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ALLEVIATION OF GLADIOLUS (*GLADIOLUS GRANDIFLORUS*) CORM DORMANCY THROUGH APPLICATION OF 6-BENZYLAMINOPURINE AND GIBBERELLIC ACID

YASAR SAJJAD[1,2*], MUHAMMAD JAFAR JASKANI[2], ASIM MEHMOOD[3], MUHAMMAD QASIM[2] AND GULZAR AKHTAR[4]

[1]Department of Biotechnology, COMSATS University Islamabad, Abbottabad campus, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan
[3]Department of Biosciences, COMSATS University Islamabad, Sahiwal campus, Pakistan
[4]Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan
[*]Corresponding author's email: yasarsajjad@cuiatd.edu.pk

Abstract

Gladiolus is commercially grown through corms and freshly harvested gladiolus corms exhibit endo dormancy for 2-4 months or even more depending on cultivars. It creates hindrance in round the year cultivation of these corm. The present research aimed to break the dormancy of gladiolus corms in relatively short time through the application of plant growth regulators under field conditions and to observe the certain biochemical changes occurred in treated and control corm. Freshly harvested corms of two gladiolus cultivars, white prosperity and amsterdam, were descaled and soaked in 0.5 and 1mM solution of 6-benzylaminopurine (BAP) for 10 hours followed by soaking in 0.1, 0.2, 0.4 and 0.8 mM solution of gibberellic acid (GA_3) for 15 hours before planting on ridges in the field. The control corms were soaked in distilled water. Treatment (GA_3 at 0.8 mM in combination with 1 mM BAP) showed maximum germination percentage (80 % and 86.66 %) and more no. of bud $corm^{-1}$ (6.52 and 6.59) in white prosperity and Amsterdam, respectively. This treatment also showed positive changes in biochemical attributes including total soluble sugars (6.89 mg/g and 7.06 mg/g), abscisic acid (30.40 ng/g and 28.58 ng/g), gibberellic acid (105.63 ng/g and 119.97 ng/g) in treated corms (1mM BAP followed by 0.8mM GA_3) in white prosperity and amsterdam compared to control corms (2.26 mg/g and 2.90 mg/g TSS, 64.29 ng/g and 59.90 ng/g ABA, 25.08 ng/g and 32.63 ng/g GA_3), respectively. Application of BAP and GA_3 was useful to reduce the dormant period of corms of two cultivars including Amsterdam and White prosperity.

Key words: Benzylaminopurine, Bulbous plant, Dormancy, Gibberellic acid, Physiology

Introduction

Gladiolus (*Gladiolus grandiflorus*) is a perennial bulbous flowering plant belongs to Iridaceae family. The genus *Gladiolus* consists of about 260 species among which 10 species are native to Eurasia and 250 belongs to sub-Saharan Africa (Goldblatt & Manning, 1998; Manning & Goldblatt, 2008). Gladiolus is commercially cultivated and propagated asexually through its underground corms. A corm is an underground modified stem that provides nutrients during sprouting (Ghamsari *et al*., 2007). The cultivated corm produces corm and also cormels which are usually harvested after the initiation of senescence of above ground parts. Corms and cormels are unable to germinate soon after lifting from soil even under favorable environmental conditions for certain period of time due to occurrence of dormancy.

Dormancy is defined as the temporary failure of a viable seed to germinate, after a specified length of time, in a particular set of environmental conditions that later evoke germination when the restrictive state has been terminated by either natural or artificial means (Simpson, 1990). The dormancy prevails in many plants including ornamental bulbous plants, as Kamerbeek *et al*., (1972) divided the bulb plants into three groups on the basis of occurrence of dormancy, group I included Lilium, Allium, and Gladioli, exhibiting dormancy over extended periods of time, group II consists of Tulipa, Hyacinthus, and Narcissus which have dormancy for short periods of time, group III expresses no sign of primary dormancy and is best symbolized by *Iris hollandica*.

The termination of dormancy occurred in a natural way which takes long period, but it can be alleviated artificially through different methods including scarification, stratification and use of different chemicals including the plant growth regulators depending on the type of dormancy. Gladiolus corm usually take 2 to 4 months to alleviate dormancy naturally, but the depth of dormancy depends on the cultivar (Imanishi, 1981). Application of GA_3 had been reported to break the dormancy of ornamental bulbous plants including, lilly, tulip, hyacinth, Helianthus, and liatris (Lin *et al*., 1975; Van Bragt & Van Ast, 1976; Saniewski *et al*., 1977; Wanjao & Waithaka, 1983; Ruttanaprasert *et al*., 2018). GA_3 is considered an alternative for cold treatment and effective in shortening the dormancy period of gladiolus corms (Bhattacharjee, 1984; Dua *et al*., 1984; Ginzburg, 1974; Tonecki, 1980).

The physiological events which lead to breakdown dormancy are not yet completely understood (Halaly *et al*., 2008). However, there are certain physiological parameters which are helpful in explaining this phenomenon, as dormancy regulation is usually associated with the fluctuation in levels of growth regulators and growth inhibitors (Wareing, 1977). Different physiological changes occurred during the shifting of plant organ from the dormant condition to the non dormant state. The endogenous levels of abscisic acid (Djilianov *et al*., 1994; Kim *et al*., 1994; Yamazaki *et al*., 1995) and sucrose (Hobson & Davies, 1978; Aguettaz *et al*., 1990) are associated with development and release of dormancy in bulbs. The endogenous level of ABA decreased during breakdown of dormancy in ornamental

bulbous plants like Lillium, tuberose and tulip (Nagar, 1995; Xu *et al*., 2006). Changes in the level of phenolic compounds are also associated with the release of dormancy. There is evidence in many plants including dormant potato buds (Cvirkova *et al*., 1994), Pistachio (Zahra *et al*., 2009), inner buds of onion bulbs (Benkeblia & Selselet-Attou, 1999) which have high phenolic contents during dormancy but decrease during sprouting of buds. Starch is a stored form of carbohydrates in plants, and conversion of starch into sucrose and reducing sugars was observed in sprouting of bulbs (Matsuo and Mizuno, 1974; Miller & Langhans, 1990). The release of dormancy is also linked to the combined effects of accumulation of soluble sugars by mobilization of starch and decline in concentration of abscisic acid and other germination inhibitors (Xu *et al*., 2006).

The freshly lifted gladiolus corms can be planted in an area having favorable environmental conditions if dormancy is alleviated through exogenous application of plant growth regulators. The aim of this study is to evaluate the efficacy of gibberellic acid and benzylaminopurine to break the dormancy of freshly harvested gladiolus corms under field conditions. Furthermore, biochemical changes occurred in corms after application of treatments will also be analyzed.

Materials and Methods

Plant material and treatments: Freshly harvested corms of two Gladiolus cultivars, white prosperity and amsterdam, were taken to the laboratory for cleaning, grading and descaling. The descaled dormant corms were soaked in solution of 6-benzylaminopurine (BAP) at 0.5 and 1 mM concentration for 10 hours followed by in 0.1, 0.2, 0.4 and 0.8 mM solution of GA_3 for 15 hours. The control corms were soaked only in distilled water for 10 hours. The corms were planted on ridges at plant to plant distance of 6 inches and row to row distance of 24 inches in the floriculture area of Institute of Horticultural Sciences, University of agriculture Faisalabad, Pakistan. The data of environmental conditions including temperature and humidity of the experimental site was obtained from Agricultural Meteorology Cell of university, Faisalabad and described in figure 4.

Morphological attributes: The germination percentage was calculated after 3 weeks of planting corms in the field while the number of sprouted buds $corm^{-1}$ were also counted.

Biochemical analysis: The biochemical parameters were also quantified after 3 weeks of experiment. Total soluble sugars, reducing sugars and nonreducing sugars were measured spectrophotometrically according to the protocol of Riazi *et al*., (1985). The corms were extracted in 95% methanol for total phenolic contents and measured by using spectrophotometer according to protocol of Ainsworth & Gillespie (2007). The corms were homogenized in 80% ethanol and supernatant was discorded after centrifugation at 3500 rpm for 3 minutes and residue was washed thrice with ethanol to remove soluble sugars. The residue was digested in 52% perchloric acid to prepare sample and Spectrophotometric measurement of starch content was done by following the method of Malik & Srivastava (1982).

The extraction and analysis of gibberellic acid and abscisic acid was done according to protocol of Kelen *et al*., (2004). The corms were quickly frozen by using liquid nitrogen immediately after their harvesting and stored at -80°C until analysis. For extraction, the corm tissue was homogenized in 70% methanol by using homogenizer. The samples were placed on shaker for overnight stirring at 4°C. After that extract was filtered by using Whatman filter paper No. 43. The methanol was evaporated under vacuum from filtrate and aqueous phase was collected in separate glass tube. The pH of aqueous phase was adjusted at 8.5 by using 0.1 M phosphate buffer. The aqueous phase was washed and separated three times with ethyl acetate and then pH of solution was adjusted at 2.5 with 1N HCl and again washed and separated with diethyl ether for three times by using separating funnel. Finally, the ether was evaporated, and dried sample was solubilized in methanol. The 20 µl sample was injected in HPLC (Shimadzu-LC-10A) having C18 column (25cm x 4.6 mm) with flow rate of 0.8 ml min^{-1} at 30°C column temperature and gibberellic acid was detected by using UV detector at 208 nm. For abscisic acid, the flow rate was adjusted at 1ml min^{-1} and detected at 265 nm wavelength.

Statistical analysis

The factorial experiment was laid out in randomized completely block design (RCBD) with three replications. Collected data was statistically analyzed through Analysis of Variance (ANNOVA), and treatment means were further compared through least significant difference (LSD) test. All the statistical analysis were performed by using SPSS software at significance level of $p \leq 0.05$ value.

Results

The results of analysis of variance (ANNOVA) including significance level of effect of treatments, varieties and their interactions are shown in Table 1.

Germination % and number of sprouted buds: The application of 0.5mM benzylaminopurine in combination with gibberellic acid (0.1mM or 0.8mM) increased germination percentage from 28.88% to 57.78% in White Prosperity and 31.11% to 68.88% in Amsterdam, respectively. Maximum germination percentage (79.99% and 86.66%) was observed in treatment of 0.8 mM GA_3 in combination with I mM BAP in White Prosperity and Amsterdam, respectively. No germination was observed in control corms after three weeks of sowing in the field (Fig. 1A). In control corms of white prosperity and amsterdam, 1.52 and 1.75 buds per corm sprouted while treatment (0.8 mM GA_3 in combination with I mM BAP) yielded highest buds $corm^{-1}$ 6.52 and 6.58, respectively (Plate 1). Gibberellic acid in combination with 0.5 mM benzylaminopurine had less effect on increasing the number of buds sprouted compared to 1mM benzylaminopurine (Fig. 1B).

Table 1. Analysis of variance of different parameters in response to treatments.

Dependent Variables	Source	df	Sum of squares	Mean square	F value
Germination %	Treatment	8	35064.337	4383.042	143.53**
	Variety	1	642.114	642.114	21.03**
	Treatment × Variety	8	240.283	30.538	0.984
Buds per corm	Treatment	8	151.464	18.933	28.48**
	Variety	1	0.024	0.024	0.04
	Treatment × Variety	8	2.73	0.341	0.51
Reducing Sugars	Treatment	8	2.939	0.367	22.19**
	Variety	1	0.004	0.004	0.27
	Treatment × Variety	8	0.181	0.023	1.36
Non reducing sugars	Treatment	8	86.315	10.789	42.24**
	Variety	1	3.471	3.471	13.59**
	Treatment × Variety	8	1.458	0.182	0.71
Total soluble sugars	Treatment	8	117.439	14.68	73.56**
	Variety	1	3.32	3.32	16.64**
	Treatment × Variety	8	1.636	0.204	1.03
Starch	Treatment	8	772.853	96.607	12.09**
	Variety	1	34.752	34.752	4.35*
	Treatment × Variety	8	6.513	0.814	0.10
Total phenolics	Treatment	8	225239.407	28154.926	303.29**
	Variety	1	49913.152	49913.152	537.67**
	Treatment × Variety	8	15636.889	1954.611	21.06**
Abscisic acid	Treatment	8	5374.31	671.789	26.46**
	Variety	1	14.664	14.664	0.58
	Treatment × Variety	8	54.144	6.768	0.27
Gibberellic acid	Treatment	8	34462.607	4307.826	43.01**
	Variety	1	1698.82	1698.82	16.96**
	Treatment × Variety	8	148.841	18.605	0.186

* $p<0.05$; ** $p<0.001$

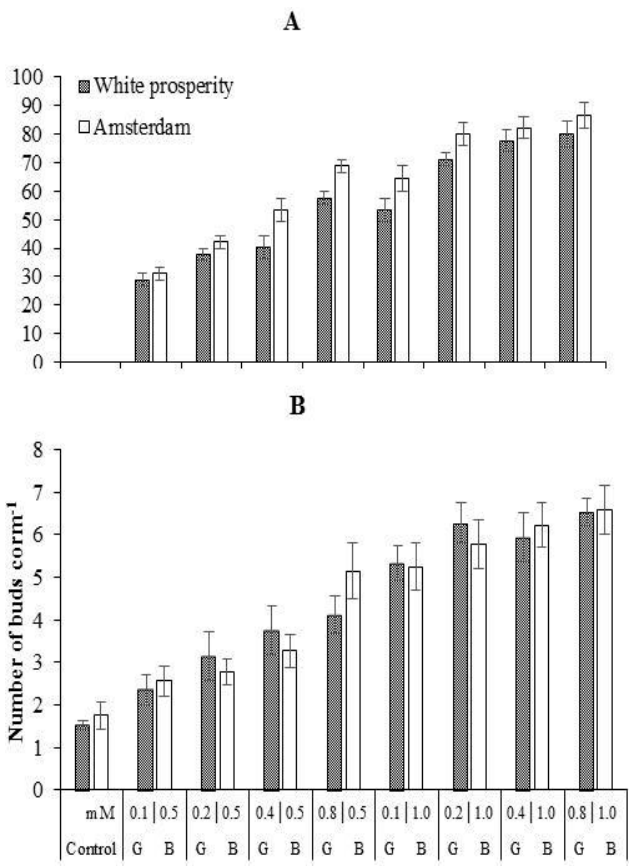

Fig. 1. Effect of plant growth regulators on germination and number of sprouted buds in dormant corms, ± shows SE of three replicates.

Reducing sugars: The quantity of reducing sugars increased in corms after application of gibberellic acid and benzylaminopurine. Gibberellic acid at 0.1 mM concentration in combination with 0.5 mM Benzylaminopurine gave 0.48 mg/g reducing sugars which increased to 0.94 mg/g at 0.8 mM concentration of gibberellic acid while 1.20 mg/g reducing sugars were measured at 0.8 mM gibberellic acid along with 1 mM Benzylaminopurine. Almost similar trend was observed in Amsterdam (Fig. 2A).

Non reducing sugars: Corms of amsterdam treated with 0.8 mM gibberellic acid along with 1 mM benzylaminopurine exhibited 5.97 mg/g non reducing sugars which was the highest among all treatments (Fig. 2B). In combination of gibberellic acid (0.1 mM) and benzylaminopurine (0.5 mM), the non-reducing sugars were recorded as 2.42 mg/g, 3.67 mg/g in white prosperity and Amsterdam, respectively. The control corms of Amsterdam showed more amount of non- reducing sugars (2.49 mg/g) compared to White Prosperity (1.78 mg/g).

Total soluble sugars: Total The total soluble sugars content in non-treated corms was 2.26 mg/g and 2.90 mg/g which were increased to maximum value of 6.89, 7.06 mg/g in treatment (0.8 mM GA_3 along with 1 mM BAP) in white prosperity and Amsterdam, respectively. gibberellic acid (0.8 mM) in combination with 0.5 mM benzylaminopurine yielded 3.84 mg/g, 4.50 mg/g total soluble sugars in 1 mM in white prosperity and Amsterdam, respectively (Fig. 2C).

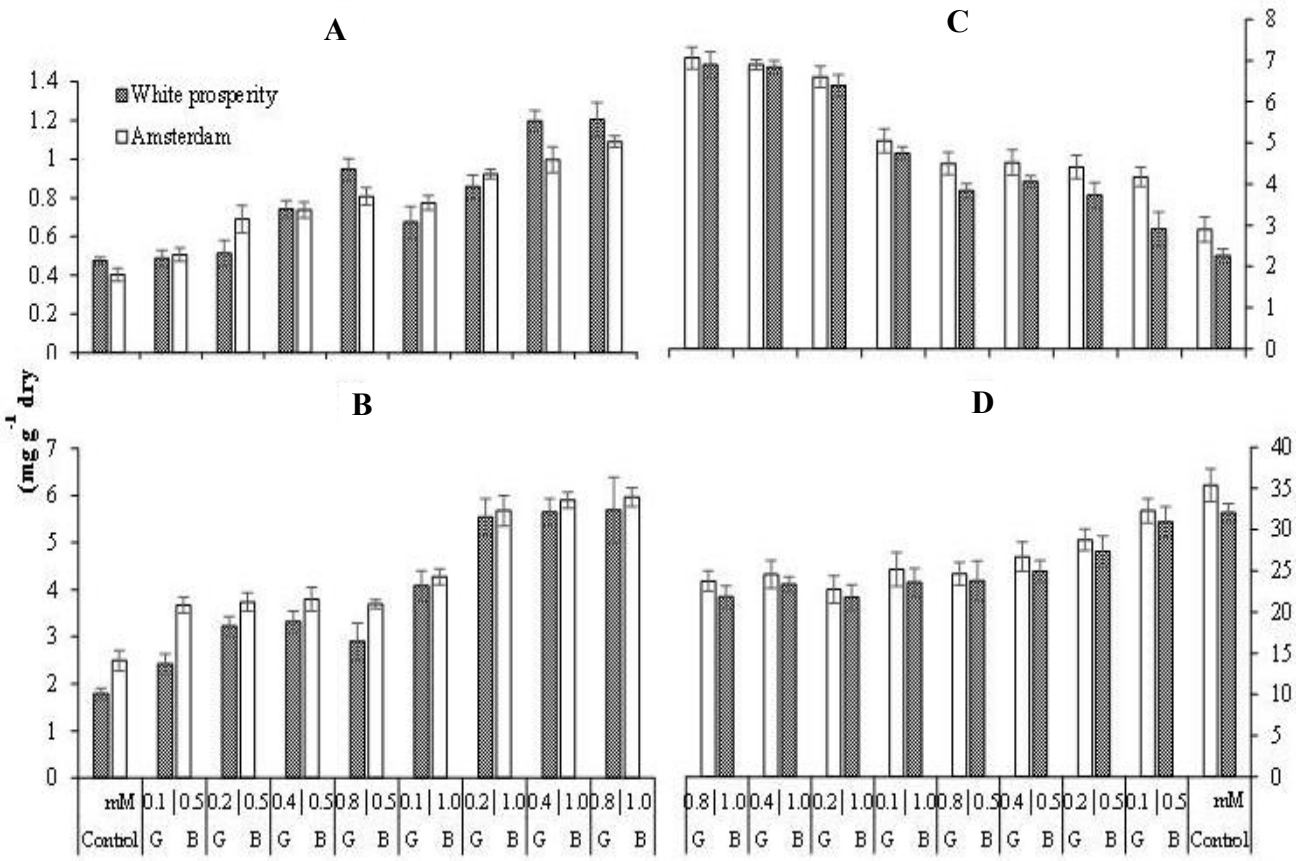

Fig. 2. Effect of plant growth regulators on reducing sugars (A), non-reducing sugars (B), total soluble sugars (C) and starch content (D) of corms, ± shows SE of three replicates.

Starch content: Figure 2D showed the higher amount of starch (32.05 mg/g and 35.38 mg/g) in control corms and started to decrease (30. 89 mg/g and 32.30 mg/g) in treatment (0.1 mM GA_3 along with 0.5 mM BAP) in white prosperity and Amsterdam, respectively. The decreasing trend of starch content continued and minimum value (21.82 mg/g and 23.75 mg/g) was recorded in white prosperity and Amsterdam at 0.8 mM gibberellic acid in combination with 1 mM benzylaminopurine.

Total phenolics: Among treatments, the control corms showed 312.54 µg/g, 254.04 µg/g total phenolic content which was decreased to 114.04 µg/g, 97.57 µg/g in response to treatment (0.8 mM GA_3 in combination with 1 mM BAP) in white prosperity and amsterdam, respectively. In overall results, the corms of amsterdam yielded less amount of total phenolic acid content in all the treatments compared to corms of white prosperity (Fig. 3A).

Abscisic Acid: Highest value of abscisic acid content (64.28 and 59.90 ηg/g) was observed in control corms of white prosperity and amsterdam. A gradual decrease in abscisic acid content is recorded in treated corms compared to control corms. Treatment of 0.4 mM gibberellic acid in combination with 1 mM benzylaminopurine showed minimum decrease (28.77 and 25.86 ηg/g) in abscisic acid in corms of white prosperity and Amsterdam (Fig. 3B).

Gibberellic acid: Gibberellic acid content tended to increase after treatment as control corms yielded 25.08 and 32.63 ηg/g in white prosperity and Amsterdam (Fig. 3C). The increase in gibberellic acid is positive indication to break dormancy of corms and increase germination percentage. The highest increase (105.63 and 119.93 ng/g) was recorded in treatment of 0.8mM gibberellic acid in combination with 1mM benzylaminopurine in white prosperity and amsterdam, respectively.

Discussion

In the present study, the application of BAP in combination with gibberellic acid was effective in breaking the dormancy of corms and also increasing the number of sprouted buds. These results supported the findings of Zafarian & Houshmand (2013) in that the combined application of GA_3 and BAP which resulted in increased germination of dormant seeds of *Kelussia odoratissima*. Similarly, Rossouw (2008) reported that the application of cytokinin in combination with GA_3 broke the dormancy of potato tubers in a short time and also resulted in increasing the number of sprouts. Gibberellins act synergistically with cytokinins as mentioned by Gardner *et al*., (1985). This may lead to the assumption that low concentration of plant growth regulators may be effective and also economical when used in combination.

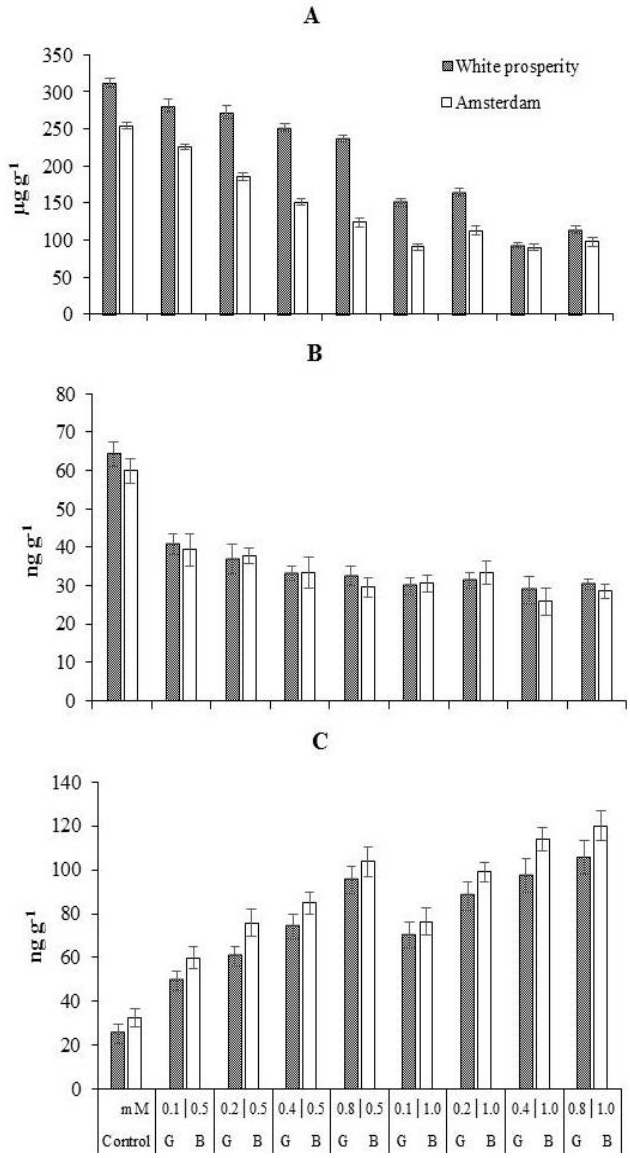

Fig. 3. Effect of plant growth regulators on total phenolics (A), abscisic acid (B) and gibberellic acid (C) content of corms, ± shows SE of three replicates. mg g^{-1} dry wt

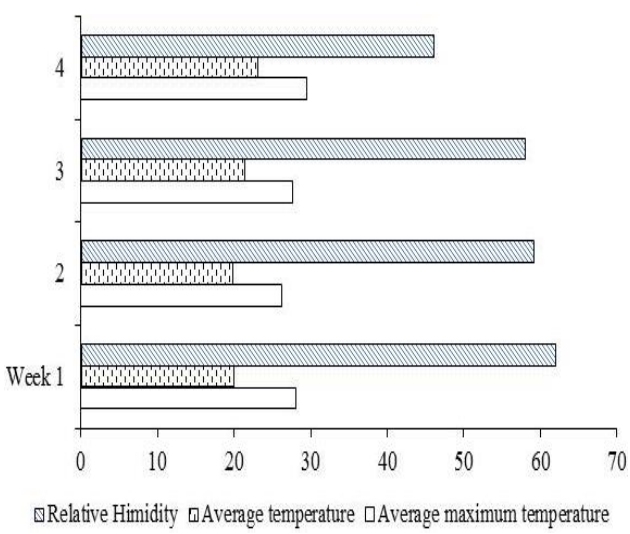

Fig. 4. Average weekly temperature (°C) and relative humidity (%) at the field during experiment.

In bulbous plants, starch is the dominant complex carbohydrate found in the underground parts and serves as a source of energy during bud sprouting (Orthen, 2001). The Gladiolus corms which were treated with different concentrations of GA_3 in combination with BAP showed a decrease in starch content indicating the mobilization of starch. The corms which showed an increase in germination had low quantities of starch compared to non-treated corms. Hence a positive correlation exists between the mobilization of starch and the sprouting of buds. Bowen & Pate (1993) further strengthened the evidence that when plants moved towards sprouting, the mobilization of carbohydrates occured which acted as source of energy to the germinating bud. According to Ohyama *et al*., (1998) starch was the predominant complex carbohydrate reserve in the mother bulb scales of tulips and decreased continuously which finally resulted in their sprouting. The initiation of process of breakdown of starch is also linked to the release of dormancy in plants as the breakdown of stored food reserves in plants is connected with the termination of dormancy and utilization of the mobilized food reserve is essential for initial growth of plants (Miller, 1992). The breakdown of starch has also been noted in underground parts such as tubers during release of dormancy and sprouting of buds (Burton, 1978; Davis & Ross, 1984). The treated corms also showed an early sprouting in this study which indicates the relationship between the amount of soluble sugars and breakdown of dormancy or initiation of sprouting. This relation has also been studied by different scientists as reported by Panneerselvam & Jaleel (2008) that the total soluble sugars was increased during transition from dormant to non dormant state. A similar trend was also reported in corms of *Crocus sativus* (Chrungoo & Farooq, 1985) and in tubers of potato (Copp *et al*., 2000). The increase in soluble sugars is due to breakdown of starch which occurs during release of dormancy. The conversion of starch to soluble sugars is initiated even before the alleviation of dormancy (Hariprakash & Nambisan, 1996).

The treated corms showed a decrease in phenolic content and an increase in germination which suggests the role of phenolic contents in controlling of dormancy. This trend establishes the inhibiting effect of phenolics on germination and our results are in line with the findings of Cvirkova *et al*., (1994) who described the inhibiting action of phenolic compounds in buds of potato tubers and noted a decrease in their level during dormancy break. Konoshima *et al*., (1973) measured three classes of growth inhibitors from dormant corms of Gladiolus, the inhibitors class 1 which showed an inhibiting effect on growth of gladiolus were identified as phenolic compounds. Benkeblia & Selselet-Attou (1999) reported a correlation between levels of phenolic compounds with the release of dormancy. They found an increase in the levels of phenolics which inhibited sprouting, when level of phenolics decreased, resulted in release from dormancy. Esmaeili *et al*., (2011) concluded that the induction of dormancy in corms of saffron was due to increased levels of phenolic compounds at the end of the waking stage which again suggested the involvement of phenolics in induction of dormancy.

The endogenous levels of growth regulators and growth inhibitors have a key role in the regulation of dormancy. Wareing (1977) found a similar relationship between dormancy and growth regulators. He stated that fluctuations in the level of growth regulators and growth inhibitors were related with the regulation of dormancy. Abscisic acid was among the growth inhibitors measured from dormant corms of gladiolus by Konoshima *et al*., (1973). Uyemura & Imanishi (1987) demonstrated a direct relationship between levels of growth inhibitors including the abscisic acid in controlling the dormancy of Freesia corms. Abscisic acid has been found as prominent factor linked with dormancy in seeds (Suzuki *et al*., 2000; Corbineau *et al*., 2002; Jacobsen *et al*., 2002; Ali-Rachedi *et al*., 2004), and bulbs (Yamazaki *et al*., 1999). The corms treated with GA_3 and BAP showed reduced content of abscisic acid but increased endogenous levels of gibberellic acid in our study. The decrease in the level of abscisic acid is directly linked with a breakdown of dormancy. Our results agreed with Xu *et al*., (2006) who found that the free abscisic contents decreased during breakdown of dormancy in *Lillium rubellum*.

Plate 1. Effect of plant growth regulators on dormancy breaking and multiple bud sprouting in white prosperity under field conditions, (A. dormancy breaking and sprouting of multiple buds, B. uprooted and descaled corm), compared to control corms (C. delayed sprouted corm, D. uprooted and descaled corm).

Conclusions

Dormancy period of corms of two Gladiolus cultivars including white prosperity and Amsterdam was shorten by application of GA_3 in combination with BAP. Treatment of corms with 0.8mM GA_3 in combination with 1 mM BAP was the best treatment to induce maximum sprouting in the dormant corms of both cultivars. The biochemical analysis of germinated corms showed the high content of total soluble sugars, gibberellic acid while control corms showed high content of phenols and abscisic acid which can be responsible for dormancy induction.

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