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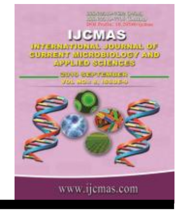

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Studies on the Exogenous Application of Plant Growth Regulators on Morphological and Biochemical Changes in Gladiolus (*Gladiolus grandiflorus* L.) Leaf

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A B S T R A C T

An experiment was conducted following Randomized Block Design at the instructional field of the Department of Floriculture, Medicinal and Aromatic Plants, Uttar Banga Krishi Viswavidyalaya, Pundibari, CoochBehar, West Bengal, India from October, 2013 to April, 2014. Three different plant growth regulators *viz*., Gibberellic acid (GA_3), N^6-Benzyladenine (BA) and Triacontanol each at 3 different levels (25ppm, 50ppm and 100ppm) were applied on Gladiolus cv. American Beauty and the effect was compared to control (distilled water). Each treatment was replicated thrice. In the present experiment, application of GA_3 25ppm increased the initial leaf length (10.05 cm) and leaf chlorophyll content (62.30 SPAD value) but GA_3 50ppm increased the phenol content of leaves (1.84 mg/ g of fresh wt.), whereas, GA_3 100ppm increased the leaf protein content (2.49 mg/ g of fresh wt.). Exogenous application of BA 25ppm improved the reducing sugar content of leaves (4.230 mg/g of fresh weight) while, BA 100ppm improved leaf enzymatic activity (1.27 Δ490 nm/min/g fresh weight). Application of Triacontanol 50ppm improved initial plant height (14.49 cm), initial leaf production (3.08), initial and final leaf width (1.68 cm and 2.63 cm respectively), leaf enzyme activity (1.27 Δ490nm/min/g fresh weight) and final leaf length (57.96 cm). Triacontanol 100ppm improved final leaf production (11.61) and plant height (75.67 cm). Results revealed that Triacontanol 50ppm and 100ppm improved the vegetative growth of Gladiolus which is the integral activity towards improvement of the yield attributing characters and hence may be recommended for commercial cultivation of gladiolus in the Terai region of West Bengal.

Introduction

Flowers are symbols of beauty, love and peace. It is one of the stunning gifts to the mankind from the end of the nature that brings joy and happiness. They form the soul of garden and convey the message of nature to man (Havale *et al.*, 2008). Gladiolus is one of the most popular cut flowers in the world for its majestic spikes (Sinha and Roy, 2002). Cut flower trade has become a profitable enterprise for many flower growing countries.

Gladiolus hold fourth place as a cut flower in international market after Rose, Carnation and Chrysanthemum (Farhat, 2004). In Netherland and other European countries, it ranked second after tulip as a cut flower among the bulbous flowers (Singh, 2006). The name of Gladiolus is derived from the Latin word '*Gladius*', because of its sword like leaves and therefore, it is called as 'Sword lily'. *G. illyricus* is found wild as weed in the corn field in Europe, that's why, it is named as 'Corn flag'. The leaves are attached to the stem in opposite direction, i.e., two-ranked. Fewer roots may also originate from the base of the aerial shoot (Mukhopadhyay, 2002). In West Bengal, the major Gladiolus producing districts include Darjeeling, Midnapore (East & West), Nadia, Jalpaiguri, Howrah and North 24-Parganas (Anon., 2014). Commercial production of Gladiolus showed a gradual increase in past few years (Memon, 2009). In West Bengal, gladioli hold second position among the cut flowers and Darjeeling district shared highest area, production and productivity within the state (Anon, 2014). Plant growth regulators are organic substances with important functions in regulating growth depending on its concentration and other intrinsic characteristics of the plant (Marcos *et al.,* 2011). They enhance the source-sink relationship or stimulate the translocation of photo-assimilates thereby influencing the plant growth, flower formation, fruit development, seed development and ultimately improve the productivity of the crops (Amanullah and Vincent, 2010). Among exogenous gibberellins, GA_3 is commonly responsible to increase the height of plants, vegetative growth and internodal distance, through altering cell division, elongation and expansion (Da Silva *et al.,* 2011; Shankar, 2011) and ultimately increase the total plant growth (Umrao *et al.,* 2007). It also promoted the sprouting, stem elongation, increased leaf production in different floricultural crops (Rani, 2013). Exogenous application of BA improved the yield attributes in field crops seemed to be due to increased availability of assimilates which in turn might cause greater chlorophyll synthesis as observed by Reddy *et al.,* (2009) in Cowpea. Another important plant growth regulator is Triacontanol. According to Mandava (1979), it is a secondary plant growth substance and cannot be considered as a phytohormone. Such types of growth regulators enhance the physiological efficiency of the cells. In India most of the studies involving Triacontanol are confined to natural extracts. Triacontanol improves better root development resulting effective utilization of nutrient as well as improved the photosynthetic activity in crops thus increasing enzyme activities, free amino acids, reducing sugars and soluble protein of plants (Naeem *et al.,* 2011).

Materials and Methods

An experiment was conducted at the instructional field of the Department of Floriculture, Medicinal and Aromatic plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, CoochBehar, West Bengal, from October, 2013 - April, 2014 to study the effect of corm dipping as well as exogenous spraying of plant growth regulators on the morphological and biochemical changes occurred in Gladiolus leaves.

Three potent plant growth regulators namely Gibberellic Acid (GA_3), N^6- Benzyl Adenine and Triacontanol each at three different levels (25, 50 and 100ppm) were applied twice - as corm dipping treatment for six hours at one day prior to planting as well as spraying on standing crop of Gladiolus at three weeks after planting and the effects were compared to control (distilled water dipping and spray) plants following Randomized Block Design with 10 treatments replicated thrice. Healthy, disease-free corms of Gladiolus cv. American

Beauty having diameter of 37.05 - 40.56 mm and 20 - 26 g in weight were selected for the experiment and treated with Carbendazim 50 WP (Bavistin) @1g/ lit of water for an hour at one week before planting. The treatment details is presented below -

T_0 - Control (Distilled Water) T_1 - GA_3 @ 25ppm
T_2 - GA_3 @ 50ppm T_3 - GA_3 @ 100ppm
T_4 - BA @ 25ppm T_5 - BA @ 50ppm
T_6 - BA @ 100ppm T_7 - Triacontanol @ 25pp m
T_8 - Triacontanol @ 50ppm T_9 - Triacontanol @ 100ppm

Treatment and replication-wise average data of plant height (cm), number of leaves per plant, leaf length (cm), leaf width (cm) each at 21 and 63 days after planting, canopy temperature (^{0}C), leaf chlorophyll content (SPAD Value) using chlorophyll meter SPAD 502 at 10:00 AM on clear sunny day after 60 days of planting, reducing sugar content of leaf (mg per g of fresh weight) following the procedures of Nelson (1944) and Somogyi (1945) at 620 nm, non-reducing sugar content of leaf (mg per g of fresh weight) following the procedures of Nelson (1944) and Somogyi (1945) at 620 nm, leaf protein content (mg per g of fresh weight) following the procedure of Lowry (1951) with Folin–Ciocaltaeu reagent and Bovine serum albumin standard curve, leaf phenol content (mg per g of fresh weight) following the procedure of Malick and Singh (1980) with Catechol at 650 nm and enzyme (peroxidase) activity (Δ490nm/min/g fresh wt.) following the procedure of Sadasivam and Manickam (1996) with pyrogallol and hydrogen peroxide were collected and analyzed according to the Fisher's analysis of variance techniques using mstatC software.

Results and Discussion

The effect of plant growth regulator applications was found statistically significant in all the parameters studied except initial plant height at 21 days after planting, initial leaf width at 21 days after planting and non-reducing sugar content of Gladiolus leaves cv. American Beauty. Corms and plants treated with 50ppm Triacontanol showed the maximum plant height (14.49 cm) at 21 days after planting (Table -1). Plant height was significantly increased at 63 days after planting of gladiolus when treated with Triacontanol 100ppm (75.67 cm) which was statistically at par with the effect of 100ppm GA_3 (71.67 cm). The minimum plant height at this stage (51.12 cm) was noticed with 50ppm BA treatment.

Application of plant growth regulators increased the leaf production of gladiolus both at 21 and 63 days after planting. Initially all the treatments performed better except BA and Triacontanol at higher level (100ppm) with values of 1.96 and 2.08 respectively. Though Triacontanol 50ppm performed better initially (3.08 leaves) but at 63 days after planting Triacontanol 100ppm treatment produced maximum number of leaves (11.61) which was at par with GA_3 100ppm (10.83 leaves). The negative effects were observed finally (table - 1) with GA_3 50ppm and Triacontanol 25ppm (6.17 leaves both).

Initially GA_3 25ppm showed highest leaf length (10.05 cm) which was at par with Triacontanol 50ppm (9.24 cm) but afterwards Triacontanol 50ppm produced the maximum leaf length (57.96 cm) of gladiolus at 63 DAP which was at par with GA_3 100ppm (57.72 cm), BA 100ppm (56.06 cm) and Triacontanol 100ppm (52.00cm). The minimum leaf lengths were observed initially with BA 50ppm (4.06cm) and finally with Triacontanol 25ppm (39.81cm) treatments (table -1).

Application of Triacontanol 50ppm significantly increased leaf width both at 21 (1.68cm) and 63 (2.63cm) days after planting. Use of BA 50 ppm (0.48cm) and BA 100ppm (1.14cm) proved inhibitory regarding leaf width at 21 and 63 days after planting of Gladiolus respectively. The maximum

(25.38°C) Canopy temperature (Figure - 1) was found with GA_3 100ppm treatment. Control plants showed better effect (22.77°C) over some of the PGR treatments like all levels of Triacontanol and BA 50 and 100ppm. Plants treated with GA_3 25ppm resulting the maximum leaf chlorophyll content (62.30 SPAD) in Gladiolus, which was statistically significant and on par with the effect of BA 50ppm (61.47 SPAD). All the plant growth regulator applications produced higher leaf chlorophyll content in Gladiolus (Figure - 2) over control (57.33 SPAD).

Corms and plants of Gladiolus cv. American Beauty when treated with BA 25ppm recorded maximum reducing sugar content in leaves (4.230 mg/g of fresh weight). All the plant growth regulator treatments showed better result over control (4.163 mg/g of fresh weight) in this aspect (Table - 2) but, control plants produced the maximum non-reducing sugar content (0.39748 mg/g of fresh wt.) in leaves which was found statistically non-significant. Treatment with GA_3 100ppm generated the maximum leaf protein content (2.49 mg/g of fresh weight) which was on par with the effect of control (2.45 mg/g of fresh weight), GA_3 25ppm (2.40 mg/g of fresh weight), GA_3 50ppm (2.34 mg/g of fresh weight) and Triacontanol 100ppm (2.31 mg/g of fresh weight). The minimum leaf protein (1.84 mg/g of fresh weight) was obtained from Triacontanol 25ppm treatment. Leaf phenol content was found highest with GA_3 50ppm (1.84 mg/g of fresh weight) and lowest with GA_3 100ppm (0.27 mg/g of fresh weight). The effect of plant growth regulators on the enzyme activity of leaves was found statistically significant (Table –2). Corms and plants treated with BA 100ppm and Triacontanol 50ppm recorded maximum enzyme activity of leaves (1.27 Δ490nm/min/g fresh weight) which were at par with BA 50ppm, GA_3 100ppm and control (1.20 Δ490nm/min/g fresh wt.) treatments in this regard. Corms and plants treated with Triacontanol 25ppm showed the least effect (1.07 Δ490nm/min/g fresh weight).

Plant growth regulators often called as plant bio-regulators are the compounds which are applied exogenously to modify the plant physiological processes to produce a gainful output. Improvement of growth of gladiolus through exogenous application of GA_3 can be attributed to enhance the source-sink relationship or to stimulate the translocation of photo-assimilates was also observed by Khan *et al.*, (2013) in gladiolus. In the present experiment application of GA_3 25ppm increased the initial leaf length. These observations are in conformity with the earlier reports of Sudhakar and Kumar (2012) in Gladiolus and this might be due to the growth promoting effect of GA_3 in stimulating and accelerating cell division and/or cell enlargement or both, photosynthetic and metabolic activities, developing plants having taller leaves (Sajid *et al.*, 2015). GA_3 25ppm also increased the chlorophyll content of leaf. The result is in accordance with the findings of Janowska and Jerzy (2003) that higher chlorophyll content (100 mg L^{-1}) clearly indicating the dominating effect of GA_3. The result might be due to the ability of gibberellic acid to prevent the degradation of photosynthetic pigment, i.e. the chlorophyll content in plants (sajjad *et al.*, 2014). Application of GA_3 50ppm increased the phenol content of leaves. Sardoei *et al.*, (2014) found that GA_3 resulted highest total phenolic content on *Calendula officinalis*. The reduction in phenol content with higher level reflected the dose-specificity of the crop (Sharaf-Eldin *et al.*, 2007). Whereas, GA_3 @ 100ppm increased the leaf protein content, canopy temperature.

Table.1 Effect of plant growth regulators on plant height, leaves per plant, leaf length and leaf width of Gladiolus cv. American Beauty

Treatment	Plant Height at 21 DAP (cm)	Plant Height at 63 DAP (cm)	Leaves per plant at 21 DAP	Leaves per plant at 63 DAP	Leaf Length at 21 DAP (cm)	Leaf Length at 63 DAP (cm)	Leaf Width at 21 DAP (cm)	Leaf Width at 63 DAP (cm)
T_0	7.26	59.30	2.20	6.83	5.06	45.43	0.77	1.57
T_1	12.72	58.26	2.50	7.44	10.05	46.55	0.98	2.12
T_2	10.26	56.06	2.39	6.17	5.46	44.88	0.90	1.99
T_3	13.17	71.67	2.78	10.83	7.91	57.72	0.96	2.26
T_4	8.52	58.96	2.50	6.86	7.49	48.81	0.78	1.75
T_5	3.65	51.12	2.83	6.28	4.06	40.70	0.48	1.70
T_6	7.76	65.43	1.96	7.89	8.04	56.06	0.73	1.14
T_7	10.42	55.92	2.94	6.17	5.41	39.81	0.95	1.82
T_8	14.49	64.31	3.08	7.61	9.24	57.96	1.68	2.63
T_9	9.16	75.67	2.08	11.61	6.51	52.00	0.72	1.87
S. E_m±	*2.91*	*3.73*	*0.24*	*0.65*	*0.41*	*2.89*	*0.24*	*0.18*
C.D at 5%	**NS**	**11.08**	**0.70**	**1.92**	**1.21**	**8.58**	**NS**	**0.54**
C.V.	51. 83.						46.38	

Table.2 Effect of plant growth regulators on reducing sugar, non-reducing sugar, protein content, Enzyme activity and phenol content of leaf in Gladiolus cv. American Beauty

Treatment	Reducing Sugar content of Leaf (mg/g of fresh weight)	Non-Reducing sugar content of Leaves (mg/g of fresh weight)	Leaf protein content (mg/g of fresh weight)	Enzyme activity of leaf (Δ490nm/min/g fresh weight)	Leaf phenol content (mg/g of fresh weight)
T_0	4.163	0.39748	2.45	1.20	0.71
T_1	4.173	0.39742	2.40	1.13	1.25
T_2	4.173	0.39744	2.34	1.13	1.84
T_3	4.180	0.39738	2.49	1.20	0.27
T_4	4.230	0.39742	1.87	1.13	0.31
T_5	4.217	0.39742	1.59	1.20	0.88
T_6	4.210	0.39744	2.16	1.27	0.68
T_7	4.207	0.39744	1.84	1.07	1.28
T_8	4.196	0.39742	1.94	1.27	1.01
T_9	4.190	0.39746	2.31	1.13	1.07
S. E_m±	*0.01*	*0.0000296*	*0.11*	*0.03*	*0.15*
C.D at 5%	**0.03**	**NS**	**0.32**	**0.1**	**0.44**
C.V.		0.01			

Fig.1 The effect of plant growth regulators on canopy temperature of Gladiolus

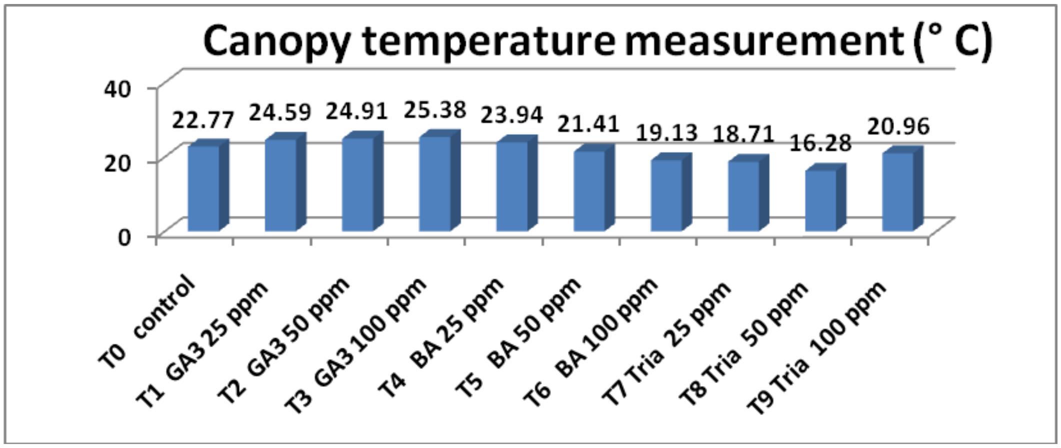
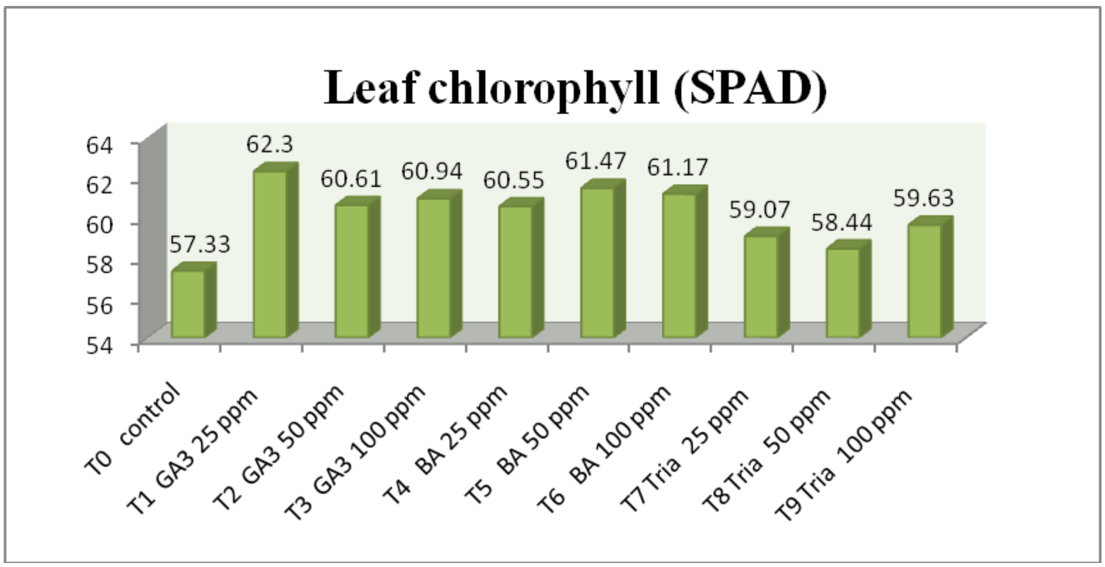


Fig.2 The effect of plant growth regulators on leaf chlorophyll estimation of Gladiolus



The findings were in agreement with the findings of Bhalla and Kumar (2008) and Dogra *et al.*, (2012) in Gladiolus.

Exogenous application of BA 25ppm improved the reducing sugar content of leaf (4.230 mg/g of fresh weight). BA delayed senescence of gladiolus spikes probably by retarding the rate of breakdown of synthesised protein and increasing the soluble sugar (Faraji and Basaki, 2014). Tricontanol is a growth promoter for several crop plants like ginger (Singh *et al.*, 2012), hyacinth beans (Naeem *et al.*, 2009), tomato (Khan *et al.*, 2009), mungbean (Reddy *et al.*, 2002), rice (Pandey *et al.*, 2001) etc. Exogenous application of Tricontanol improved the growth in plants might be due to the triggering effect on photosynthesis. Triacontanol increased the growth and quality of *Chrysanthemum morifolium* (Skoren *et al.*, 1982). In the present experiment application of Triacontanol @ 50ppm improved initial plant height (14.49 cm), initial leaf production (3.08), initial and final leaf width (1.68 cm and 2.63 cm respectively), leaf enzyme activity (1.27 Δ490nm/min/g fresh weight) and final leaf length (57.96 cm). The increase in plant height using Tricontanol and GA_3 through increase in cell elongation in apical meristem resulting increased intermodal length (Sainath, 2009). Triacontanol 100ppm improved the final plant height (75.67 cm), final leaf production per plant (11.61). Triacontanol resulted better root development which is further helpful for utilization of nutrient as well as improved the photosynthetic activity in crops thereby developed better flowering, minimizing flower and fruit drop, improvement in nitrogen fixation, enzyme activities, free amino acids, reducing sugars and soluble protein in plants as observed by Ries (1991). Triacontanol-mediated improvement in growth, yield, photosynthesis, protein synthesis, uptake of water and nutrients, nitrogen-fixation, enzymatic activities and contents of free amino acids, reducing sugars, soluble protein, and active constituents of essential oil were observed in various crop plants [Idrees *et al.*, (2010); Naeem *et al.*, (2011)]. It enhances the physiological efficiency of the cells and exploits the genetic potential of plant to a large extent. Actually, it increased free amino acids, reducing sugars, and soluble protein in rice and maize (Ries, 1991).

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