg/lit) and benzyladenine (0, 100, 150, 200 mg/l) and solely for 6 hours then planted The bulbs were dismantled from November individual bulb weight (gr) was estimated.

*2.1.1. Geographical conditions*

Functions city of Arak in Markazi province, Altitude 1700m, Mean precipitation is 280 mm, climate is cold semiarid.

*2.2. Biochemical assays*

*2.2.1. Soluble carbohydrates content*

The samples were taken from both of petal and leaves of gladiolus after 1, 5 and 10 days of experiment. 0.25 g chopped material of perianth tissue was fixed in the ethanol. Then the material was macerated and centrifuged (3500 X, 10 min). Finally, the supernatants were pooled and used for the estimation of carbohydrate content. Carbohydrates were measured by the method of Pakqain and Lechacer (Paquan and Lechasseur, 1976). Antron was used as the standard solution.

*2.2.2. Protein content*

The samples were taken from both of petal and leaves of gladiolus after 1, 5 and 10 days of experiment. Proteins were extracted from both leaves and petals through an extraction buffer (0.01 Mtris-HCL) and protein assay was carried out according to method of Bradford (Bradford, 1976).

*2.2.3. Chlorophyll content*

To measure the leaves chlorophyll content, at each stage 0.5 gr chopped material of perianth tissue was fixed in deionized water. The tissue was homogenized in 10 ml acetone. Then the

material was macerated and centrifuged (3500 X, 10 min) and the supernatants were pooled and used for the estimation of chlorophyll content. According to Ausati absorbance of extracts was measured using a spectrophotometer (WAPS 105). The leaf chlorophyll content was determined as absorbance of these extracts at 663 and 645 nm (Osati, 1980).

*2.3. Data analysis*

The collected data were subjected to analysis of variance and the means were separated by the LSD test at *P*=0.01. All statistical analysis was performed using SAS Ver. 9.1 (SAS institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSION

Analyses of variance showed that emergency of bulb, time of flowering, diameter floret, vase life and weigh bulbs affected by PGR treatments. Incorporation of IAA and BA in the pulsing solution considerably stimulated germination bulbs.BA in concentration 200 mg/lit and IAA in concentration 200 mg/lit on germination bulb determined 15 day. IAA in concentration 200 mg/lit and BA in concentration 150 mg/lit significantly stimulated onset stalk flower in comparison with other studied treatments (table1).

In this study IAA and BA application enhanced diameter florets and stalk elongation. In bulb flower auxin is also necessary for stalk elongation. Removal of two major auxin sources, the flower bud and leaves, before the rapid elongation of the floral stalk reduces floral stalk elongation considerably, whereas application of IAA reverses this effect (14, 11,21 pakestan). IAA and BA both of them in concentration of 200 mg/lit enhanced diameter of florets, effectively (Tables 1 and 2). Not only the PGR avoid water loss of plant, but also the water uptake enhances and water balance improved (Emongor and Shwenyane, 2004). It was found that IAA and BA increase in cut flower longevity. There was significant difference (p≤01) in soluble sugar content among treatments. The highest sugar content in both petals and leaves were observed after 5 days (table 2). A significant effect (p≤01) obtained on flowers treated with IAA and BA.IAA does by 200 mg/lit and BA by 200mg/lit had high sugar content in petals and leaves (figures 1 and 2). IAA in concentration of 150 and 200mg/lit resulted in high sugar content in the leaves and petals, respectively (tables 3 and 4) Carbohydrate concentration was the highest amount in the leaves in the first day after harvest while the highest amount in the petals was 10 days after harvesting (figures 2 and 4). Maybe transport of carbohydrate from leaves towards the flowers accounted for changes (Jones *et al.*, 1994; Swart, 1986). Vital processes of plant like respiration, syntheses and etc. required the substrates like carbohydrates. According the results, sugar content which presented in petals affects the vase life of cut flowers. Also, there was positive correlation between sugar content in petals and vase life, whereas it was negative correlation between sugar content and ethylene production (Kazoo and Kenichi, 1999). The results showed IAA and BA in concentration of 200 mg/l significantly enhanced protein content in the leaves and petals in comparison with other studied PGR (tables 3 and 4). Protein content in the petals was higher than the leaves in 1 and 5 days after harvesting while petals had lower protein concentration than the leaves in 10 days after harvesting (figures 3 and 4). These trials showed IAA significantly enhanced protein content in the leaf. Also in BA protein content in the petals was higher than the leaves on the 10$^{th}$ day after harvest benzyladenin increase vase life in cut flower by delay breakdown degradation protein (7isi). These trials showed BA significantly raised protein content in the petal. This could be protein transport towards the flower. IAA and BA prevent of chlorophyll degradation in leaves of cut flowers (Han, 2001). Result with application of BA which delay senescence of diminishing of internal concentrations of phytohormones may be associated with senescence processes in cut flower (Ferrant *et al.*, 2003) plant growth regulators is due to retarding the rate of breakdown of protein synthesis. Although prevented accelerated protein loss that was typical in detached leaves (Ho and Nichols, 1997).

**Table 1:** the effect of application IAA on germination, early flower, stalk elongation, diameter floret,

| Application | Germination (day after sowing) | Onset pedicel (day after sowing) | Stalk elongation | Diameter floret (millimeter) | Vase life (day) |
|---|---|---|---|---|---|
| Control | 22a | 74a | 62.3a | 85c | 8c |
| 100ppmIAA | 16b | 61b | 62a | 117.71a | 11ab |
| 150ppm IAA | 16b | 60b | 61a | 88.1bc | 10bc |
| 200ppmIAA | 15b | 58.3b | 62.3a | 93.6c | 11.6bc |

Values with different superscripts along columns are significantly different (p<0.01)

**Table 2:** the effect of application IAA on germination, early flower, stalk elongation, diameter floret

| Application | Germination (day after sowing) | Onset pedicel (day after sowing) | Stalk elongation | Diameter floret (millimeter) | Vase life (day) |
|---|---|---|---|---|---|
| Control | 23.33a | 74a | 59a | 82.4b | 8c |
| 100ppmIAA | 16.33b | 63b | 65a | 94.4a | 11abc |
| 150ppm IAA | 15.66c | 62c | 65a | 95.5a | 12ab |
| 200ppmIAA | 15c | 61c | 66a | 95.7a | 14a |

Values with different superscripts along columns are significantly different (p<0.01)

**Table 3:** Effect of pretreatment bulbs with IAA on soluble sugars and protein content of petals and leaves of Gladiolus

| IAA(mg/lit) | protein petal | protein leaf | carbohydrate petal | carbohydrate leaf | chlorophyll leaf |
|---|---|---|---|---|---|
| 0 | c3.8 | c4.5 | d100 | d100.2 | c0.18 |
| 100 | c6.5 | c5.5 | b140 | c95.8 | b0.39 |
| 150 | b8.9 | b9.3 | a150 | b180 | a0.42 |
| 200 | a10.3 | a9.8 | c115 | a224.3 | a0.42 |

Values with different superscripts along columns are significantly different (p<0.01)

**Table 4:** Effect of pretreatment bulbs with BA on soluble sugars and protein content of petals and leaves of Gladiolus

| BA(mg/lit) | protein petal | protein leaf | carbohydrate petal | carbohydrate leaf | chlorophyll leaf |
|---|---|---|---|---|---|
| 0 | c3.8 | c4.5 | d100 | d100 | c 0.18 |
| 100 | b6.5 | b 5.9 | c 110 | b 140 | b0.26 |
| 150 | b6.7 | b5.9 | b 157 | b 140 | a0.28 |
| 200 | a7.5 | a 7.6 | a 159.7 | a 150 | a0.28 |

Values with different superscripts along columns are significantly different (p<0.01)

In this study, IAA and BA affected the chlorophyll content of gladiolus. Treatment with100 mg/l IAA and BA was the most effective one in retarding chlorophyll degradation as evidenced by the retention of high leaf chlorophyll content (figures 3 and 4). Plant growth regulators could delay the degradation of chlorophyll by possibly delaying the breakdown of protein used in the synthesis of chlorophyll (Hafman, 1988). Vase life per treatment was enhanced due to the IAA treatments while control plant exhibited a mean of only 8 day whereas the IAA and BA treated with 200mg/l dose had 12 day (figures 1 and 3). Plant grow regulator delaying the senescence of flower and reduced the effect of ethylene in promoting. There is a possibility of Plant grow regulator by either quality the sensitivity of the tissue ethylene or by delaying the natural rise in ethylene production (Mayak and Halevy, 1980; Jones *et al.*, 1973).

Finally, BA and IAA in concentration of 200 mg/l has the potential to be used as a commercial cut flower preservative for prolongs the vase life and postharvest quality of Gladiolus cut flowers.

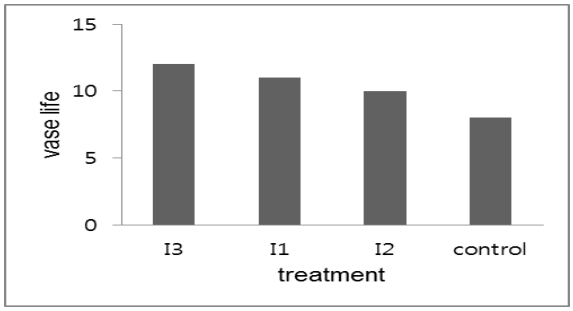


**Figure 1:** effects of various IAA per treatment bulbs on the vase life

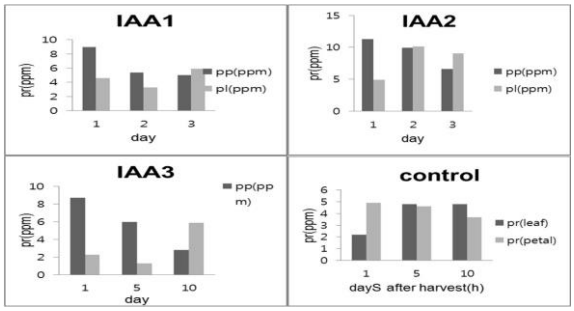


**Figure 2:** effects of various IAA per treatment bulbs on the change soluble carbohydrate of the upper most petal and leaf during vase life in gladiolus

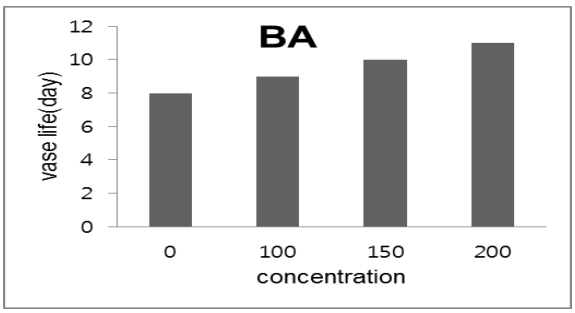


**Figure 3:** effects of various IAA per treatment bulbs on the vase life

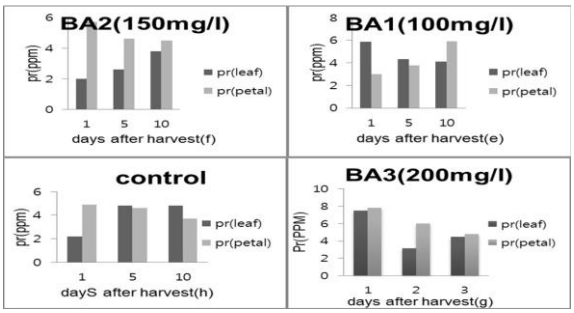


**Figure 4:** effects of various BA per treatment bulbs on the change soluble carbohydrate of the upper most petal and leaf during vase life in gladiolus.

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