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Gibberellic Acid (GA_3) and Benzyl Adenine (BA) Effects on the Vase Life of Cycad's Cut Foliage

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Abstract

Cycad is one of the most important cut foliage plants with several usages. This experiment was focused on increasing the vase life of cycad's cut foliage in normal storage condition. Experiment was based on a CRD with three replications. Treatments were conducted for four different levels of gibberellic acid (GA_3) and benzyl adenine (BA) each one with 0, 50, 100 and 150 mg L^{-1}, according to mentioned statistical design. In all treatments except control, silver nitrate ($AgNO_3$) with 40 mg L^{-1} and 3% sucrose were used. The measured traits were; vase life, chlorophyll and carotenoid content, water loss and some external quality factors. Results showed that GA_3 had significant and positive effects on increasing the vase life of cycad's cut foliage and kept the external quality in good condition. However, unlike the gibberellic acid, treatment with high concentration of BA decreased vase life of cycad's cut foliage. Also, relation between storage time, BA and GA_3 on most of measured external factors like leaflet dryness and fragility, were significant. The best treatments were selected as 50 mg L^{-1} GA_3 without BA which increased the vase life of cycad's leaves to 101 days in storage condition.

Keywords: Carotenoid, Chlorophyll, Cycad, Treatments, Water loss.

INTRODUCTION

Beautiful leaves of cycad (*Cycas* sp.) are used for bunch of flowers arrangement in many ceremonies. Unfavorable environmental conditions of leaves may reduce vase life of cut foliage significantly.

Postharvest senescence is major factor in marketing management of most cut flowers and leaves. Several researches have been done to show the effect of different chemicals on increasing vase life of cut flowers (Da silva, 2003). Today, quality of cut flowers during post-harvest process is a critical issue. Treatment of flowers with preservative materials (flower preservative solutions) is recommended during postharvest preservation (Ebrahimzade *et al.,* 2003).

The cut flowers will keep their freshness in preservative solution. These solutions should be prepared according to guidance on the provided label of package. Most of the solution compounds are prepared for similar goals and similar flower species (Nowak and Mynett, 1985). The importance of preservative solutions is related to their effects on delaying senescence processes. Main compositions of preservative solutions are carbohydrates, anti-bacteria's, anti-microbes, anti-ethylene, hormones and nutrients (Nowak and Mynett, 1985). Preservative solutions can be different for variable species and in cases for different variety.

In general, to increase the vase life of cut foliage, various treatments can be used, in which sucrose is one of the most common of them. Beginning of cellular and carbohydrates deterioration is a primary sign of senescence stage in harvested plant tissues, and truly existence of carbohydrates would strengthen the vase life (Ebrahim zade *et al.,* 2003). The application of 0.25% sucrose to vase solutions significantly enhanced vase life in *Eucalyptus crenulata* and *Eucalyptus gunnii* (Jones *et al*., 1993). $AgNO_3$ (20 mg L^{-1}) with sucrose (4%) delays bent neck (10 days) and petals abscission (7 days) of cut gerbera compared to control flowers. $AgNO_3$ (20 mg L^{-1}) and sucrose 6% prevent from petals' pale about 16.7 days, in comparing with 8 days in control (Nair *et al*., 2003).

GA_3 and BA are recommended to increase the vase life of young cut flowers and delaying their senescence in which has been showed in the finding of Pinto *et al*. (2007) on the cut foliage of *Calathea louisae*. Their results showed that a combination of GA_3 or BA (250 and 500 mg L^{-1}) significantly extended the longevity of cut foliage. It has been also showed that GA_3 and BA (250 and 500 mg L^{-1}) maintained leaves' green color and brightness for a longer time compared to control.

BA (100 mg L^{-1} in plunge and spray method) increased the vase life of two varieties of *Anthurium, Heliconia psittacorum* "Andromeda", *H. chartacea* "Sexy Pink" and *Alpinia perpurata*, but it wasn't effective on the vase life of *Zingiber spectabilis, Strelitzia* and *Dicranopteris linearis*. Difference *Anthurium* varieties show different vase life from 20% decrease to more than twice increase, which is depends on BA treatment (Paul and Chantrachit, 2000). Vase life of cut foliage of *Ctenathe setosa* significantly extended when treated with GA_3 or BA treatments (6 days compared to control). Moreover in the mentioned study, leaves color and brightness significantly increased compared to control. So, in the present research, there has been more focus on the usage of different preservative solutions on the vase life of cycads' cut foliage.

MATERIALS AND METHODS

Plant materials

Experiment was carried out in Horticultural Department of Gorgan University of Agricultural Sciences and Natural Resources (GUASNR). Cut foliage of cycad (*Cycas* sp.) were prepared from a greenhouse near the Gorgan. After transferring the cut foliage to the laboratory, the leaves were put into pots which their mouth were covered by plastic film to prevent evaporation of the vase solution from pots. Light density, temperature and relative humidity were kept close to the room conditions and were recorded every day. Average of temperature, light and relative humidity in the storage during the study were 20°C, 865 lux (12/12 day- night) and 70 %, respectively. Chlorophyll a,b and ab, carotenoid, water loss and external quality of cut foliage were measured during the experiment.

Chemicals

Chemical solutions were prepared and set in the pots in appropriate concentrations. Each pot contained 1 L of solution. GA_3 and BA were used in 4 levels (0, 50, 100 & 150 mg L^{-1}) in a mutual experiment design, so finally there were 16 treatments with 3 replications. Moreover a control treatment (just distilled water) was considered for comparing effect of various treatments.

Chlorophyll and carotenoid, leaf quality and vase solution uptake

Leaf chlorophyll and carotenoid were measured based on Arnon method (Arnon, 1956). To do this, the amount of one gram of leaves in each treatment, cut and then carefully weighed and each sample was powdered. Then with acetone 80% reached to 100 ml. After this step, samples located within the Satryfyvzh and then the chlorophyll was measured by Spectrophotometer.

Six characteristics were considered for measurement of external quality of leaves which were included; leaflet burning, getting tube like form, paleness, bent angle of main vein and finally dryness and fragility of leaves. Each one of these was measured for each leaf separately. The scoring method was based on 1-10 Amerin method (Amerin *et al*., 1956). The method was based on observable external change. Each cut foliage was given score of 10, when it was intact with any external negative change. But when some external changes were seen during the storage period, scores were then decreased. Final given score was obtained from average of five qualitative factors as already mentioned (Fig. 1). Durability of cut foliage and remained vase solution have been other factors which were measured.

To measure the volume of the internal solution, pots were graded before filling with solution, by adding water by one -liter flask and were graded to ten episodes (each episode 100 ml). Measuring internal volume of the solution was generally 8 times. At each stage, the remaining solution per each container were measured and recorded. Leaf chlorophyll and carotenoids were measured as Mazumdar and Majumdar (2003) method.

Research design was based on a randomized completely design. Results were analyzed using SPSS.17 software and LSD test at 5 percent level.

These treatments a control treatment (just distilled water) was considered for comparing various treatments effects.

RESULTS

General results

Analysis of variance showed that the effects of gibberellic acid and benzyl adenine are statistically significant at the 5% level and increased vase life of cycads cut foliages.

The best treatments were selected as 50 mg L^{-1} of GA_3 without BA which increased the vase life of cycad's leaves to 101 days in storage condition.

Fig. 1. Scoring the leaves during vase life. left to right: 6-8, 9-10, 4-5, 1-3.

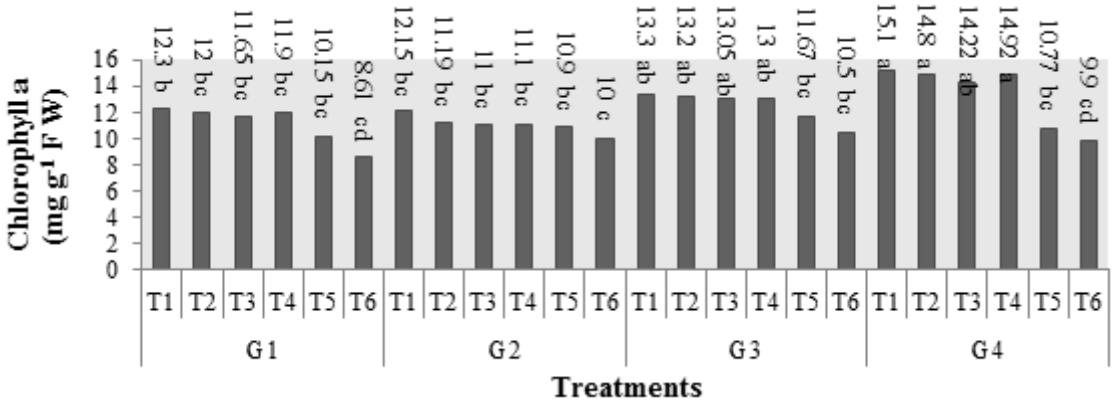

Fig. 2. Mutual effect of GA_3 and time on the chlorophyll a of cycad cut foliage.

T1= 12 Nov.
T2=22 Nov.
T3=7 Dec.
T4=18 Dec.
T5=31 Dec.
T6=9 Jan
G1=Control
G2=50 mg L^{-1}, Gibberellic acid
G3=100 mg L^{-1}, Gibberellic acid
G4=150 mg L^{-1}, Gibberellic acid

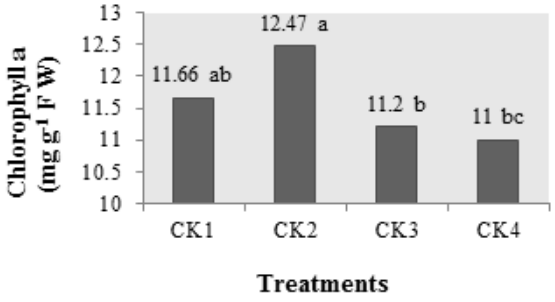

Fig. 3. Effect of cytokinin (benzyl adenine) on chlorophyll a of cycad's cut foliage

Ck1=Control
Ck2 =50 mg L^{-1}, cytokinin
Ck3 =100 mg L^{-1}, cytokinin
Ck4=150 mg L^{-1}, cytokinin

Chlorophyll and carotenoid of cut foliage

The lowest reduction in the content of chlorophyll and carotenoid was observed in the vases in which contained 50 and 100 mg L^{-1} GA_3 without BA (Fig. 3). Instead most demolished cut leaves were seen in treatments with high concentration of benzyl adenine; in other word by increasing the BA concentration, speed of leaf demolition increased significantly in compare with control during the time.

During the period of study, chlorophyll a, b and carotenoid were decreased (Fig. 1, 4, 5, 6 and 7). Until fourth measurement, no changes were observed in chlorophyll content of leaves (Fig. 4). Contrary to that at 5 and 6 stages the amount of chlorophyll was decreased signifi-

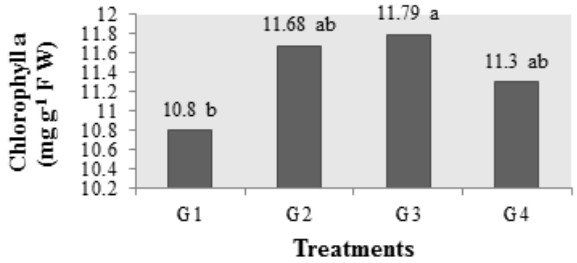

Fig. 4. Effect of GA_3 on chlorophyll a of cycad cut foliage.

G1=Control = 0 mg L^{-1}, Gibberellic acid
G2=50 mg L^{-1}, Gibberellic acid
G3=100 mg L^{-1}, Gibberellic acid
G4=150 mg L^{-1}, Gibberellic acid

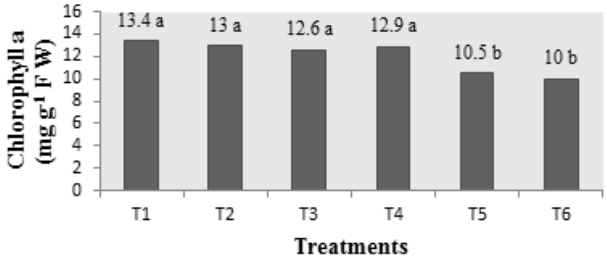

Fig. 5. Effect of preserved time on the chlorophyll a of cycad's cut foliage.

T1= 12 Nov.
T2=22 Nov.
T3=7 Dec.
T4=18 Dec.
T5=31 Dec.
T6=9 Jan.

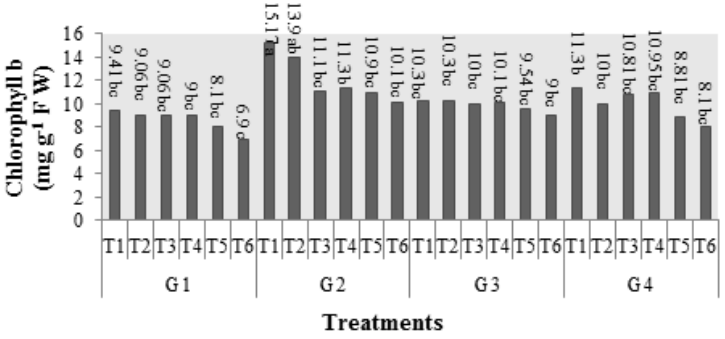

Fig. 6. Mutual effect of GA_3 and time on the chlorophyll b of cycad cut foliage.

T1= 12 Nov.
T2=22 Nov.
T3=7 Dec.
T4=18 Dec.
T5=31 Dec.
T6=9 Jan.
G1=Control
G2=50 mg L^{-1}, Gibberellic acid
G3=100 mg L^{-1}, Gibberellic acid
G4=150 mg L^{-1}, Gibberellic acid

cantly. Fig. 3 shows that the highest amount of chlorophyll a was recorded in vases contained 100 mg L^{-1} GA_3 (11.79 mg g^{-1} in compared with control 10.54 mg g^{-1}). It seems that the 100 mg L^{-1} of GA_3 increases the life of chlorophyll molecule. Contrast to GA_3, the highest chlorophyll a was observed in the pots in which treated with 50 mg L^{-1} of BA with to 12.47 mg g^{-1} leaf fresh weight chlorophyll a that presented in Fig. 2. High concentration of BA was resulted low amount of chlorophyll content. So it can be concluded that concentration of 50 mg L^{-1} of BA could be the best used treatment to preserve the chlorophyll a content of cycad's cut foliage during preservation period.

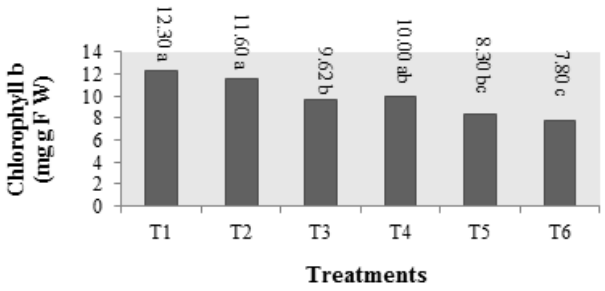

Fig. 7. Effect of preserved time on the chlorophyll b of cycad's cut foliage.

T1= 12 Nov.
T2=22 Nov.
T3=7 Dec.
T4=18 Dec.
T5=31 Dec.
T6=9 Jan.

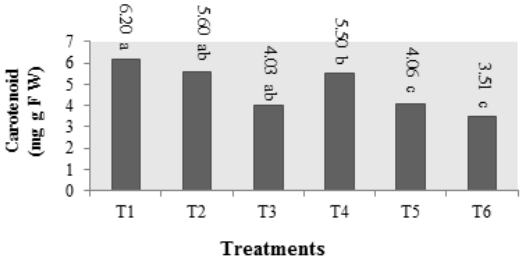

Fig. 8. Effect of preserved time on the carotenoid of cycad cut foliage.

T1= 12 Nov. T4=18 Dec.
T2=22 Nov. T5=31 Dec.
T3=7 Dec. T6=9 Jan.

Absorbed preservative solution

The treatment which absorbed most preservative solution during the time was treatment without any growth regulator which just had sucrose and $AgNO_3$. Next ones were treatment of 100 mg L^{-1} GA_3 without BA and also control, which absorbed more than 500 cc of pot preservative solution (Fig. 8 and 9). Generally treatments with GA_3 and without BA or with low concentrations of BA absorbed more solution, so it can be concluded in hormonal treatments that increasing the

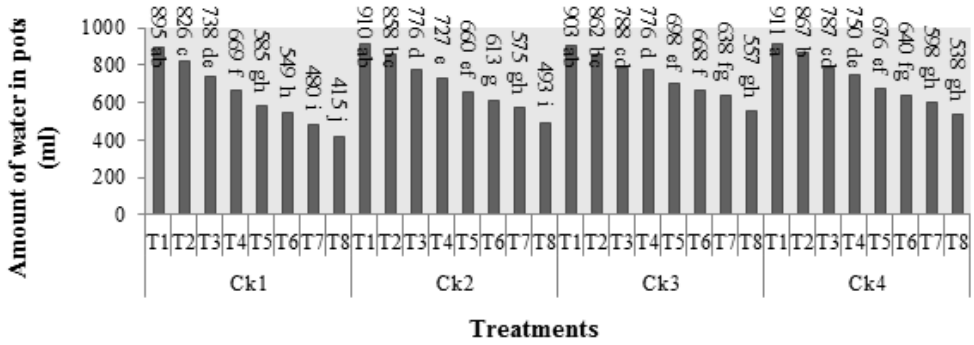

Fig. 9. Mutual effects of cytokinins (benzyl adenine) and time on the water-loss in pots.

T1= 13 Nov. T7= 14 Dec.
T2= 18 Nov. T8= 31 Dec.
T3= 23 Nov. Ck1=Control
T4=17 Nov. Ck2 =50 mg L^{-1}, cytokinin
T5= 4 Dec. Ck3 =100 mg L^{-1}, cytokinin
T6= 8 Dec Ck4=150 mg L^{-1}, cytokinin

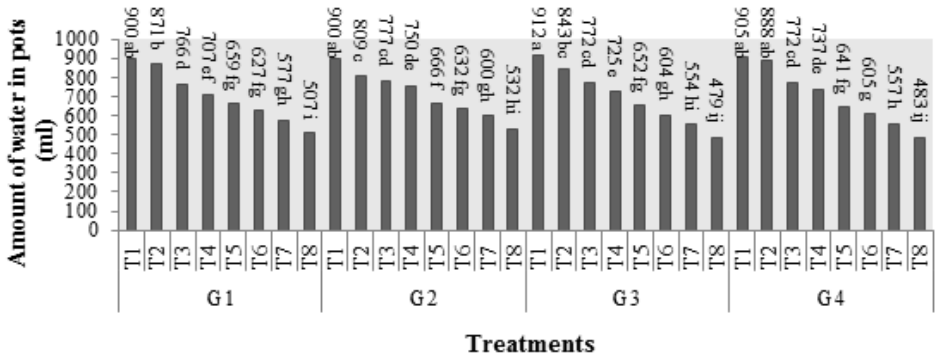

Fig. 10. Mutual effects of GA_3 (Gibberellic acid) and time on the water-loss in pots.

T1= 13 Nov. T7= 14 Dec.
T2= 18 Nov. T8= 31 Dec.
T3= 23 Nov. G1=Control
T4=17 Nov. G2=50 mg L^{-1}, Gibberellic acid
T5= 4 Dec. G3=100 mg L^{-1}, Gibberellic acid
T6= 8 Dec. G4=150 mg L^{-1}, Gibberellic acid

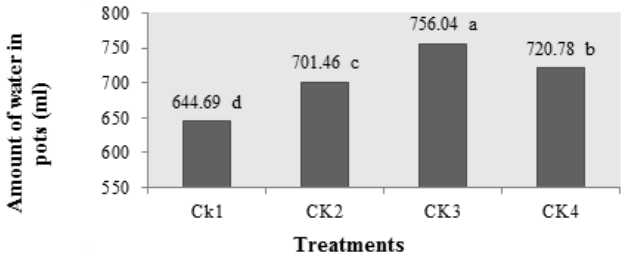

Fig. 11. Effect of cytokinins (benzyl adenine) on water loss in pots.

Ck1=Control
Ck2 =50 mg L^{-1}, cytokinin
Ck3 =100 mg L^{-1}, cytokinin
Ck4=150 mg L^{-1}, cytokinin

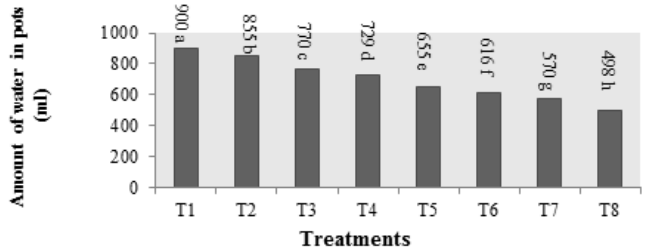

Fig. 12. Process of water absorption during the time.

T1= 13 Nov.
T2= 18 Nov.
T3= 23 Nov.
T4=17 Nov.
T5= 4 Dec.
T6= 8 Dec.
T7= 14 Dec.
T8= 31 Dec.

absorption of preserving solution causes increase in the vase life of cut foliage (Fig. 10). When no treatment is used in preservative solution it can't be effective on increasing the vase life of cut foliage. Generally process of absorbing the vase solution by leaves was decreased gradually during the time (Fig. 11).

Changes in external quality of cut foliage

Another important factor was change in external quality of cut foliage which included five measured indexes containing leaflet burning, getting tube form, paleness, bent angle of mid-ribe, dryness and fragility which among them first change appeared in the Cycad's preserved cut leaves was paleness. Next appeared symptom in cut leaves was dryness and fragility. It means cut leaves starting to dry soon after paleness. Then percentage of fragility was increased and simultaneously the overall capacity of bacterial concentration in vase solution was decreased. Next negative symptom was bending of midrib in cut Cycad leaves. Bending was appeared in the middle of foliage to the right or left direction. By passing the time, bent angle became more, even in some cases, this leaf angle reached to over 90° of deviation. Next change was getting tube shape by curving leaflets toward midrib and the last symptom was leaflet burning. The last symptom happened less than others. For each leaf the overall given score was the average of 5 given scores separately to all above indexes. Given score was according to the following scales of leaf condition: 10=Excellent, 9=Very good, 8=Good, 7=Acceptable, 6=Average, 5=A little bad, 4=relatively bad, 3=Bad, 2=Not acceptable, 1=Very bad. A leaf condition which reached to the average scale of 5 (stage of a little bad), was a leaf which wasn't desirable and actually passed its normal vase life. In high concentration of benzyl adenine, leaf demolition was seen faster than control, and their vase life were short. In eleventh stage of measurement, the vase life of cut leaves was finished practically, but in treatments of GA_3 and low concentrations of benzyl adenine, demolition was happened more slowly. When concentrations of 50, 100 and 150 mg L^{-1} of GA_3 were used without benzyl adenine (Fig. 12), most leaf durability and leaf permanence was seen, so it can be concluded even with

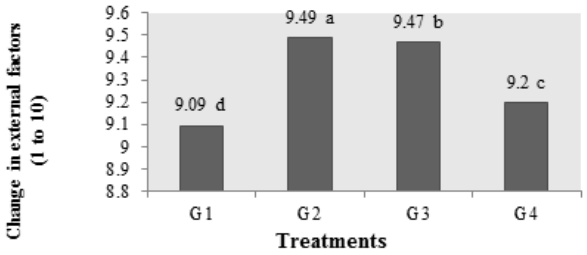

Fig. 13. Effect of GA_3 on changing external factors of cycad cut leaves.

G1=Control
G2=50 mg L^{-1}, Gibberellic acid
G3=100 mg L^{-1}, Gibberellic acid
G4=150 mg L^{-1}, Gibberellic acid

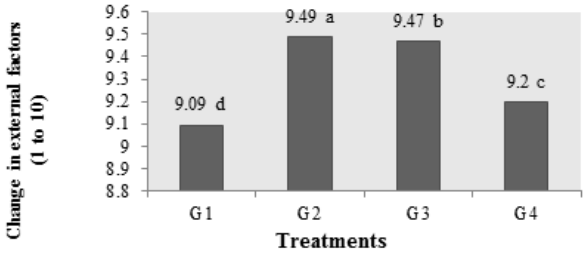

Fig. 14. Mutual effect of cytokinin (benzyl adenine) and gibberellic acid on change the external factors of cycad cut leaves.

G1=Control
G2=50 mg L^{-1}, Gibberellic acid
G3=100 mg L^{-1}, Gibberellic acid
G4=150 mg L^{-1}, Gibberellic acid

presence of gibberellic acid, increasing the level of BA causes more leaf demolition. Least leaf permanence was happened in treatment 100 & 150 mg L^{-1} benzyl adenine. In these concentrations leaves characters were measurable even until stage 14 (30 of January) but least demolition was happened when concentrations of BA were 0 and 50 mg L^{-1}. So it can be concluded that low concentration of BA has different effects on permanence of Cycad's cut leaves and caused longer preservation of the leaves (Fig. 13 and 14). The best concentrations of GA3 were 50 and 100 mg L^{-1} which caused leaf durability and permanence until stages of 15 and 16 (90 and 101 days after starting the experiment). Since the stage 11 of measurements (64 days after starting the experiment) large decrease was seen on cut leaves' vase life which was particularly related to the applied treatments but in some treatments with attention to the kind of treatment, leaves were preserved till stage of 16 (Fig. 15). Generally the best result was seen from concentration 50, 100 and 150 mg L^{-1} of GA_3 and concentration of 0 and 50 mg L^{-1} of benzyl adenine.

Durability of cut leaves

Best durability was seen in treatment of 50 mg L^{-1} GA_3 and after that, treatment of 100 mg L^{-1} of GA_3 without using BA which caused vase life of the leaves reach to 101 and 90 days respectively.

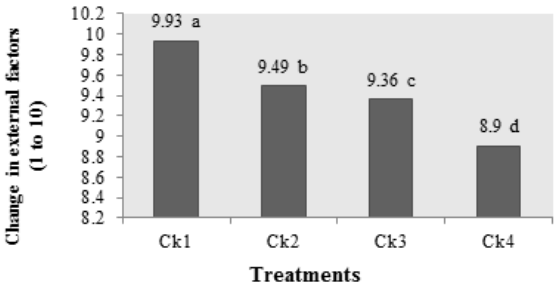

Fig. 15. Effect of cytokinins (benzyl adenine) on external factors of cycad cut foliage.

Ck1=Control
Ck2 =50 mg L^{-1}, cytokinin
Ck3 =100 mg L^{-1}, cytokinin
Ck4=150 mg L^{-1}, cytokinin

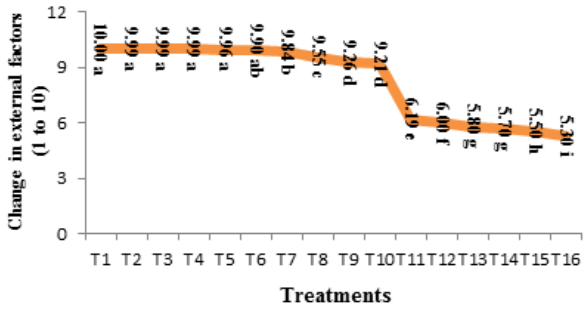

Fig. 16. Effect of preserved time on change the external factors of cycad cut foliage.

T1= 11 Nov. T9=28 Dec.
T2=20 Nov. T10=3 Jan.
T3=24 Nov. T11=9 Jan.
T4=27 Nov. T12=16 Jan.
T5=3 Dec. T13=23 Jan.
T6=6 Dec. T14=30 Jan.
T7=12 Dec. T15=6 Feb.
T8=23 Dec. T16=15 Feb.

Based on results, GA_3 is a suitable hormone for preserving the cut leaves of cycad, but high concentrations of BA aren't fit for this aim. It can be concluded that gibberellic acid extended the longevity of the leaves because of protecting the cells, preventing chlorophyll loss, affecting chloroplast and membrane, increasing photosynthesis and so delaying leaf senescence. Permanence of green color in leaves is a sign of increasing the vase life of cut leaves which treated with gibberellic acids. Such results were not seen in treatments containing levels of benzyl adenine.

DISCUSSION

Result of this experiment shows GA_3 is so useful in extending the vase life and durability of Cycad's cut leaves. It preserved chlorophyll and carotenoid contents of the cut leaves and also strengthen their delicateness and succulence. Gibberellic acid in comparison with benzyl adenine, caused absorption of more vase solution by leaves and so it preserved leaf freshness (succulence) during the post-harvest period. However when $AgNO_3$ and sucrose were used, vase life of leaves were increased (Ketsa *et al*., 1995; Philosoph Hadas *et al.*, 2010). But it wasn't so significant in comparison with using the growth regulators which increased the vase life of leaves, and when both of them were used it was significant. First negative symptom which decreased leaf quality was paleness. Other symptoms were leaf dryness, fragility, bent angle, getting tube form and finally leaflet burning respectively. Among the applied concentrations of gibberllic acid, the best and most suitable concentrations were 50 and 100 mg L^{-1}, so use of these concentrations is advisable for preserving the Cycad's cut leaves (Kjonboon and Kanlayanarat., 2005). Results of these experiment shows high concentrations of BA could not increase the Cycad's cut leaves permanence, because it caused earlier leaf demolition and faster chlorophyll demolition. Moreover this results show that preserving environmental conditions of current study like average humidity of 70%, temperature of 20°C and light intensity of 865 Lux were relatively good situation for preserving Cycad's cut leaves. In current research, the best treatment for longer durability of the Cycad cut leaves was treatment of 50 mg L^{-1} GA_3 plus 40 mg L^{-1} $AgNO_3$ and 3% sucrose. It is necessary to be mentioned that natural postharvest life of cut leaves of cycad in flower shops with no controlling atmosphere (light, temperature and humidity) is too short. As the vase life of control in current study were about 30 days in compare with 12 days in no controlled condition (open environment under shade) so the above claim will be confirmed. Results showed that GA_3 had significantly positive effect on vase life and leaf quality of Cycad as achieved on some other studies (Pinto *et al*., 2007; Danayi *et al.*, 2009; Skutnik *et al.*, 2001; Mutui *et al*., 2006). But unlike the expectation, BA in high concentrations had destructive effect and shortens the vase life of the cut leaves, accelerating leaf dem-

olition and chlorophyll loss during preservation (Pinto *et al.*, 2007; Paul and Chantrachit., 2000; Danayi *et al.*, 2009). The best results were earned from treatments of GA_3 50 mg L^{-1} and GA_3 100 mg L^{-1} without added BA which caused Cycad vase life reach to 101 and 90 days respectively. These are sort of record in preserving the cut leaves. Results showed that the relation between time, BA and GA_3 on chlorophyll, carotenoid and water loss were non-significant but on external leaf factor was significant, instead mutual effects, between gibberellic acid-BA and BA-time on chlorophyll and carotenoid were non-significant but on other factors were significant.

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