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INCREASING DROUGHT TOLERANCE OF GLADIOLUS PLANTS THROUGH APPLICATION OF SOME GROWTH REGULATORS

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ABSTRACT: **Two pot experiments were carried out under the conditions of open field of the National Research Centre, Dokki, Giza, Egypt during the two successive seasons of 2002/2003 and 2003/2004 to study the effect of some growth regulators; namely, spermine(sp.) and paclobutrazol(pac.) on overcoming the harmful effects of drought on flowering and some physiological aspects of gladiolus plants. Drought has been found to cause a significant reduction in the spike length and number of florets per spike, while the time of vase life was significantly increased under decreasing water supply as compared with those normally irrigated. Spraying plants with 20 ppm of spermine and 30 ppm of paclobutrazol significantly increased the vase life. Paclobutrazol treatments decreased spike length and number of florets per spike, while spermine increased spike length when compared with untreated plants. On the other hand, spermine had no significant effect on number of florets per spike. Both drought and growth regulators treatments significantly increased cell sap concentration and consequently its osmotic pressure as well as proline content in comparison with the control.**

Key words: Gladiolus, drought, spermine, paclobutrazol.

INTRODUCTION

Drought stress affects nearly every process in plant, but not all processes are affected equally by a particular degree of stress. Loos of turger is probably the process most sensitive to water deficit. The result is a decrease of growth rate, stem elongation, leaf expansion and stomatal operture (Hale and Orcutt, 1987).The reduction in photosynthesis, inhibition of translocation and disturbance of nitrogen metabolism all added to the effect of reduced turgor in reducing growth (Simpson, 1981). According to Turner (1986), plants responded to drought either by delaying dehydration where the plant maintained relatively high plant water potential or by tolerating dehydration where the plant continued to function but at lower plant water potentials. Plants that delayed dehydration often exhibited reduced transpiration by reducing stomatal conductivity, thereby maintaining higher plant water potential. Plants that relied on tolerating dehydration experience lower plant water potentials but exhibited active osmotic adjustment that maintained turgidity and supports transpiration.

Various workers pointed out the role of growth regulators to improve qualitatively and/or quantitively the yield of many crop plants. The preliminary reports in this regard showed that, the affected natural hormones balance and also cell division and elongation, in terms of promotion plant growth, flowering, bud dormancy breaking as well as the increment in plant yield of flowers, corms and cormels. (Zizzo and Fascella, 2000). Furthermore, the use of growth regulators in order to control the growth and development of different plant species especially for increasing plant tolerance to water stress has been reported by several investigators (Abo El-Kheir, 2000 and Laz and Ismail, 2005).

Therefore, the present study was undertaken to determine the efficiency of spermine and paclobutrazol to overcome the harmful effects of water stress on flowering, some physiological processes of gladiolus plants.

MATERIALS AND METHODS

This study was carried out under the conditions of open field of the National Research Centre, Dokki, Giza, Egypt during the two successive seasons of 2002/2003 and 2003/2004 to study the effect of some growth regulators; namely, spermine(sp.) and paclobutrazol (pac.) on overcoming the harmful effects of drought on flowering and some physiological aspects of *Gladiolus* *gandavensis* plants cv. Yellow Top.

Each experiment included fifteen treatments which were the combination between three levels of water supply and five concentrations of growth regulators. The treatments were arranged in a complete randomized block design in three replicates, each replicate contained four pots and each pot contained four plants. Plastic pots (30 cm diameter) were field with 20 kg of air-dried loamy sand soil (Physical and chemical analysis of the soil is illustilated in Table 1) which determined according to Singh (1980), Jackson (1967) and Black and Hartage (1986).

Uniform corms (20g in weight and 3cm diameter) were planted on October 8th in the two seasons at the rate of four corms per pot.

After one month from planting gladiolus plants were subjected to three levels of soil moisture. They were irrigated after depletion of 35, 50 and 65 % of field capacity. These treatments reflecting conditions achieved as optimum level of water supply, moderate and severe water stress, respectively. The pots were weighed daily and the needed amount of water was added to each pot. At the same time, the pots of each soil moisture treatment were subdivided into five groups and plants of each group were sprayed with one of the used growth regulators concentrations. These concentrations were 10 and 20 ppm of spermine or 30 and 60 ppm of paclobutrazol in addition to distilled water as control. Spraying was conducted twice throughout plant development; i.e., at 30 and 60 days from planting. The spraying was maintained till drip. All treatments received the same agricultural practices.

Representative plant sample was collected from three replications for each treatment after 90 days from planting and the following constituents were determined:

1-Water content (%) was calculated according to the following equation

$$\text{Water content (\%)} = \frac{\text{Fresh weight - Oven dry weight}}{\text{Fresh weight}} \times 100$$

2-Cell sap concentration and the corresponding values of osmotic pressure (Atm) were determined according to Gusev (1960).

3-Proline content (μ moles/g fresh weight) was determined according to the method adopted by Bates *et al.* (1973).

The flowering stems were cut when the basal floret became colored leaving three leaves on each plant in which spike length (cm), number of florets per spike and vase life were recorded.

The data of the experiments were subjected to statistical analysis as factorial experiment in complete randomized blok design according to **Snedecor and cochran (1980).**

RESULTS AND DISCUSSION

Effect of Water Regime, Growth Regulators and Their Interaction on Flowering Parameters

Spike length

Data presented in Table 2 reveal that the spike length was significantly decreased with increasing soil moisture stress resulted from irrigation with either 50 or 35% of F.C. in the two experimental seasons when compared with the control (irrigation with 65% of F.C.). The data indicate further that spraying spermine remarkably increased the spike length. On the contrary, the spike length significantly decreased with paclobutrazol treatment in the two experimental seasons when compared with the untreated plants.

Concerning the interaction effects, the data in Table 2 show that spermine had no significant effect on spike length of plants that normally irrigated (irrigation with 65% of F.C.), while under soil moisture stress (irrigation with 35% of F.C.), spraying with 20 ppm of spermine significantly increased spike length in the two

Table 1. Physical and chemical analysis of the soil

Sand (%)	Silt (%)	Clay (%)	Textural class	Field capacity (%)	Soil pH (1:2.5)	Electric conductivity (dS_m^{-1})	Calcium carbonate (%)
62.7	24.0	13.3	Loamy sand	20	7.81	1.95	2.11

experimental seasons in comparison with untreated plants. On the other hand, paclobutrazol treatments caused additional retarding effect to that caused by drought on spike length.

Number of florets

Data in Table 2 show that increasing soil moisture stress by irrigation with 50% or 35% of F.C led to a significant reduction in the number of florets per spike in the two experimental seasons in comparison with the control (irrigation with 65% of F.C). The data also reveal that spermine had no significant effect on the number of florets per spike in the two experimental seasons. On the other hand, the number of florets/ spike was decreased by 60 ppm of paclobutrazol application in the two experimental seasons in comparison with untreated plants.

As for the interaction effect, the data indicate that growth regulators used did not significantly affect the number of florets/ spike of plants that normally irrigated (65% of F.C.), while under moderate stress(irrigation with 50% of F.C.) spermine and paclobutrazol increased the number of florets per spike when compared with untreated plants (Table 2).

Vase life

Data in Table 2 show that decreasing soil moisture content through irrigation with 35% of F.C. led to a significant increase in the time of vase life in the two experimental seasons in comparison with the control (irrigation with 65% of F.C.). The data in the same table reveal that treated plants with 20 ppm of spermine and 30 ppm of paclobutrazol significantly increased the vase life.

Concerning the interaction effects in Table 2, it could be noticed that spraying with spermine and paclobutrazol increased the vase life in plants irrigated with the higher levels of field capacity; i.e, 65 or 50% but under severe water stress (irrigation with 35% of F.C.) the time of vase life was decreased in comparison with untreated plants.

The results of the flowering stem measurements showed that spike length and number of florets per spike were significantly decreased as a result of increasing soil moisture stress. Similar results were obtained by Chimonidou-Pavlidou (1996) and D' Andria *et al.* (1996). The depression in these parameters could be explained on the basis of the loss of turger which affected the rate of cell

Table 2. Effect of water regime and growth regulators (spermine and paclobutrazol) treatments on some flowering parameters of gladiolus plants during the two seasons of 2002/2003 and 2003/2004

Spermine (Sp.) and paclobutrazol (Pac.) (ppm)	Water supply levels (%F.C)											
	Spike length (cm)				Number of florets / spike				Vase life (day)			
	65	50	35	M	65	50	35	M	65	50	35	M
First season												
Control	57.3 e	42.3 c	32.3 a	44.0 C	8.33 b	7.00 ab	6.33 ab	7.22 B	7.33 ab	6.67 a	14.3 c	9.44 A
Sp 10	56.3 e	42.6 c	40.0 bc	46.3 D	8.33 b	7.00 ab	7.00 ab	7.44 B	11.33 b	7.00 ab	10.3 b	9.55 A
Sp 20	56.0 e	41.0 bc	42.6 c	46.5 D	8.33 b	7.00 ab	7.00 ab	7.44 B	9.67 b	11.0 b	12.0 bc	10.8 B
Pac 30	51.0 d	41.0 bc	32.3 a	41.4 B	7.33 b	7.00 ab	7.00 ab	7.11 A	9.33 ab	9.67 b	11.6 b	10.2 B
Pac 60	43.0 c	38.0 b	30.6 a	37.2 A	7.00 ab	6.33 ab	5.67 a	6.33 A	7.33 ab	9.00 ab	8.00 ab	8.11 A
M	52.7 C	41.0 B	35.6 A		7.87 B	6.87 A	6.60 A		9.00 A	8.67 A	11.2 B	
Second season												
Control	56.6 j	53.0 h	39.6 c	49.7 C	8.66 c	8.00 bc	7.66 b	8.44 B	8.00 a	8.33 ab	11.3 c	9.22 A
Sp 10	56.0 j	54.6 i	41.3 d	50.7 D	8.33 bc	9.33 d	8.33 bc	8.55 B	9.66 b	9.33 b	9.66 b	9.55 B
Sp 20	56.3 j	51.3 g	42.0 d	49.8 C	8.33 bc	9.00 cd	8.33 bc	8.22 B	9.00 ab	9.33 b	11.0 c	9.77 B
Pac 30	55.6 ij	48.6 f	35.6 b	46.6 B	8.33 bc	9.00 cd	8.33 bc	8.00 B	8.66 ab	8.66 ab	11.3 c	9.55 B
Pac 60	53.6 hi	43.6 e	33.6 a	43.6 A	7.33 ab	8.33 bc	6.66 a	7.21 A	8.33 ab	8.33 ab	9.33 b	8.66 A
M	55.6 C	50.2 B	38.4 A		8.39 B	7.99 A	7.86 A		8.73 A	8.79 A	10.5 B	

Values having the same alphabetical letter (s) did not significantly differ at the 0-05 level according to Duncan's multiple range test.

expansion and ultimate cell size. In this concern, Simpson (1981) showed that water stress resulted in disturbance in most of the physiological process; e.g. photosynthetic protein synthesis, enzyme activity and these affect the metabolites transpiration to the end product. In addition, Robinson *et al.* (1983) reported that water stress decreased the mobilizing ability of the inflorescence and increased that of the corm, reduced $^{14}CO_2$ fixation and, to a lesser degree, delayed assimilate translocation out of the source leaves.

It is clear from the results that spraying gladiolus plants with spermine increased spike length and number of florets/ spike. The opposite trend can be obtained in case of paclobutrazol application. Spraying with either spermine or paclobutrazol increased vase life. These results were in harmony with those obtained by several investigators. (Maloupa *et al.*, 1999 and Ali. 2002). The positive effect of spermine on flowering parameters noticed in the present study was in accordance with the findings of Kakkar *et al*. (2000). In this concern, Lee *et al.* (1997) reported that spermine delayed the senescence of cut carnation flowers and reduced ethylene production.

Effect of Water Regime, Growth Regulators and Their Interaction on Water Conditions.

Water content

Examination of Table 3 reveal that the water content of irrigated plants with 35% of F.C. was the lowest in the two seasons in comparison with the control (irrigation with 65% of F.C.). With regard to the effect of spraying with growth regulators, data indicate that spermine significantly increased water content comparing with control in the first season but there was no significant effect in the second season. On the other hand, spraying gladiolus plants with paclobutrazol reduced water content in the two seasons.

As for the interaction affect, Table 3 indicate that spraying plants with spermine especially under severe water stress significantly increased water content in comparison with untreated plants in the two seasons.

Osmotic pressure of the cell sap

Data in Table 3 show that increasing soil moisture stress by irrigation with 50% or 35% of F.C. resulted in significantly increased osmotic pressure of the cell sap in comparison with the control (irrigation with 65% of F.C). Concerning the effect of growth regulators, data indicate that spraying gladiolus plants with spermine in the two seasons especially at 20 ppm significantly increased the osmotic pressure in comparison with the control. With regard to paclobutrazol in the two seasons generally significantly increased the osmotic pressure in comparison with the control.

As for the interaction between different treatments, the data indicate that growth regulators treatments caused addition increasing to that caused by drought on osmotic pressure of the cell sap. The highest value was detected in plants irrigated with 35% of F.C. and sprayed with 20 ppm of spermine.

Proline content

Data in Table 3 show that decreasing soil moisture stress content by irrigation with 35% of F.C led to significant increase in proline content in the two seasons in comparison with the control (irrigation with 65% of F.C.). The data indicate also that spraying plants with spermine up to 20 ppm and paclobutrazol up to 60 ppm significantly increased proline content in comparison with the untreated plants.

As for the interaction between different treatments, the data indicate that the highest accumulation of proline content was recorded in plants irrigated with 35% of F.C and sprayed with 30ppm of paclobutrazol.

The collected data in the present investigation show that the decrease of soil moisture caused a decrease in water content and increase in cell sap concentration and proline content in the leaves. Similar results were reported by several investigators. Bohnert and Jensen (1996) reported that the ability of plants to tolerate water deficit was determined by multiple biochemical pathways that facilitate retention and /or acquisition of water, protect chloroplast functions and maintained ion homeostasis. Essential pathways included those that lead to synthesis of osmotically active metabolites and specific proteins that control ion and water flux. Singh and Patel (1996) found that leaf water potential, osmotic pressure and relative water content decreased whereas, accumulation of proline increased with water stress. Furthermore, Ali *et al.* (1999)

Table 3. Effect of water regime and growth regulators (spermine and paclobutrazol) treatments on water conditions of gladiolus plants after 90 days from planting during the two seasons of 2002/2003 and 2003/2004

Spermine(Sp.) and paclobutrazol (Pac.) (ppm)	Water supply levels (%F.C)											
	water content (%)				osmotic pressure (atm)				proline content (µmoles/g fresh wt.)			
	65	50	35	M	65	50	35	M	65	50	35	M
						First season						
Control	76.0 d	66.5 bc	54.7 a	65.7 C	8.09 a	9.54 b	10.6 bc	9.42 A	17.0 a	18.1 a	38.1 e	24.4 A
Sp 10	76.7 d	67.8 c	56.3 a	66.9 C	8.27 ab	9.99 bc	10.6 bc	9.63 A	17.5 a	18.6 a	39.9 e	25.3 A
Sp 20	75.7 d	65.1 bc	66.4 bc	69.0 D	8.93 ab	10.6 bc	11.4 c	10.2 B	25.0 b	29.6 c	47.2 f	34.0B
Pac 30	68.2 c	57.5 a	63.9 bc	63.2 B	8.20 a	9.82 bc	11.0 c	9.68 A	25.5 b	33.1 d	52.3 g	37.0 C
Pac 60	63.8 b	55.3 a	58.4 a	59.2 A	8.29 ab	9.96 bc	11.2 c	9.82 A	32.7 cd	34.8 d	57.6 h	40.6 D
M	72.1 C	62.4 B	59.9 A		8.36 A	9.99 B	10.8 C		23.5 A	26.8 B	46.4 C	
						Second season						
Control	80.0 e	76.2 d	68.7 ab	74.9 C	8.09 a	8.64 a	10.0 c	8.92 A	7.2 a	17.6 a	28.5 c	21.1 A
Sp 10	80.2 e	77.3 de	69.4 b	75.6 C	8.35 a	9.41 bc	8.92 bc	8.89 A	18.5 a	18.6 a	33.8 d	23.6 B
Sp 20	78.8 de	74.2 cd	71.0 bc	74.6 C	8.84 b	10.9 d	9.84 c	9.86 B	21.7 b	23.7 bc	40.5 e	28.6 C
Pac 30	76.2 d	71.7 bc	64.9 a	70.9 B	7.65 a	9.68 bc	10.5 cd	9.30 A	22.9 bc	25.8 c	43.2 f	30.6 D
Pac 60	72.9 c	68.8 b	62.0 a	67.9 A	7.84 a	10.1 cd	10.4 cd	9.47 B	28.5 c	26.8 c	48.2 g	34.5 E
M	77.6 C	73.6 B	67.2 A		8.15 A	9.75 B	9.96 B		21.8 A	22.5 A	38.8 B	

Values having the same alphabetical letter (s) did not significantly differ at the 0-05 level according to Duncan's multiple range test indicated that soil drying decreased leaf growth thereby reducing leaf water status in addition to accumulation of organic solutes to osmotic adjustment which in turn inhibited the incorporation of small substrate molecules into the polymers needed to grow new cells. Rodrigues *et al.* (1980) studied the effect of different levels of soil moisture on gladiolus plants .They observed that the relative water content was positively correlated with soil moisture content.

The results of this study indicated that, spraying gladiolus plants with either spermine or paclobutrazol increased osmotic pressure, proline content, whatever there was no detected trend can be obtained on the effect on water content in the leaves comparing with control. In this connection, Frakulli and Voyiatzis (1999) found that treated olive trees with paclobutrazol increased water potential and decreased stomatal resistance and water saturation deficit. Moreover, Anju *et al.* (1998) found that treating olive plants with paclobutrazol increased the accumulation of osmolytes such as amino acids and soluble sugars compared with in untreated plants. In addition, Yue *et al.* (1998) observed that decreases in water potential and relative water content were least in leaves treated with spermine comparing with untreated plants. According to the present data, it was indicated that the response of cell sap concentration to spermine or paclobutrazol treatments depended upon both the dose applied and the status of water regime.

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زيادة تحمل نباتات الجلاديولس للجفاف باستخدام بعض منظمات النمو

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تم تنفيذ هذا العمل لدراسة كفاءة بعض منظمات النمو(الأسبرمين و الباكلوبترازول) فى التغلب على التأثيرات الضارة للجفاف على الازهار وبعض العمليات الفسيولوجيه لنباتات الجلاديولس ، تم اجراء التجربة فى اصص فى صوبة المركز القومى للبحوث بالدقى- الجيزه خلال موسمى ٢٠٠٢/٢٠٠٣، ٢٠٠٣/ ٢٠٠٤. حيث عرضت النباتات إلي ثلاثة مستويات من الإمداد المائي (٦٥، ٥٠، ٣٥% من السعة الحقلية) بالاضافة إلى خمسة تركيزات من منظمات النمو (١٠و٢٠ جزء فى المليون من الاسبرمين. ٣٠و ٦٠ جزء فى المليون من الباكلوبترازول ، بالإضافة إلى الرش بالماء للمقارنة) وقد دلت النتائج المسجله خلال الموسمين على ما يلي:

أدي نقص محتوى التربة من الماء (الري بمستوى ٣٥% من السعة الحقليــة)الــى نقص معنوي في طول الشمراخ الزهرى و عدد الازهار على الشمراخ بينما سجلت فترة بقاء اكثر للزهرد فى الماء مع الري بمستوى ٣٥% من السعة الحقلية مقارنة بالكنترول (الري بمستوى ٦٥% من السعة الحقلية). كما أوضحت النتائج ايضا أن اضافة الاسبرمين كان له تأثير محسن على الصفات الانتاجية للشمراخ.على النقيض، فقد أدي اضافة الباكلوبوترازول إلى نقص معنوي في طول الشمراخ الزهرى و عدد الازهار على الشمراخ مقارنة بالنباتات غير المعاملة، كما أدي كلا من الجفاف ومعاملات منظمات النمو الى زيادة معنوية فى كلا من الضغط الأسموزى للعصير الخلوى ومحتوى البرولين فى الأوراق مقارنة بالكنترول.