

## EFFECT OF COLCHICINE ON GROWTH AND FLOWERING OF *BOUGAINVILLEA GLABRA CHOICY* VAR *SANDERIANA*

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### ABSTRACT

A series of pot experiment was conducted at the Nursery of Hort. Res. Inst., ARC., Giza, Egypt during 2004/2005 and 2005/2006 seasons to detect the response of *Bougainvillea glabra choicy* var. *sanderiana* transplants cultivated in 16-cm- diameter plastic pots filled with about 1.5 kg of loam, sand and peatmoss mixture (1:1:1, by volume) to the various concentrations of colchicine (0.00, 0.02, 0.04 and 0.08%), application method (either as a soil drench or as a foliar spray), and also to their interaction. The obtained results showed that plant height (cm) and number of leaves /plant were progressively decreased with increasing colchicine concentration, whereas leaf area was argumentatively increased. Stem diameter was non-significantly improved in response to the various level of colchicine, with the mastery of 0.04% level which gave the highest means. Number of branches/plant was only increased due to the low (0.02%) and medium (0.04%) concentrations, but the high one (0.08%) significantly declined it. Number of peduncles/plant, as well as number of florets/ peduncle were markedly elevated with raising colchicine rate, with the superiority of the high rate (0.08%) that unexpectedly gave the utmost high records in this regard. Peduncle length, on the contrary was gradually depressed as the concentration of colchicine raised. Gradual increment in the content of chlorophyll a, b and carotenoids (mg/g F.W.) was observed in the leaves of treated plants with increasing colchicine level. The prevalence was unexpectedly attributed to the high level (0.08%) that gave the highest content of chlorophyll a and carotenoids, while for chlorophyll b content, the medium level, (0.04) was the superior. Total indoles and total phenols, on the other side were decreased as a result of elevating colchicine level. Application of the colchicine as a soil drench resulted a significant improvement in the measurements of plant height, peduncle length and total of both indoles and phenols, while foliar spray method surpassed in number of both leaves and peduncles/plant. However application method had no significant effect on other parameters.

According to the aforementioned results, it could be summarized that drenching or spraying *B. glabra choicy* var. *sanderiana* transplants with either 0.04% or 0.08% of colchicine aqueous solution, twice with one month interval may gave stunted, compact and floriferous plant suitable for selling as flowering pot plants. In addition, occurrence of some reverse responses may suggest the incidence of some changes in response pattern, which may lead to some variations in the gene expression. So, the second part of such trial will be deviated for documenting this inference.

**Key words:** *Bougainvillea*, colchicine, mutations, soil drench, foliar spray, vegetative growth, flowering, chemical composition.

### INTRODUCTION

Induction of artificial genetical changes accompanied with selection is still one of the most important ways to get new phenotypes or races with desirable characters. Also increasing the vegetative growth and flowering at the lower doses.

Among plant species may be valid in this concern, *Bougainvillea glabra choicy*, var.

*sanderiana* plants (Fam. *nyctaginaceae*), which are using now on a wide scale for beautification and landscaping the different gardens and places in tropical and subtropical areas. It is a very floriferous and worthy plant for both pot culture and as a shrub or climber. Often blooms at 1 fb. height, even in a very small pot, but reaches a height of 10-20 fbs. (Huxley et al., 1992).

give excellent and secure results. Among these chemicals, colchicine. It is a highly poisonous alkaloid, originally extracted from plants of the genus *Colchicum* (specially *C. autumnale*, Meadow Saffron) and corms of *Gloriosa spp.* It has a medicinal use in the treatment of gout and as anticancer drug. It is also used for inducing polyploidy in plant cells by inhibiting chromosome segregation during meiosis. It was affirmed that subjecting several plant species to various concentrations of colchicine for different durations exhibited a pronounced fluctuation in the morphogenetic parameters and in the endogenous constituents. In this regard, Wang *et al.* (1989) indicated that proliferated plantlets on scales of *Lilium davodii* were decreased by colchicine (1-4 mg/L). Also variations in leaf thickness, leaf colour and bulb size were appeared. Endo *et al.* (1997) found that treated shoot tips of chrysanthemum with colchicine (0.2%) for 24 or 48 h resulted doubled chromosome plants, which were obviously dwarfed with less number of branches, leaves and flower buds.

On *Melaleuca armillaris* Youssef *et al.* (1998) mentioned that low concentration of colchicine (100 ppm) gave the greatest shoots fresh and dry weights, while a high concentra-

tion (400 ppm) increased leaf area and leaf length and width. All concentrations (100-400 ppm), however reduced chlorophyll a content to a low level. Han *et al.* (1999) reported that that an increment in concentration and duration of colchicine resulted in a decrease in survival rate and shoot regeneration, while low concentrations (0.25 and 0.50 mM) for 48h increased diploid cells and shoot regeneration.

Similarly, were those results of Xing Cui *et al.* (2000) on *Melaleuca alternifolia*, Youssef (2003) on *Yucca elephantips* and *philodendron scandens* Nassor (2003) on cassava (*Manihot estulenta*), Youssef and Abou-Dahab (2006) on *Populus alba* and Sayed *et al.* (2007) who revealed that low level of colchicine (0.05 mg/L) promoted survival capacity and shootlet length of *Solidago altissima* var. "Tara" but higher levels depressed the shootlet and root formation.

This study, however aims to produce some genetical variations by all increasing the vegetative growth in *B.glabra* var. *sanderiana* transplants by means of the different levels of colchicine application method and their interaction.

## MATERIALS AND METHODS

A trial was performed at the Nursery of Hort. Res Inst., A.R.C., Giza, Egypt throughout 2004/2005 and 2006/2006 seasons to study the effect of colchicine at various levels, method of application and their interaction on growth, flowering and chemical composition of *Bougainvillea glabra* choicy. var. *sanderiana* transplants.

Therefore, cuttings of *Bougainvillea glabra* choicy. var. *sanderiana* with a long ranged between 12-15 cm and 1-1.2 cm diameter, were taken from the middle parts of woody branches of five- years- old shrub and dipped in a fungicide solution of 0.2% Topsin M-70% WP (produced by Sumitomo chemical Co., Ltd., Osaka, Japan) for 15 minutes. Then, they were planted on July, 26<sup>th</sup> for both seasons in 8-cm-diameter plastic pots (one cutting/pot) filled with an equal mixture of loam, sand and peatmoss, (1:1:1 by volume).

All cuttings were successfully rooted in the proper time. After rooting by about two

months, they were transplanted into 16-cm-diameter plastic pots filled with about 1.5 kg of the same previous mixture. On December, 26<sup>th</sup>, pots were divided into two main groups; the first was treated with colchicine as a soil drench, while the second treated with the same chemical as a foliar spray. Each main group, however was divided into four sub-groups to receive the different concentrations of colchicine aqueous solution, which were: 0.00 (referred to as control), 0.02, 0.04 and 0.08% (referred to as low, medium and high level, respectively).

Applications were repeated once again after one month later. The layout of such factorial experiment was a completely randomized design (Das and Giri, 1986) consisting of 8 treatments in 3 replicates, as each replicate contained 5 pots.

At the end of experiment (on 26<sup>th</sup> of stumper), the following Data were recorded: plant height (cm), stem diameter at the base

(cm), number of branches and leaves, plant, leaf area (cm<sup>2</sup>) number of peduncles/plants, peduncle length (cm) and number of florets/peduncle.

In fresh leaf samples, however chlorophyll a, b and carotenoids (mg/g F.W.) according to the method of Moran (1982), as well as total indoles (Selim *et al.*, 1978) and total phe-

nols (Daniel and George, 1972) were determined in the second season only.

Data were then statistically analyzed according to SAS program (1994) using Duncan, s Multiple range test to verify the significance level among means of various treatment described by Duncan (1955).

## RESULTS AND DISCUSSION

**Effect of colchicine level, application method and their interaction on:**

### **Vegetative growth:**

According to data presented in Table (1), it could be concluded that plant height and number of leaves /plant were gradually decreased with increasing colchicine concentrations in the two seasons to reach the least averages due to the high rate (0.08%), which registered the utmost low means in these two parameters. On the contrary, leaf area (cm<sup>2</sup>) was progressively increased with raising colchicine concentration to reach the maximum values in response to applying the high level (0.08%) in both seasons. Stem diameter (cm) was non-significantly improved as a result of treating with the various levels of the chemical mutagen, with the superiority of 0.04% level that recorded the highest means in the two seasons.

As for number of branches/plant, it was significantly increased in response to the low (0.02%) and medium (0.08%) declined it to the least values in the first and second seasons. With regard to application method, it was noticed that soil drench technique significantly improved plant height and leaf area traits, while foliar spray method only hastened number of leaves/ plant with significant differences in the two seasons.

Application method, however had no significant effect on both stem diameter and number of branches/plant in both seasons. In general, the best performance of bougainvillea transplants gained from applying the medium level of colchicine (0.04%) which gave a height in harmony with leaf area and number of both branches and leaves/plant. The previous results may be interpreted and discussed as done before in case of sodium azide effects on vegetative growth of this plant (part 1).

### **Flowering:-**

Data in Table (2) show that number of peduncles produced by treated plants was significantly higher than those of untreated ones, especially for the plants sprayed or drenched with the highest concentration of colchicine (0.08%), which unexpectedly gave in the two seasons the utmost high values in this concern. A similar trend was also gained regarding number of florets/peduncle, as the concentration of 0.08% unexpectedly vaised such parameter to the highest averages in both seasons.

Peduncle length (cm), on the other hand was cumulatively depressed as the rate of colchicine was elevated with significant differences in comparison to the control in the two seasons.

However, effect of the chemical mutagen as a foliar spray surpassed its effect as a soil drench in the matter of peduncles number trait in both seasons, but the opposite was the right concerning peduncle length in the first season only.

Such findings, however could be explained and discussed as previously mentioned in case of the effect of sodium azide, application method and their interaction on flowering of this plant at part 1.

### **Chemical composition**

It is clear from data in Table (3) that chlorophyll a, b and carotenoids content (mg/g F.W.) in the leaves were significantly increased in response to the different levels of colchicine. The highest level (0.08%) unexpectedly scored the highest means in the content of both chlorophyll a and carotenoids, whereas the medium one (0.04%) registered the highest means in chlorophyll b content only. Application method had no significant effect on either of pigments determined in this study.

On the other hand, total indoles and total phenols content (mg/g F.W.) were gradually decreased as colchicines concentration increased with the prevalence of soil drench method, which gave significantly higher values than foliar spray one.

**Table (1): Effect of colchicine treatments, application methods and their interaction on some vegetative growth parameters of *Bougainvillea glabra* choicy. var *sanderiana* transplants during 2004/05 and 2005/06 seasons.**

Colchicine treatments	Plant height (cm)			Stem diameter (cm)			No. branches/plant			No. leaves/plant			Leaf area (cm <sup>2</sup> )		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
<b>First season: 2004/05</b>															
0.00%	38.33 a	36.76 a	37.55 a	1.67d	1.46c	1.57c	2.00c	2.00c	2.00 b	20.33 a	22.00 a	21.17 a	10.00 d	9.73 d	9.87 e
0.02%	37.50 a	33.83 b	35.67 a	1.73c	1.67b	1.70b	2.67a	2.71a	2.69 a	15.67 c	18.33 b	17.00 b	15.92 b	2.42 d	4.17b
0.04%	29.67 b	29.68 b	29.68 b	2.00 a	1.83a	1.92a	2.33b	2.30b	2.32 ba	14.33 c	14.70 c	14.52 c	15.83 b	13.53 c	4.68 b
0.08%	26.88 c	23.46 d	25.17 c	1.81b	1.67b	1.74b	1.67d	1.56d	1.62 c	4.00 c	13.18 d	13.59 d	18.00 a	13.75 c	5.88 a
Mean	33.10 a	30.93 b		1.80 a	1.66b		2.17a	2.14a		16.08 b	17.05 a		14.94 a	12.36 b	
<b>Second season: 2005/06</b>															
0.00%	39.65 a	35.88 a	37.77 a	0.90c	0.90d	0.90d	2.00c	2.00c	2.00 b	22.67 a	22.81 a	22.74 a	9.78 c	10.08 c	9.43 e
0.02%	35.00 a	35.00 a	35.00 a	0.98cb	1.00c	0.99c	2.33b	2.76a	2.55 a	17.26 b	17.10 b	17.18 b	3.25 cd	3.33 cd	3.29 b
0.04%	33.12 b	33.56 b	33.34 b	1.33a	1.65a	1.50a	2.50a	2.63b	2.57 a	15.80 c	17.33 b	6.57 b	14.00 b	16.83 a	5.42 a
0.08%	25.76 c	24.27 c	25.02 c	1.07b	1.50b	1.29b	1.82d	2.00c	1.91 b	14.67 c	15.00 c	14.84 c	16.29 a	4.96 b	5.63 a
Mean	33.38 a	32.18 b		1.07b	1.27a		2.16b	2.35a		17.60 b	18.06 a		13.33 b	13.80 a	

**Table (2): Effect of colchicines treatments, application methods and their interaction on flowering of *Bougainvillea glabra* choicy var *sanderiana* transplants during 2004/05 and 2005/06 seasons.**

Colchicine treatments	No. beduncles/plant			Peduncle length (cm)			No. florets/peduncle		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
<b>First season: 2004/2005</b>									
0.00%	1.00 d	1.00 d	1.00 b	19.33 a	18.96 a	19.15a	3.33 c	3.33 c	3.33c
0.02%	1.00 d	1.33 c	1.17 b	18.50 ba	18.67 ab	18.59ba	5.00 ab	4.28 b	4.64 b
0.04%	1.00 d	1.33 c	1.17 b	18.37 ba	17.98 b	18.18b	5.16 a	4.76 ba	4.96 a
0.08%	1.67 b	2.33 a	2.00 a	15.00 c	13.51 d	14.26c	5.50 a	5.00 ab	5.25 a
Mean	1.17 b	1.50 a		17.80 a	17.28 b		4.75 a	4.34 a	
<b>Second season: 2005/2006</b>									
0.00%	1.00 d	1.00 d	1.00c	22.10 a	22.33a	22.22a	3.00 c	3.00 c	3.00c
0.02%	1.00 d	1.67 b	1.34bc	22.00 a	19.76 b	20.88ba	4.00 b	3.33 cb	3.67bc
0.04%	1.33 c	1.70 b	1.52b	17.33 cd	18.00 c	17.67bc	4.26 b	4.33 b	4.30b
0.08%	1.78 b	2.67 a	2.23a	16.80 d	17.10 dc	16.95c	4.67 ab	5.00 a	4.84a
Mean	1.28 b	1.76 a		19.56 a	19.30 a		3.98 a	3.92 a	

Decrement of both indoles and phenols made the ratio between them constant under the various treatments of colchicine and

the different methods of application. These gains, however may be interpreted and discussed as those attained in case of sodium

azide effects on chemical composition of this plant (part 1).

In conclusion, drenching or spraying *B. glabra* var. *sanderiana* transplants with colchicine aqueous solution at either medium (0.04%) or high (0.08%) levels, twice with one month interval can be recommended to obtain the best performance, compact and floriferous

plants suitable for marketing as flowering pot plants. However, getting some of unexpected responses may suggest that some changes in the response pattern may have involved. This means, consequently that some variations in the gene expression could be inferred.

Hence, the second part of such trial will be devoted for proving this expectation.

Table (3): Effect of colchicines treatments, application methods and their interaction on some constituents of *Bougainvillea glabra* choicy var *sanderiana* transplants during 2004/2005 and 2005/2006 seasons.

Colchicine treatments	Chlorophyll a (mg/g. F.W)			Chlorophyll b (mg/g. F.W)			Carotenoids (mg/g. F.W)			Total indoles (mg/g. F.W)			Total phenols (mg/g. F.W)		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
0.00%	0.41c	0.48cb	0.45c	0.12c	0.17cb	0.15c	0.21 d	0.23d	0.22c	1.53 a	1.38ba	1.46a	5.64a	5.08cb	5.36a
0.02%	0.62ab	0.69a	0.66b	0.18 bc	0.21b	0.20b	0.37 c	0.42bc	0.40b	1.46 a	1.31b	1.39ab	5.32b	4.79 c	5.06b
0.04%	0.54b	0.58ba	0.56cb	0.29 a	0.33a	0.31a	0.44 b	0.48b	0.46b	1.31 b	1.18c	1.25b	4.33ed	3.90 e	4.12c
0.08%	0.68a	0.73 a	0.71a	0.17 cb	0.18bc	0.18bc	0.53a	0.58a	0.56a	0.85 d	0.88d	0.87c	4.48d	4.02 e	4.24c
Mean	0.56a	0.62a		0.19 a	0.22 a		0.39a	0.43a		1.29 a	1.19b		4.94a	4.45 b	

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### تأثير المعاملة بالكولشيسين على نمو وأزهار الجهنمية

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قسم بحوث نباتات الزينة-معهد بحوث البساتين -مركز البحوث الزراعية -الجيزة-مصر

أجريت سلسلة من تجارب الإصص بممثل معهد بحوث البساتين ،مركز البحوث الزراعية ،الجيزة، مصر خلال موسمي ٢٠٠٤/٢٠٠٥ ، ٢٠٠٥/٢٠٠٦ للتعرف على مدى استجابة شتلات الجهنمية *Bougainvillea glabra choicy* صنف ساندريانا النامية في أصص بلاستيك قطرها ١٦ سم ملأت بحوالي ١,٥ كجم من مخلوط متساوي من الطمي، الرمل والبيتموس (١:١:١ حجماً) لمحلول الكولشيسين (مطفر كيميائي) بتركيزات: صفر، ٠,٠٢، ٠,٠٤، ٠,٠٨ % تم إضافتها إما للتربة أو رشاً على الأوراق وكذلك التفاعل بينهما. ولقد أظهرت النتائج المتحصل عليها مايلي:-

أن طول النبات وعدد الأوراق /نبات انخفضا بشكل متصاعد بزيادة تركيز الكولشيسين، بينما زادت مساحة الورقة بشكل تصاعدي كلما زاد تركيز المحلول المضاف. ولقد أحدثت التركيزات المختلفة من الكولشيسين تحسناً طفيفاً في قطر الساق مع تفوق المستوى (٠,٠٤ %) والذي أعطى أعلى القيم في هذا القياس. أما عدد الأفرع /نبات فقد زاد معنوياً نتيجة للمعاملة بالتركيز المنخفض (٠,٠٢ %) والمتوسط (٠,٠٤ %)، بينما انخفض معنوياً بالتركيز المرتفع (٠,٠٨ %). ولقد زاد عدد العناقيد الزهرية /نبات، وكذلك عدد الزهيرات /عنقود زهرى تدريجياً كلما ارتفع تركيز الكولشيسين مع تفوق التركيز المرتفع (٠,٠٨ %) والذي أعطى بشكل غير متوقع أعلى القيم في هذين القياسين -على العكس فان طول العنقود الزهرى قد انخفض تدريجياً مع زيادة تركيز المادة المطفرة. - ولقد لوحظ أيضاً حدوث زيادة في محتوى الأوراق من كلوروفيل اعب والكاروتينويدات (ملجم/جم وزن طازج) بزيادة تركيز الكولشيسين، حيث أحدثت التركيز المرتفع تقوفاً على باقى المعاملات في محتوى الأوراق من كلوروفيل ب عزيت الى التركيز المتوسط. على الجانب الاخر، فان محتوى الأوراق من الإندولات الكلية والفينولات الكلية قد انخفض تدريجياً بزيادة تركيز الكولشيسين .

أدت إضافة محلول المادة المطفرة للتربة الى أحداث تحسناً معنوياً فى ارتفاع النبات، طول العنقود الزهرى و محتوى الأوراق من الإندولات الكلية والفينولات الكلية. بينما تفوقت طريقة رش المحلول على الأوراق في إعطاء أكبر عدد من الأوراق والعناقيد الزهرية/نبات. هذا ولم يكن لطريقة الإضافة أى تأثير معنوي على باقي القياسات الأخرى.

طبقة النتائج المذكورة سابقاً، يمكن ايجاز الآتى: القول أنه برى أو رش شتلات الجهنمية صنف ساندريانا بالمحلول المائي للكولشيسين أما بتركيز ٠,٠٤ أو ٠,٠٨ % مرتين وبفاصل شهر بينهما، يمكن الحصول على نباتات مقدمة، مندمجة غزيرة الأزهار تصلح لتسويقها كنباتات إصص مزهرة.

أيضاً، فإن حدوث بعض الاستجابات المعاكسة للكولشيسين، يوحى بتوقع حدوث بعض التغيرات فى نمط الاستجابة، والتي قد تؤدي بالتالى إلى بعض التغيرات فى التعبير الجينى.