

Effects of benzyl adenine and gibberellic acid pre-treatments on dormancy release, flowering time and multiplication of oriental lily (*Lilium longiflorum*) bulbs

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Abstract

Dormancy in Oriental lily bulbs (*Lilium* spp) is a major bottleneck in lily flower production by small scale farmers because they cannot afford expensive chilled bulbs that have been induced to break dormancy. Thus for developing alternative and low cost dormancy mitigation techniques, the study investigated the effects of lily bulb pre-treatments with benzyl adenine (BA) and gibberellic acid (GA_3) on dormancy breaking, emergence rates, time to flowering and bulb multiplication. Bulbs were pre-soaked for 24 hours in prepared solutions of various concentrations of BA and GA_3 (0; 25; 50; 100 and 150 mg/L) and their combinations, plus a positive control of chilled bulbs. An unbalanced factorial arrangement in a randomized complete block design with three replications was used. The experiment was repeated in two seasons. Results showed that treating bulbs with BA and GA_3 significantly influenced dormancy breaking in both the trials and was comparable with the chilling treatment. The highest sprouting was observed in bulbs treated with 50 mg/L BA (92%) and 50 mg/L GA_3 (96.67%) in both trials; compared to chilled bulbs with 100% sprouting. The number of days to 50% bulb emergence was significantly reduced in trial 1 with various combinations of GA_3 and BA (50 mg/L:100 mg/L; 150 mg/L:100 mg/L and 150 mg/L :150 mg/L). Combining the plant growth regulators also decreased the number of days to flowering; with 25 mg/L BA + 150 mg/L GA_3 ; 50 mg/L BA + 100 mg/L GA_3 ; 50 mg/L BA + 150 mg/L GA_3 and 100 mg/L BA + 100 mg/L GA_3 , respectively, significantly decreasing the number of days to flowering to 124 compared with 132 for the control in trial 2.

Key words: Lily, bulb, dormancy, gibberellic acid, benzyl adenine, chilling, flowering

Introduction

The floriculture industry is a key foreign exchange earner for the economy of Kenya. In 2012, the subsector contributed Ksh 39.7 billion accounting for 18% of the domestic value of horticulture. The area under floriculture was 4,039 ha with a production of 878,067 tons in 2012 signifying a 25.26% increase compared to the previous year (HCDA, 2012). In the export market, Kenya is a major supplier of over 35% of cut flowers and ornamentals to the world's largest market - the European Union. The trend towards a greater variety of flowers, is increasing demand for less traditional varieties of both temperate and tropical flowers (UN, 2001). Florists are in search of new flowers to create new designs that appeal to clients who are looking for novelty (Patrick, 2003).

To satisfy the demand for new and different flowers, a range of bulbous ornamental plants are being forced either in greenhouses or outdoors to flower. These bulbous flowers are also referred to as specialty cut flowers used on special occasions. Among the bulbous plants, lily cultivation has gained tremendous potential over the past 15 years in Kenya and globally (UN, 2001). It is now the second largest bulbous flower grown in the Netherlands. Lily has become a popular cut flower throughout the world. The bulk production of lily bulbs occurs in ten countries, the Netherlands with the largest production area of 4,280 hectares (77%), followed by France with 401 ha (0.8%), Chile with 205 ha (0.4%), the U.S with 200 ha (0.4%), Japan with 189 ha (0.3%) and New Zealand with 110 ha (0.2%) (Dutch Marketing Board for Agriculture, 2010). Currently, Kenya is producing Oriental and Asiatic hybrid

lilies. Bulbous plant/flower production *per se* is very well adapted to small scale farmers in Kenya because most of the plant species do not require expensive greenhouse structures to grow.

Dormancy in lily bulbs is the most binding constraint to their commercial production by small scale flower farmers because of the requirement for chilling treatments. Proper chilling treatments require that bulbs are exposed to low temperatures (4-5°C) for 6-8 weeks. This requires expensive refrigeration equipments and electrical power supply systems that are prohibitively expensive for these resource poor farmers. Thus, low cost dormancy breaking alternatives need to be sought.

The use of growth regulators has been reported as a substitute for chilling in some plants that are largely propagated using underground storage structures such as corms and tubers (Ohkawa, 1979). These structures share some physiological and functional similarities with bulbs. Therefore, the objective of this study was to investigate the potential of exogenously applied plant growth regulators in breaking the dormancy of oriental lily bulbs as an alternative to chilling treatment.

Materials and methods

Two trials to study the effect of hormone treatments on performance of Oriental lily bulbs were set up at James Finlay, Lemotit Farm *viz.*, September 2009 - January 2010 (trial 1) and January 2010 - April 2010 (trial 2). The farm is located approximately at latitude 0°22' South and longitude 35°18' East with average annual rainfall of 1386 mm/year. The annual mean

maximum and minimum temperatures are 24 °C and 9 °C, respectively, with an average relative humidity of 85%. During the experimental seasons mean soil temperatures measured at a depth of 15 cm were 16 °C for season 1 and 22 °C for season 2.

Oriental lily bulbs of the variety 'Tiber' were used. Selected bulbs of 12 cm diameter were soaked for 24 hours in buckets containing various concentrations of GA₃ and BA (*viz.* 0 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L) and their respective combinations. Following treatment, the bulbs were kept under shade in a well ventilated store for 12 days before planting. The experimental design was a factorial in a randomized complete block design with three replications. The field layout was row plots of 26 treatments *i.e.* 25 treatment combinations of GA₃ and BA plus one treatment of chilled bulbs. Each treatment had 10 bulbs and each replicate of the experiment contained all GA₃: BA treatment combinations and 10 chilled treatment bulbs served as positive control. The untreated bulbs (*i.e.* 0 mg/L GA₃ and 0 mg/L BA) served as the negative control. The experiment was done in tunnels with shade net cover. The land was ploughed deeply and the soil clods were broken using a harrow to a fine tilth. Beds of 1m wide, raised to a height of 15 cm and separated with 0.5 m wide paths, were made. Guard rows were planted all around the layout.

The bulbs were planted in rows 20 cm apart and 20 cm between rows. Data collection commenced a week from the date of planting. Data collected included percentage sprouting of bulbs, days to 50% bulb emergence, number of days to flowering and number of bulblets formed as an indicator of bulb multiplication. The number of visible sprouts after emergence was recorded in each plot. This was used to calculate the percentage of bulbs that had sprouted. This is important to growers because a high percent sprout indicates that the breaking of dormancy was effective. In addition, the number of days to 50% bulb emergence per plot was determined by counting the number of days from planting till when there was 50% sprouting in each plot. Number of days to flowering was determined by counting the number of days from planting till there was 50% flowering observed in each plot. Number of bulblets formed per mother bulb was counted after lifting the mother bulbs when the top plant parts had dried. Harvesting began as soon as the first bud had shown a change in colour and opened at the apex. All flower buds harvested were free from diseases and pests and fell within the required grade specifications.

Data were analyzed using Analysis of Variance (ANOVA) under the General Linear model (GLM) procedure of the SAS statistical package; and means for significant treatments separated using LSD at $P \leq 0.05$.

Results and discussion

Bulb sprouting: The different levels of BA and GA₃ had significant effects on the sprouting of lily bulbs (Table 1). The highest percentages of sprouting (80 and 73.3%) were observed when bulbs were treated singly with 150 mg/L of BA and 50 and 100 mg/L GA₃, respectively in trial 1. These were not significantly different from the results obtained with chilling treatment where 98.67% sprouting was attained. There was a general increase in sprouting response as the GA₃ concentrations increased from 0 mg/L to 100 mg/L in trial 1. Similar results where sprouting

increased with plant growth regulator treatments have been reported in various plant species propagated by specialized structures. Kirad *et al.* (2001) reported high sprouting responses in gladiolus following corm treatment with GA₃. Rahman *et al.* (2006) also reported maximum sprouting of garlic after GA₃ treatment, where soaking of cloves in 50 ppm proved to be the optimum treatment for breaking dormancy. Similarly, Barman *et al.* (2004) obtained 100% sprouting in gladiolus corms that had been soaked for 24 hours in GA₃. However, in a comparative study, Goo *et al.* (1998) noted that BA was better at stimulating sprouting of *in vitro* produced gladiolus corms.

In the present study, the highest performing single treatments of BA and GA₃ were not significant in terms of percentage sprouting but were better performing compared with the control. In trial 1 there was a decline in sprouting emergence at concentrations above 100 mg/L while in trial 2 there were no noticeable trends in the response. Similar findings were reported by Wanjao and Waithaka (1983) on *Liatris* where GA₃ replaced cold treatment in breaking dormancy but high concentrations proved detrimental. In general the treatments did not show significant differences in the sprouting response in trial 2. All single treatments consistently recorded high sprouting responses with a range of 70-76% and 86.6 -100% for BA and GA₃, respectively. In all treatments with BA, 96.67% sprout emergence was observed compared to the negative control where only 23.33% sprouting was recorded in trial 1. Various combination levels of BA and GA₃ produced significant effects on sprouting with the highest mean response (96.67%) achieved with a combination of 50 mg/L BA and 100 mg/L GA₃.

The results of the present study indicate that lily bulbs require low concentrations of plant growth regulators to promote high sprouting. Gibberellic acid and BA play an important role in cell elongation and cell division, respectively. BA is known as an activator of cell division and plays a part in promoting meristematic activity. In this study, the cells stimulated to multiply by BA, were also induced to elongate by the activity of GA₃ hence an overall accelerated sprouting response. Kiyoshi (2003) reported similar responses with *Lilium speciosum* where sprouting responses were enhanced with various combinations of GA₃ and BA.

Number of days to 50% emergence: The rate of sprout emergence in bulbs was measured by determining the number of days to 50% sprouting. In trial 1, the time to 50% sprout emergence was significantly influenced by the treatments with means ranging from 13 to 23 days in trial 1 and 9 to 11 days in trial 2, respectively, for the various single treatments of BA (Table 2). A significant reduction of sprouting time was observed when BA was used at 50 mg/L. In trial 2, a great improvement in time to 50% sprouting was observed. All the BA treatments applied recorded less than 10 days or less to 50% sprouting which was not significantly different from the chilling treatment (Table 2).

The effect of GA₃ levels on the time to 50% emergence followed a similar pattern to that of BA. Specifically, in both seasons, GA₃ levels up to 50 mg/L significantly reduced the time to 50% emergence compared to the controls (Table 2). There was a significant reduction in number of days to 50% emergence when various combinations of BA and GA₃ were used. The lowest

number of days to 50% emergence in trial 1 and 2 were recorded when bulbs were treated with a combination of 100 mg/L of BA and 50 mg/L of GA₃, giving means of 8.67 and 9 days, respectively. Overall, observations showed a marked decrease in the number of days to 50% emergence in trial 2 compared to those observed in trial 1. Previous studies have indicated that exogenous applications of GA₃ cause a sudden increase in endogenous gibberellin concentrations leading to accelerated sprouting (Sonnewald, 2001). This has been ascribed to the effect of GA₃ in facilitating movement of cytokinins to newly formed buds leading to increased cell division and accelerated bud break (Sonnewald, 2001).

GA₃ effects also correlate with increased cell division due to its ability to mobilize cytokinins translocation for cell division. Taiz and Zeiger (2002) suggested that the newly produced cells may act as sinks for carbohydrates and that invertase activity in them provokes starch breakdown resulting in sugars being transferred to the sprouts, resulting in new growth. In the present study similar reactions may be presumed where bulbs were treated with PGRs leading to the accelerated growth responses. It is noteworthy in this study that combinations of GA₃ and BA further increased the rate of sprouting, suggesting a synergistic effect. For instance, when the two PGRs were combined at rates of 100 mg/L BA and 50 mg/L GA₃, the mean number of days to 50% sprouting was reduced to 9. This effect was greater in season 2 compared to season 1. Prevailing higher soil temperatures in trial 2, though not evaluated, could have contributed to the greater response of bulbs in trial 2 when considered together with the pre-treatments applied.

Flowering time: The various levels of individual PGR treatments did not significantly affect the flowering time of the treated bulbs in trial 1. In all single PGR treatments the number of days taken for bulbs to flower was not statistically different from the untreated controls. In this season, the chilled bulbs recorded significantly shorter periods to flowering (Table 3). In season 2, some significant differences were observed between some treatments. The highest levels of GA₃ and BA (150 mg/L) recorded shortest time to flowering. There was a decreasing trend in the length of time to flowering as the PGR levels increased. A similar trend has been reported in *Zantedeschia elliottiana* when rhizomes were soaked in 50-100 mg/L solutions of BA (Tjia, 1986). BA has also been implicated in the accelerated flowering of poppy anemone (Janowaska *et al.*, 2009).

According to Ouzounidou *et al.* (2008, 2011), Yamaguchi (2008) and Yu *et al.* (2009), gibberellins are also implicated as playing a major role in diverse growth processes including organ elongation,

Table 1. Effect of various levels of BA and GA₃ and their combinations on the sprouting percentages of Oriental lily bulbs

Trial 1		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	23.33g	70abcdef	73.33abcde	73.33abcde	56.67cdef
	25	56.67cdef	60bcdef	83.33abc	46.67def	43.33fg
	50	70abcdef	90a	86.67ab	96.67a	80abcd
	100	56.67cdef	90a	96.67a	76.67abcd	86.67ab
	150	80abcd	70abcdef	70abcdef	73.33abcde	53.33def
Chilling		98.67a				
Trial 2		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	36.67d	93.33ab	86.67abc	100a	90abc
	25	76.67bc	83.33abc	100a	100a	100a
	50	70c	93.33ab	100a	96.67ab	100a
	100	76.67bc	100a	96.67ab	90abc	96.67ab
	150	70c	100a	100a	96.67ab	96.67ab
Chilling		100.00a				

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

Table 2. Effect of various levels of BA and GA₃ and their combinations on the number of days to 50% emergence of Oriental lily bulbs

Trial 1		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	32.00a	21.33bcd	18.67cdef	13.67defg	14.33defg
	25	16.67def	16.33defg	20.00bcde	31.67a	16.00defg
	50	13efg	11.67fg	20.00bcde	26.00abc	15.33defg
	100	21.33bcd	11.33fg	8.67g	13.67defg	13.33efg
	150	33.00a	27.33a	13.67defg	16.33defg	15.00defg
Chilling		20.00bcde				
Trial 2		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	9.33bcde	8.00e	9.67abcd	8.67de	8.67de
	25	9.33bcde	9.00cde	9.00cde	9.00cde	9.33bcde
	50	9.00cde	9.67abcd	10abcd	9.67abcd	10abcd
	100	10.00abcd	9.33bcde	9.00cde	10.67ab	9.67abcd
	150	9.33bcde	9.00cde	9.33bcde	11.00a	10.33ab
Chilling		10.27ab				

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

Table 3. Effect of various levels of BA and GA₃ and their combinations on the number of days to flowering of Oriental lily bulbs

Trial 1		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	131.33cdef	132.00cde	132.33bcd	134.00ab	133.00abc
	25	132.00cde	132.00cde	131.33cdef	132.00cde	131.33cdef
	50	131.67cdef	132.00cde	131.33cdef	132.00cde	132.00cde
	100	133.00abc	130.00f	131.00def	134.00ab	130.33ef
	150	132.67bcd	131.67cdef	132.33bcd	134.67a	132.33bcd
Chilling		130.00ef				
Trial 2		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	132.67a	127.33defg	124.33ij	123.67j	124j
	25	131.33abc	129de	126.67efghi	125.33fghij	124.67hij
	50	132.67a	127efghi	125ghij	125ghij	124j
	100	132ab	128.33de	127.67def	125.67fghij	125ghij
	150	131bcd	129.67cde	128.33de	126.67efghi	125.33fghij
Chilling		129.33cde				

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

senescence and control of flowering time. Similarly, Sun and Gubler (2004) indicated that in most species, the transition to floral development is stimulated by GAs. However, the actual effect of GAs on flowering is not clear. Ben-Tal and Erner (1999) reported that GAs had effects on flowering date in many plant species but the effect was not similar; in some species, it enhanced earlier flowering while in others it delayed flowering.

Table 4. Influence of various levels of BA and GA₃ and their combinations on bulblet formation of Oriental lily bulbs

Trial 1		GA ₃ (mg/L)				
		0	25	50	100	150
BA (mg/L)	0	0.00c	0.00c	0.00c	0.00c	0.00c
	25	0.00c	0.00c	0.00c	1.00bc	0.00c
	50	0.00c	1.00bc	0.00c	0.00c	0.00c
	100	0.00c	1.67b	4.00a	0.00c	1.00bc
	150	0.00c	1.00bc	0.00c	0.00c	1.00bc
Chilling	0.33					
Trial 2						
BA (mg/L)	0	0.00g	1.33cdefg	2.00bcdef	2.00bcdef	5.67a
	25	1.00defg	1.00defg	3.00bc	2.00bcdef	2.33bcde
	50	0.67efg	0.33fg	1.00defg	1.33cdefg	2.33bcde
	100	3.33b	2.00bcdef	3.00bc	2.00bcdef	2.67bcd
	150	2.67bcd	2.00bcdef	3.00bc	2.33bcde	1.67bcdefg
Chilling	0.33					

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test

In trial 1, no observable trends were observed when bulbs were treated with various combinations of BA and GA₃, however, in trial 2, BA at 25 mg/L combined with GA₃ 150 mg/L significantly decreased the number of days to flowering to 124.67 days compared to 132.67 days in the untreated control (Table 3). Similar observations were made in the combinations, 50 mg/L BA: 100 mg/L GA₃; 50 mg/L BA: 150 mg/L GA₃; and 100 mg/L BA: 100 mg/L GA₃, respectively. From these results it can be concluded that the two plant growth regulators promote early flowering in Oriental lily bulbs and can be used by growers as a crop scheduling and manipulation tool to target specific marketing periods.

Bulblet multiplication: Bulb multiplication is an important trait where additional planting materials are desired from the production system. In the present study, the number of bulblets formed was inconsistent in trial 1. Only a few PGR combinations produced extra bulbs. A notable observation was the combination of 100 mg/L BA and 50 mg/L GA₃ where a mean of 4 bulblets were formed (Table 4). In all the other combination treatment where bulblets were observed, a mean of 1 bulblet was recorded. In trial 2, various PGR treatments significantly affected bulblet multiplication in comparison to both non treated and chilling treatment. A general increase in the number of bulblets was found with increase in the levels of BA and GA₃. The highest number of bulblets were obtained with BA and GA₃ 150 mg/L, respectively. Growth regulators combination treatments variously affected bulblet formation for instance, in the combination of 50 mg/L BA and 25 mg/L GA₃ and 50 mg/L BA and 50 mg/L GA₃, 1 or less bulblets was recorded. However, other combinations recorded significantly higher number of bulblets ranging from 2.0 to 3.0 bulblets. Similar findings were reported by Rana *et al.* (2005) in gladiolus corms and Singh (1999) in tuberose bulbs. The increase in bulb multiplication may be attributed to the high concentrations of BA stimulating axillary bud proliferation and the promotion of sink activity in these buds by GA₃ (Rana *et al.*, 2005) leading to increased carbohydrate translocation and accumulation under the influence of the warmer temperatures experienced in trial 2.

In conclusion, the results obtained from this study revealed that BA and GA₃ pre-treatments of dormant oriental lily bulbs strongly influence sprouting (dormancy release), flowering time and bulb multiplication. It is also revealed in this study, that the prevailing

temperature and rainfall regimes during the two trials could have influenced the results. Weather data collected during the experimentation indicates cooler temperatures and high rainfall in trial 1 as opposed to warmer temperatures and lower rainfall obtained in trial 2. This could have led to a synergistic effect of the warm temperature and PGR treatment hence greater responses to the parameters measured in trial 2. Weighed against the conventional and expensive chilling treatments used to break bulb dormancy, these pre-treatments could be adopted by small scale lily producers to cut production costs and reduce flowering times of the crop. Further experiments to fine tune the proper combinations of the two PGRs and other pre-culture conditions are underway to recommend a cheaper dormancy breaking regime to suit small scale farmers.

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