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EFFECTS OF BENZYL ADENINE (BA) AND GIBBERELLIC ACID (GA_3) ON PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF GLADIOLUS (*GLADIOLUS GRANDIFLORUS* L.) SPIKES

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Abstract

The uniform sized corms (3.5-4.0 cm dia) of *Gladiolus grandiflorus* L. were dipped in various concentrations of BA (50,75, 100 and 125 mg/l) and GA_3 (50 , 75 , 100 and 125 mg/l) for 24 hrs. The Membrane stability index (MSI), Relative water content (RWC), total soluble sugars and total protein content of florets increased with the increased concentrations of BA and GA_3. The post harvest quality parameters also improved with BA and GA_3 treatments as compared to control but GA_3 had more significant effect than BA. The treatment with 125 mg/l GA_3 significantly hastened the opening of basal floret, increased the floret size, number of florets open at one time, per cent of opening of florets, water absorbed and led to maximum reduction in pH that ultimately led to improvement in vase life.

Introduction

Gladiolus (*Gladiolus grandiflorus*), also known as 'queen of bulbous flowers' has gained prominence in the floriculture industry due to its alluring and pretty florets found on majestic spikes. It is propagated through corms and thus quality of spikes is highly dependent on the quality of corms and cormels. Corms produce standard flower spike and daughter cormels after a period of two to three seasons (Bhande *et al.* 2015).

The post harvest management of spikes is a key factor in determining the net profitability of the produce to the growers as flowers are highly perishable in nature. After harvest, florets face various stresses that lead to increased respiratory activity, decreased water uptake ability, lowered carbohydrate content etc. (Gul *et al.* 2012). The reserves of carbohydrates, proteins, water and sensitivity to ethylene determine the longevity of cut flowers (Singh and Srivastava 2006).

The use of growth regulators especially cytokinins and gibberellins have received attention due to their multifaceted physiological effects that could improve all aspects of growth from sprouting till production of corms in gladiolus (Emongor 2004). Cytokinins are highly preferred by horticulturists to improve the post harvest life of crops by causing a significant delay in senescence. Likewise, gibberellic acid is also known for delaying processes like fading of flower colour and postponing wilting of flowers by suppressing proteolysis at senescence (Eason *et al.* 2002). The growth regulators at different concentrations are used as pulsing or holding solutions that also improve the keeping quality of gladiolus or other cut flowers (Mohammadi *et al.* 2014).

Thus, the present study was planned to evaluate the effect of dip treatments of corms in cytokinin i.e. BA and GA_3 on post-harvest quality of gladiolus spikes.

Materials and Methods

The uniform sized corms (3.5-4.0 cm dia) of gladiolus var. Punjab Glance and Priscilla were dipped in various concentrations (50, 75, 100 and 125 mg/l) of benzyl adenine and gibberellic acid for 24 hrs. The corms without dipping in any solution/water served as control. Thus, there were total of ten treatments- T_1= 50 mg/l, BA, T_2= 75 mg/l, BA, T_3= 100 mg/l, BA, T_4=125 mg/l, BA.

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T_5=50 mg/l, GA_3, T_6=75 mg/l, GA_3, T_7=100 mg/l, GA_3, T_8=125 mg/l, GA_3, T_9 = Water and T_{10}= Control. A stock solution of 125 mg/l of BA/GA_3 was prepared by dissolving 125 mg/l of BA/GA_3 in small quantity of ethanol, followed by addition of distilled water to make the final volume to 1 litre. Then, stock solution of BA/GA_3 was diluted to prepare different concentrations. All the treatments were replicated thrice with three spikes per replication and nine spikes per treatment. The corms were raised in the Research Farms of Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana following recommended agronomical practices to obtain spikes. The spikes were harvested at the commercial harvest stage i.e. the tight bud stage (when basal 1-2 florets showed colour). After harvesting, the spikes were grouped in bundles of 3 each and each bundle was placed in vertical position in vase containing water so that basal 5-7 cm portions of the spikes were dipped in distilled water. The observations recorded at tight bud stage for post harvest quality parameters were vase life, days to opening of basal floret, floret size, maximum florets open at one time, per cent of opening of florets, change in pH of vase solution, total water absorbed and physiological loss in weight.

The experiment was conducted in a factorial completely randomized design (CRD). Data were subjected to statistical analysis of variance (ANOVA) using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). Mean comparisons to calculate significant differences were performed using Least Significant Differences (LSD) test at 0.05 level of probability.

Results and Discussions

The post harvest life of spikes is interdependent upon different quality parameters and among these, vase life is the most crucial parameter as it adds to profitability of farmers. Corm soaking treatments significantly increased the vase life of gladiolus spikes as from 8.16 days in control to 15.16 days with 125 mg/l GA_3 (T8) and 13.33 days with 125 mg/l BA (T4). Among all the treatments, 125 mg/l GA_3 (T8) recorded maximum vase life in both varieties (Fig. 1). The present study is in agreement with the findings of Chopde *et al.* (2012) who also reported enhancement of vase life by treatment with BA and GA_3 via delaying lipid peroxidation and ion leakage.

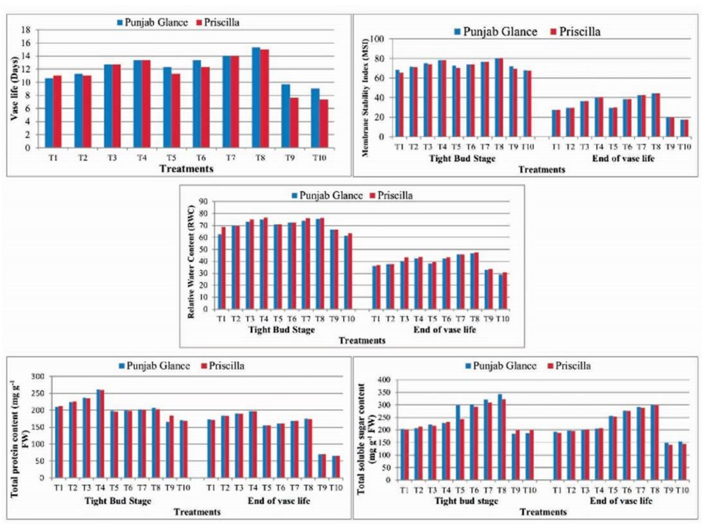

Fig. 1. Effects of BA and GA_3 on vase life, MSI, RWC, TPC and TSS of *Gladiolus grandiflorus*.

Days to the opening of basal floret is an important parameter influencing the vase life of spikes. The spikes resulting from the corms treated with 125 mg/l BA took maximum days (3.91

days) for the opening of basal floret whereas spikes from the corms treated with 125 mg/l GA_3 took minimum time (1 day) for the opening of basal floret (Table 1). Baskaran *et al.* (2014) reported delayed flowering in gladiolus with BA treatments. GA_3 corm treatment with 125 mg/l resulted in spikes with maximum floret size of 11.00 cm, which was 1.58 times more than the control (6.94 cm). On the contrary, corm treatment with BA resulted in reduction of size of florets. This increase could be accounted to the increased number of leaves and leaf area with GA_3 treatment that increased the photosynthetic ability of the plant and thus positively influenced the reproductive attributes (Yousif and Mahmoud 2006). The results revealed that floret size increased with increasing concentration of GA_3 and vice versa with BA treatments. Similar results of decreased floret size following BA treatments were also reported by Sajjad *et al.* (2015) in gladiolus.

Table 1. Effects of BA and GA_3 on various post harvest and biochemical parameters of *Gladiolus grandiflorus*.

Parameters	Days to the opening of basal florets			Per cent opening of florets			Size of fully expanded floret (cm)		
Treatments	Punjab glance	Priscilla	Mean	Punjab glance	Priscilla	Mean	Punjab Glance	Priscilla	Mean
50 mg/l BA (T_1)	2.16^{cdef}	2.00^{cdef}	2.08^{DE}	61.01^{h}	60.64^{g}	60.82^{D}	7.97^{efg}	8.33^{def}	8.15^{D}
75 mg/l BA (T_2)	2.50^{becd}	2.33^{cde}	2.41^{CD}	60.35^{j}	60.12^{k}	60.23^{E}	7.84^{fgh}	7.40^{ghij}	7.62^{EF}
100 mg/l BA (T_3)	2.83^{bcd}	3.00^{abc}	2.91^{B}	59.85^{l}	53.99^{q}	56.92^{G}	7.13^{hijk}	7.13^{hijk}	7.13^{G}
125 mg/l BA (T_4)	4.16^{a}	3.66^{ab}	3.91^{A}	54.66^{p}	49.73^{r}	52.19^{H}	6.45^{k}	6.70^{jk}	6.57^{H}
50 mg/l GA_3 (T_5)	2.33^{cde}	3.00^{abc}	2.66^{BC}	59.89^{m}	58.89^{l}	59.39^{F}	7.62^{fghi}	8.03^{efg}	7.82^{DE}
75 mg/l GA_3 (T_6)	2.00^{cdef}	2.33^{cde}	2.16^{DE}	62.32^{g}	66.68^{d}	64.50^{C}	8.60^{de}	9.03^{cd}	8.81^{C}
100 mg/l GA_3 (T_7)	1.83^{cdef}	2.00^{cdef}	1.91^{EF}	64.61^{f}	67.45^{c}	66.03^{B}	9.64^{bc}	10.20^{b}	9.92^{B}
125 mg/l GA_3 (T_8)	1.00^{f}	1.00^{f}	1.00^{G}	69.48^{b}	89.25^{a}	79.36^{A}	10.21^{b}	11.80^{a}	11.00^{A}
Water (T_9)	1.66^{def}	1.66^{def}	1.66^{F}	56.85^{n}	47.12^{s}	51.98^{I}	7.35^{ghij}	7.16^{hijk}	7.26^{GF}
Control (T_{10})	1.33^{ef}	1.33^{ef}	1.33^{F}	55.00^{o}	45.25^{t}	50.12^{J}	6.95^{ijk}	6.93^{ijk}	6.94^{GH}
Mean	2.18^{A}	2.23^{A}		60.96^{A}	59.91^{B}		7.97^{B}	8.27^{A}	
LSD (p = 0.05)	T=0.53	V=0.23	T×V= 0.89	T=0.01	V=0.005	T×V= 0.30	T=0.28	V=0.12	T×V= 1.09

Table 1 contd. right side

Maximum number of florets open at one time			Change in pH			Loss in physiological weight (%)			Water uptake (ml)		
Punjab glance	Priscilla	Mean	Punjab glance	Priscilla	Mean	Punjab glance	Priscilla	Mean	Punjab glance	Priscilla	Mean
7.00^{bc}	7.00^{bc}	7.00^{B}	0.45^{ghij}	0.52^{fghi}	0.49^{D}	14.60^{e}	14.46^{ef}	14.53^{C}	41.80^{gh}	40.30^{hi}	41.05^{F}
5.00^{fg}	6.33^{bc}	5.66^{D}	0.57^{efg}	0.61^{ef}	0.59^{C}	14.07^{efg}	13.41^{efghi}	13.74^{CD}	46.60^{ef}	43.50^{fgh}	45.05^{E}
5.00^{fg}	5.33^{def}	5.16^{DE}	0.70^{cde}	0.74^{bcd}	0.72^{B}	12.72^{ghjk}	12.5^{hijkl}	12.61^{EF}	48.70^{e}	49.73^{de}	49.21^{D}
4.66^{fg}	4.33^{g}	4.83^{EF}	0.81^{abc}	0.94^{a}	0.87^{A}	11.47^{klmno}	10.81^{no}	11.14^{HI}	53.86^{bc}	52.46^{bc}	54.16^{C}
4.66^{fg}	4.33^{g}	4.50^{EF}	0.41^{ijkl}	0.43^{hijk}	0.42^{E}	13.5^{efgh}	13.25^{fghij}	13.38^{DE}	44.40^{fg}	44.30^{fg}	44.35^{E}
5.66^{def}	6.66^{bcd}	6.16^{C}	0.44^{ghij}	0.55^{fgh}	0.49^{D}	12.09^{jklmn}	12.47^{hijklm}	12.28^{FG}	49.66^{de}	46.43^{ef}	48.05^{D}
6.66^{bcd}	7.33^{ab}	7.00^{B}	0.64^{def}	0.61^{def}	0.62^{C}	11.25^{lmno}	11.45^{klmno}	11.35^{GH}	60.80^{b}	56.56^{cd}	58.68^{B}
7.33^{ab}	8.33^{a}	7.83^{A}	0.84^{ab}	0.92^{a}	0.88^{A}	10.10^{o}	10.48^{o}	10.29^{I}	69.97^{a}	69.93^{a}	69.80^{A}
4.66^{g}	4.33^{g}	4.50^{EF}	0.39^{ijkl}	0.31^{kl}	0.35^{F}	32.57^{b}	27.69^{c}	31.12^{A}	37.03^{ij}	33.20^{k}	35.11^{F}
4.66^{fg}	4.33^{g}	4.33^{F}	0.37^{jkl}	0.28^{l}	0.32^{F}	34.56^{a}	24.75^{d}	28.66^{B}	34.73^{jk}	33.20^{k}	33.96^{F}
5.06^{B}	5.83^{A}					16.77^{A}	15.14^{B}		48.72^{A}	47.16^{B}	
T=0.82	V=0.36	T×V=1.17	T=0.05	V=0.02	T×V=0.14	T=0.90	V=0.40	T×V= 1.29	T=1.27	V=0.60	T×V= 3.93

The maximum number of florets open at one time increased with increasing concentration of GA_3 as 7.83 florets were open following GA_3 (125 mg/l) treatment whereas increasing concentration of BA adversely affected the opening as maximum 7 florets were open following 50 mg/l BA that declined to 4.83 florets following 125 mg/l BA treatment (Table 1). Thus, increase in number of florets open at one time with GA_3 could be related to the improvement in per cent opening of florets following GA_3 treatments. Treatment of corms with various concentrations of GA_3 improved the per cent opening of florets from 50.12 in control to 79.36 following GA_3 (125 mg/l) whereas BA treatment (50 mg/l) increased the per cent opening to 60.82.

Increase in concentration of both growth regulators led to increase in water uptake by spikes. The maximum amount of water (69.80 ml) was absorbed with 125 mg/l GA_3 followed by 58.68 ml with 100 mg/l of GA_3. The corresponding values of BA treatment were 54.16 ml and 49.21 ml, respectively, with 125 mg/l and 100 mg/l BA (Table 1).

The per cent opening of florets, floret size and maximum number of florets open at one time and total water absorbed were more than control but less than GA_3 with BA treatments. The possible explanation behind such behaviour of BA could be the promotion of sink activity of growing corms and cormels at the expense of development of flower spike (Baskaran *et al.* 2014).

Corm treatments with GA_3 and BA significantly improved per cent opening of florets, floret size, maximum number of florets open at one time and total water absorbed as compared to the control. The corresponding values were more with GA_3 as compared to BA corm treatments. This could be due to enhanced vegetative growth and metabolism with GA_3 treatments, leading to enhanced photosynthetic ability, assimilation of photosynthetic products and improved flowering attributes (Tawar *et al*. 2007). The positive influence of BA treatments as compared to control could be accounted to the promotion of sink activity of growing corms and cormels at the expense of development of flower spike (Baskaran *et al.* 2014).

The decrease in pH of vase water at the end of vase life of spikes was 0.88 with GA_3 (125 mg/l) and 0.87 with BA (125 mg/l) as compared to 0.32 in control (Table 1). The maintenance of low pH in the vase solution prevents growth of bacteria, inhibits vascular blockage and enhances conductivity of water in spikes leading to long vase life. The endogenous enzymes responsible for plugging of stems might get inhibited due to highly acidic environment, thus inhibiting microbial growth (Marousky 1971). The decrease in pH of vase water with increasing concentration of BA and GA_3 supports their promontory effect on vase life of spikes that increased with increased concentrations of BA and GA_3.

Corms treated with 125 mg/l GA_3 recorded minimum per cent loss in physiological weight (10.29), followed by 125 mg/l BA (11.14) as compared to control (28.66) (Table 1). This reduction in loss of physiological weight of spikes might be due to higher water uptake by spikes. Higher water uptake not only regulates metabolic processes but also maintains freshness and turgidity of cut flowers that further improves the vase life (Woltering *et al.* 2018). Both GA_3 and BA are known for their role in increasing the permeability of cell membrane to glucose, conversion of sucrose and starch into glucose, leading to decrease in water potential of cells. This leads to increased water uptake and reduced per cent loss in physiological weight (Salisbury and Ross 2010). In this study, there was an increase in water uptake and reduction in per cent loss in weight that could have improved vase life of spikes. Similar results have been reported by Mangave *et al*. (2013) in Heliconia.

Membrane Stability Index (MSI) in florets was high at tight bud stage and decreased with the onset of senescence indicating deterioration of membranes (Fig. 1). Highest MSI of 80.50 and 44.87 was recorded at tight bud stage and end of vase life, respectively, in florets of GA_3 (125 mg/l), which were 1.19 and 2.53 times higher than the control, respectively. Minimum MSI was recorded in florets of control spikes that further declined with the onset of senescence (67.62 at tight bud and 17.70 at the end of vase life). Among all BA treatments, 125 mg/l BA recorded maximum MSI at both stages. The results are in agreement with the results reported by Zhang and Guo (1998) in chrysanthemum. The increase in MSI with GA_3 treatment can be attributed to its role in increasing calcium uptake by cells which results in reduction in electrolyte leakage (Jonas and MacMillan 1985).

Relative Water Content (RWC) increased with increasing concentration of BA and GA_3 in both the varieties and at both stages. At tight bud stage, highest RWC was recorded with 125 mg/l GA_3 which was 1.21 times higher than control. At the end of vase life, corm treatment with GA_3 at 125 mg/l had significantly higher mean RWC. Among all the BA concentrations, 125 mg/l BA recorded maximum RWC at both stages (Fig. 1). Increase in RWC with corm treatments with GA_3 might be due to its starch and polysaccharide hydrolysing property. The production of hydrolysis products (glucose and fructose) decrease water potential in cells of floret resulting in more water uptake and increased RWC (Emongor 2004). Similar results were reported by Hashemabadi *et al.* (2014) in carnation.

At tight bud stage, maximum protein content was recorded in BA (125 mg/l) which was 1.53 times higher than control. Between the two varieties, Priscilla recorded 0.35 per cent higher protein content than Punjab Glance. Protein content of florets decreased with the onset of senescence. At the end of vase life, highest protein content was recorded with BA 125 mg/l which was 2.82 times higher than control. Low protein content in florets at the end of vase life could be due to simultaneous protein degradation and protein synthesis inhibition. High protein content in florets due to treatment with BA and GA_3 might be due to their effect in lowering the activity of protease enzyme as reported by Elanchezhian and Srivastava (2001) in chrysanthemum.

Corm treatments with BA and GA_3 significantly increased the total soluble sugar content in florets at both stages and in both varieties. At tight bud stage, maximum sugar content was recorded in treatment with GA_3 (125 mg/l), which was 1.72 times higher than the control. Between the two varieties, Punjab Glance recorded 2.80 per cent higher sugar content than Priscilla. Sugar content of florets decreased with the onset of senescence. GA_3 (125 mg/l) recorded maximum sugar content (299.57 mg/g FW) at the end of vase life (Fig. 1). These results can be explained on the basis of role of GA_3 in preventing the formation of enzymes responsible for starch hydrolysis (Ichimura *et al.* 2002). High sugar content due to treatment with BA might be due to increased activity of invertase and amylase (Han 1995). Similar results were reported by Faraji *et al.* (2011) in gladiolus.

Among both cultivars, Punjab Glance recorded minimum number of days for the opening of basal floret (1.00), minimum loss in physiological weight (10.10 g) and maximum water uptake (69.97 ml) with GA_3 (125 mg/l). Whereas, Priscilla recorded maximum per cent flowering (89.25 %), floret size (11.80 cm), maximum number of florets open at one time (8.33) with GA_3 @ 125 mg/l.

Correlation analysis was done to find the effect of various post harvest and biochemical parameters at various stages on the vase life of gladiolus spikes. The analysis revealed significant positive coefficient of correlation (r) between vase life and different parameters except days to opening of florets (Table 2). The vase life had strong positive and significant correlation with membrane stability index (r = 0.962), relative water content (r = 0.948) and total water absorbed (0.936) and significant inverse relationship with loss in physiological weight (0.81). The content of metabolic reserves viz. total soluble sugars at tight bud stage (r = 0.792) and end of vase life (r = 0.862), soluble proteins at tight bud stage (r = 0.528) and end of vase life (r = 0.782) significantly influenced the vase life as revealed by high values of correlation coefficients. The

Table 2. Correlation analysis among various post harvest and biochemical parameters of gladiolus spikes.

	Vase life	MSI at tight bud stage	MSI at end of vase life	RWC at tight bud stage	RWC at end of vase life	TPC at tight bud stage	TPC at end of vase life	TSS at tight bud stage	TSS at end of vase life	Days to opening of basal floret	Per cent opening	Floret size	Maximum No.of florets open at one time	Change in pH	Loss in weight	Water absorbed
Vase life	1															
MSI at tight bud stage	0.851882	1														
MSI at end of vase life	0.962138	0.870052	1													
RWC at tight bud stage	0.763309	0.839285	0.850889	1												
RWC at end of vase life	0.947682	0.859628	0.982133	0.879447	1											
TPC at tight bud stage	0.528501	0.475523	0.587217	0.481515	0.533275	1										
TPC at end of vase life	0.781707	0.536855	0.790099	0.585408	0.765527	0.843587	1									
TSS at tight bud stage	0.791529	0.688508	0.772086	0.698732	0.791966	0.017768	0.389263	1								
TSS at end of vase life	0.860247	0.666785	0.834887	0.696075	0.853558	0.144966	0.559175	0.955841	1							
Days to opening of basal floret	0.178911	0.166204	0.241862	0.18674	0.205071	0.782028	0.545212	-0.2407	-0.08556	1						
Per cent opening	0.682762	0.507761	0.608641	0.459316	0.622974	-0.00567	0.406314	0.696943	0.753454	-0.37205	1					
Floret size	0.615285	0.473559	0.588765	0.499936	0.633252	-0.18645	0.249394	0.798471	0.796451	-0.54255	0.899417	1				
Maximum no. of florets open at one time	0.558987	0.321211	0.54821	0.342783	0.554201	0.020741	0.406744	0.580121	0.617684	-0.43474	0.827326	0.851126	1			
Change in pH	0.804031	0.835147	0.826111	0.752712	0.798725	0.750821	0.744167	0.408156	0.471445	0.328519	0.455748	0.335315	0.392611	1		
Loss in weight	-0.81025	-0.57942	-0.8501	-0.72082	-0.85252	-0.68614	-0.93075	-0.60941	-0.73395	-0.39617	-0.47376	-0.42475	-0.47567	-0.66683	1	
Water absorbed	0.935559	0.894597	0.921169	0.788021	0.923753	0.410483	0.646195	0.803782	0.824154	-0.02474	0.731212	0.724162	0.620341	0.823158	-0.71793	1

MSI- Membrane stability index, RWC- Relative water content, TPC- Total protein content, TSS- Total soluble sugar.

amount of water absorbed had high degree of significant positive influence on membrane stability index (r = 0.921) and relative water content (r = 0.923) at the end of vase life that further played a determining role in vase life. Among all the post harvest parameters, floret size was most dependent upon days to the opening of basal floret (r = 0.89), followed by total soluble sugar content at tight bud stage and end of vase life (r = 0.798 and r = 0.796 respectively).

Thus, significant and positive correlation coefficients between post harvest quality, physiological and biochemical parameters strengthen the present findings where improved membrane stability index, relative water content, total water absorbed and higher content of total soluble sugars and soluble proteins enhanced the vase life of spikes, produced from corms treated with GA_3 and BA. The vase life of Priscilla spikes (11.56 days) was significantly higher than Punjab Glance (10.63 days).

Therefore, promontory effects of GA_3 and BA on post harvest quality parameters *viz.* water uptake, loss in physiological weight might be due to maintenance of MSI, RWC, total protein content and total soluble sugar content in the spikes that ultimately improved the vase life.

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