

## THE INFLUENCE OF LIGHT QUALITY AND BA ON *in vitro* GROWTH AND DEVELOPMENT OF *Gardenia jasminoides* Ellis

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**Abstract.** *Gardenia* is an ornamental shrub or small tree that has thick dark evergreen leaves and fragrant flowers of white colour. It is used as a garden plant in the mild climate as well as a flowering potted plant. Many studies have been taken to propagate *gardenia in vitro* and to reduce the time necessary for the introduction of new cultivars. The present study investigated the effect of different light qualities (white, blue, yellow, red) on shoot and root development and growth of *Gardenia jasminoides* Ellis. Shoot tips were obtained from aseptic *in vitro* cultures and explants were cultured on modified Murashige and Skoog medium containing BA at 5 or 25  $\mu\text{M}$  as well as on medium without growth regulators. The experiment lasted 6 weeks and was repeated twice. The promoting effect of yellow light on the length of the main shoot growing on control medium was noted. There was no significant effect of different light qualities on the number of axillary shoots obtained from tip shoots cultured on growth regulator-free medium. Yellow light increased the number of axillary shoots from explants cultivated on medium containing 25  $\mu\text{M}$  BA and it promoted shoot elongation most strongly on medium with the addition of 5  $\mu\text{M}$  BA. Red and yellow light increased the number of roots on control medium, whereas yellow light significantly promoted root elongation growth. However, yellow and white light had the most positive effect on the average fresh weight of roots.

**Key words:** cytokinin, branching, rooting, light quality, micropropagation

### INTRODUCTION

Light performs an important role in plant growth and development processes. The morphogenesis processes are affected by both light quality and intensity. Growth substances also have an enormous influence [Gabarkiewicz et al. 1995]. In *in vitro* cultures, by changing the colour of light, the propagation efficiency can be enhanced and shoot growth improved as well as the rhizogenesis can be stimulated. Latkowska and Chmiel

[1996] observed the highest number of axillary shoots in *Dendranthema grandiflora* in white and blue light in the presence of BA at  $0.6 \text{ mg}\cdot\text{dm}^{-3}$  in the medium, but this number was much lower in red light. On the other hand, Gabryszewska and Rudnicki [1997] report that in *in vitro* cultures *Ficus benjamina* developed the highest number of shoots in red light with the addition of 2iP at  $15 \text{ mg}\cdot\text{dm}^{-3}$  to the medium. A similar response to red light was observed by Gabryszewska and Rudnicki [1995] in the case of *Gerbera jamesonii* 'Queen Rebeca' on medium with kinetin at a rate of  $5 \text{ mg}\cdot\text{dm}^{-3}$ . Red light stimulated elongation growth of *Petunia hybrida* shoots [Michalczyk 2000, Witomska and Ładyżyńska 2001], and *Chrysanthemum* × *grandiflorum* 'Richmond' [Miler et al. 2005]. Rooting of *Petunia hybrida* shoots was stimulated by white light [Witomska and Ładyżyńska 2001]. White light, but also green light, promoted rooting of *Dendranthema* × *grandiflora* 'Lilac Wonder' shoots [Miler and Zalewska 2006]. Red light had a beneficial effect on the rhizogenesis of *Phlebodium aureum* [Bach and Reby 1994], and *Tibouchina urvilleana* [Kozak et al. 2010].

The aim of the present study was to determine the effect of light quality and its interaction with BA in the growth and development of gardenia shoots.

## MATERIALS AND METHODS

Shoot tips of 8–10 mm in length having at least 2 fully developed leaves were taken from aseptically grown shoots of *Gardenia jasminoides*. The explants were placed on basic Murashige and Skoog (MS) [1962] medium containing: mineral salts and thiamine –  $0.4 \text{ mg}\cdot\text{dm}^{-3}$ , pyridoxine –  $0.5 \text{ mg}\cdot\text{dm}^{-3}$ , nicotinic acid –  $0.5 \text{ mg}\cdot\text{dm}^{-3}$ , glycine –  $2 \text{ mg}\cdot\text{dm}^{-3}$ , myo-inositol –  $100 \text{ mg}\cdot\text{dm}^{-3}$ , sucrose –  $30 \text{ g}\cdot\text{dm}^{-3}$ , Agar-Agar (Sigma) –  $6.5 \text{ g}\cdot\text{dm}^{-3}$ , and supplemented with benzyladenine (BA) at 5 or 25  $\mu\text{M}$ . A medium without cytokinin was also included. The pH of the media was adjusted to 5.7 before autoclaving. The cultures were maintained at  $22^\circ\text{C} \pm 2^\circ\text{C}$ , under 16-h photoperiod at photosynthetic photon flux of  $35 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , under different light quality emitted by TLD Philips 36 W fluorescent lamps: white (TLD 33), blue (TLD 18), yellow (TLD 16), red (TLD 15). There were four replications per treatment, each consisting of 5 explants/Erlenmeyer flask. The experiment was repeated twice.

The following characters were evaluated after 6 weeks: length and fresh weight of the main shoot, fresh weight of shoot clusters, number of axillary shoots and their length, number of leaves on axillary shoots, fresh weight of shoots/explant, number, length and fresh weight of roots. The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design, while the Tukey test was used to estimate the differences between the means at the 5% level of significance.

## RESULTS AND DISCUSSIONS

When investigating the length of the main shoot of *Gardenia jasminoides* cultured on growth regulator-free medium, yellow and white light was found to have the most

beneficial effect (respectively: 30.8 mm and 26.5 mm) (tab. 1). Main shoot length was significantly lower in blue light (12.4 mm). The addition of BA at a concentration of 5 or 25  $\mu\text{M}$  resulted in a significant inhibition of shoot growth in yellow and white light, whereas the presence of BA in the medium at a rate of 5  $\mu\text{M}$  in blue and red light resulted in a slight increase in shoot length relative to control medium. A further increase in BA concentration (up to 25  $\mu\text{M}$ ) inhibited the elongation growth of the main shoot. Miler et al. [2005] obtained the highest plantlets of *Chrysanthemum*  $\times$  *grandiflorum* 'Richmond' in red and yellow light on growth regulator-free medium. Kozak et al. [2010] observed a similar response of the main shoot to light in *in vitro* cultures of *Tibouchina urvilleana*. Red light had a beneficial influence on elongation growth of shoots in *Dendranthema grandiflora* 'Escort' [Latkowska and Chmiel 1996], and *Petunia hybrida* [Michalczyk 2000; Witomska and Ladyżyńska 2001]. Norton et al. [1987] observed that length of *Potentilla*, *Spirea*, *Rhododendron* and *Vitis* shoots was less under blue light than at red or white light, at low cytokinin concentration. Blue light inhibited the elongation growth of *Ficus benjamina* 'Golden King' shoots in presence of 2iP [Gabryszewska and Rudnicki 1994].

Table 1. Effect of light colour and BA on the growth of main shoot and shoot cluster of *Gardenia jasminoides* Ellis after 6 weeks of *in vitro* culture

Tabela 1. Wpływ barwy światła i BA na wzrost pędu głównego i zespołu pędów *Gardenia jasminoides* Ellis po 6 tygodniach kultury *in vitro*

Light Światło	BA $\mu\text{M}$	Length of main shoot Długość pędu głównego mm	Fresh weight of main shoot Świeża masa pędu głównego mg	Fresh weight of shoot cluster Świeża masa zespołu pędów mg
White – Białe	0	26.5 ab	291.7 ab	422.7 cde
	5	16.8 bc	228.9 abc	505.8 bcd
	25	11.6 c	264.6 abc	410.9 cde
Blue – Niebieskie	0	12.4 c	161.9 c	222.1 e
	5	18.9 abc	207.7 bc	353.5 de
	25	10.8 c	209.3 bc	278.6 e
Yellow – Żółte	0	30.8 a	260.7 abc	416.3 cde
	5	18.9 abc	241.0 abc	678.9 ab
	25	15.2 bc	279.3 abc	679.4 ab
Red – Czerwone	0	19.3 abc	247.8 abc	355.8 de
	5	21.6 abc	273.9 abc	596.2 abc
	25	15.8 bc	314.2 a	747.1 a

\*Means followed by the same letter are not significantly different at  $\alpha = 0.05$   
Średnie oznaczone tą samą literą nie różnią się istotnie przy  $\alpha = 0,05$

The fresh weight of the main shoot of *Gardenia jasminoides* ranged from 161.9 mg on control medium in blue light to 314.2 mg on medium with the addition of 25  $\mu\text{M}$  BA in red light (tab. 1). Shoots of *Tibouchina urvilleana* in red light on medium enriched with BA at 2.5  $\text{mg}\cdot\text{dm}^{-3}$  and IAA at 0.1  $\text{mg}\cdot\text{dm}^{-3}$  were also characterized by the highest fresh weight. The fresh weight of the shoot cluster was the highest (747.1 mg), similarly

to that of the main shoot, in red light in the presence of 25  $\mu\text{M}$  BA (tab. 1). The fresh weight of the shoot cluster in yellow light on media with the addition of BA at 5 and 25  $\mu\text{M}$  also reached a high value (respectively: 678.9 mg and 679.4 mg).

Table 2. Effect of light colour and BA on the number and growth of *Gardenia jasminoides* Ellis axillary shoots after 6 weeks of *in vitro* culture

Tabela 2. Wpływ barwy światła i BA na liczbę i wzrost pędów kątowych *Gardenia jasminoides* Ellis po 6 tygodniach kultury *in vitro*

Light Światło	BA ( $\mu\text{M}$ )	Number of axillary shoots from explant Liczba pędów kątowych z eksplantatu	Length of axillary shoots Długość pędów kątowych mm	Number of leaves from axillary shoots Liczba liści na pędach kątowych	Fresh weight of 1 shoot from explant Świeża masa 1 pędu kąтового z eksplantatu mg
White – Białe	0	2.1 e	2.1 de	2.2 d	5.1 d
	5	5.8 abc	5.8 b	5.2 bc	21.0 bc
	25	5.2 bcd	2.9 d	3.4 cd	11.9 cd
Blue – Niebieskie	0	1.8 e	1.8 e	2.0 d	4.1 d
	5	6.5 ab	3.8 cd	3.5 cd	8.9 d
	25	4.2 cd	2.1 de	2.6 d	8.4 d
Yellow – Żółte	0	1.6 e	1.6 e	2.2 d	8.1 d
	5	5.2 bcd	9.2 a	5.9 b	28.6 ab
	25	7.2 a	5.6 bc	5.1 bc	23.9 b
Red – Czerwone	0	1.5 e	2.1 de	3.0 cd	11.5 cd
	5	4.8 cd	7.4 ab	5.4 bc	27.8 ab
	25	6.4 ab	6.7 b	8.2 a	37.3 a

\*Means followed by the same letter are not significantly different at  $\alpha = 0.05$

Średnie oznaczone tą samą literą nie różnią się istotnie przy  $\alpha = 0,05$

When analysing the number of axillary shoots of *Gardenia jasminoides*, light quality was found to have no effect on the number of shoots developed by explants on control medium (1.5–2.1) (tab. 2). The addition of BA resulted in a significant increase in the regeneration rate. It was the highest (7.2) in yellow light on medium with the addition of 25  $\mu\text{M}$  BA. Good regeneration results were also obtained on medium supplemented with 5  $\mu\text{M}$  BA in blue (6.5) and white light (5.8) as well as in the presence of 25  $\mu\text{M}$  BA in red light (6.4). In the studies on micropropagation of *Gardenia*, cultures were cultivated using cool white fluorescent lamps [Economou and Spanoudaki 1985, 1986; Berrios and Economou 1991; Al-Juboory et al. 1998], white and red light (50:50%) [Minas 2007], or Gro-lux fluorescent lamps (they radiate energy in the far-red, red and blue regions of the spectrum) [Chuenboonngarm et al. 2001]. White light in the presence of 2iP at 10  $\text{mg}\cdot\text{dm}^{-3}$  stimulated most strongly the formation of axillary shoots in *Dieffenbachia* ‘Compacta’ [Gabarkiewicz et al. 1997]. Latkowska and Chmiel [1996] obtained the highest number of axillary shoots in *Dendranthema grandiflora* ‘Escort’ in white and blue light in the case of shoots growing in the presence of BA at 0.6  $\text{mg}\cdot\text{dm}^{-3}$ , while Miler and Zalewska [2006] obtained the highest propagation efficiency for *D. grandiflora* ‘Lilac Wonder’ in green light. In the case of *Ficus beniamina*, the largest number of shoots developed in red light on medium with the addition of 2iP at

15 mg·dm<sup>-3</sup> [Gabryszewska and Rudnicki 1997], while for *Gerbera jamesonii* ‘Queen Rebecca’ it was in red and green light on media containing kinetin at 5 mg·dm<sup>-3</sup> [Gabryszewska and Rudnicki 1995]. Kozak et al. [2010] found no effect of light quality on the formation of axillary shoots in *Tibouchina urvilleana*, both in the presence of growth regulators and in their absence.



Fig. 1. Plants of *Gardenia jasminoides* obtained on MS regulators-free medium on the lights: 1 – white, 2 – blue, 3 – yellow, 4 – red, after 6 weeks of *in vitro* culture

Ryc. 1. Rośliny *Gardenia jasminoides* uzyskane na pożywce MS bez regulatorów wzrostu w obecności światła: 1 – białego, 2 – niebieskiego, 3 – żółtego, 4 – czerwonego, po 6 tygodniach kultury *in vitro*



Fig. 2. Shoot clusters of *Gardenia jasminoides* obtained on MS medium containing BA 5 or 25 µM on the lights: 1 – white, 2 – blue, 3 – yellow, 4 – red, after 6 weeks of *in vitro* culture

Ryc. 2. Zespoły pędów *Gardenia jasminoides* uzyskane na pożywce MS z dodatkiem BA 5 lub 25 µM w obecności światła: 1 – białego, 2 – niebieskiego, 3 – żółtego, 4 – czerwonego, po 6 tygodniach kultury *in vitro*

There was a significant effect of light quality and the presence of BA in the medium on the length of *Gardenia* axillary shoots (tab. 2). Axillary shoots characterized by the largest length were observed on medium supplemented with BA at 5  $\mu$ M in yellow and red light (respectively: 9.2 and 7.4 mm). Kozak et al. [2010] observed a similar response of axillary shoots to light in the case of *Tibouchina urvilleana*. Red light stimulated the growth of axillary shoots in *Dendranthema grandiflora* ‘Escort’ [Latkowska and Chmie, 1996]. Blue light strongly inhibited shoot elongation in *Pelargonium*  $\times$  *hortorum* ‘Penny Irene’ [Appelgren 1991], *Azorina vidalii* [Moreira da Silva and Debergh 1997] and *Zantedeschia jucunda* ‘Black Magic’ [Jao et al. 2005]. Axillary shoots of *Dieffenbachia* ‘Compacta’ were longest in the dark on medium without growth regulators [Gabarkiewicz et al. 1997].

Table 3. Effect of light colour and BA on the number and growth of *Gardenia jasminoides* Ellis roots after 6 weeks of *in vitro* culture

Tabela 3. Wpływ barwy światła i BA na liczbę i wzrost korzeni *Gardenia jasminoides* Ellis po 6 tygodniach kultury *in vitro*

Light Światło	BA $\mu$ M	Number of roots from tip explant Liczba korzeni z eksplan- tatu wierzchołkowego	Length of roots Długość korzeni mm	Fresh weight of roots from explant Świeża masa korzeni z eksplantatu mg
White – Białe	0	1.2 abc	24.1 b	24.4 a
	5	0 c	-	-
	25	0 c	-	-
Blue – Niebieskie	0	0.8 c	5.8 c	6.7 b
	5	0 c	-	-
	25	0 c	-	-
Yellow – Żółte	0	1.8 ab	33.1 a	27.8 a
	5	0 c	-	-
	25	0 c	-	-
Red – Czerwone	0	2.0 a	16.3 b	12.2 b
	5	0 c	-	-
	25	0 c	-	-

\*Means followed by the same letter are not significantly different at  $\alpha = 0.05$   
Średnie oznaczone tą samą literą nie różnią się istotnie przy  $\alpha = 0,05$

Red light showed the most beneficial effect on the number of leaves produced by *Gardenia* axillary shoots cultured on medium containing BA at 25  $\mu$ M (tab. 2). Miler and Zalewska [2006] report that light quality did not have a significant influence on the number of leaves in microcuttings of *Dendranthema grandiflora* ‘Lilac Wonder’. Gabryszewska and Rudnicki [1995] report that the formation of leaves in gerbera ‘Queen Rebecca’ was stimulated by red and green light in the presence of kinetin. The study showed a significant effect of light quality and BA on fresh weight of *Gardenia* axillary shoots (tab. 2). The fresh weight of axillary shoots obtained on media with the addition of 5 or 25  $\mu$ M BA in red light, but also in yellow light, was the highest. Red light stimulated most strongly, in the presence of growth regulators, the fresh weight of axillary shoots in *Tibouchina urvilleana* [Kozak et al. 2010]. In the case of *Dieffenba-*

*chia* 'Compacta', the fresh weight of axillary shoots was highest in red light on medium without growth substances [Gabarkiewicz et al. 1997], while for gerbera 'Queen Rebecca' in white light in the presence of kinetin. Red light significantly inhibited the increase in fresh weight of shoots on control medium [Gabryszewska and Rudnicki 1995].

Analysing the number of roots produced on tip explants of *Gardenia*, light quality and type of medium were found to have a significant influence (tab. 3). On the control medium without growth substances, the shoots produced from 0.8 to 2.0 roots, with their highest number in red and yellow light, while the shoots cultured on BA-containing medium did not produce roots. In their study on the effect of light on rooting of *D. grandiflora* 'Escort', Latkowska and Chmiel [1996] did not find any significant effect of light quality on the number of roots. The highest percentage of rooted microcuttings of *D. grandiflora* 'Lilac Wonder' was obtained in white and green light [Miler and Zalewska 2006]. In their study of *Ficus benjamina* 'Golden King', Gabryszewska and Rudnicki [1995] found a beneficial effect of red light on the formation of roots, the largest number of which formed in the presence of IAA. The beneficial influence of red light, but also green light, on the number of roots in microcuttings of *Dieffenbachia* 'Compacta' was observed by Gabarkiewicz et al. [1997]. This light also stimulated the formation of roots in *Phlebodium aureum* [Bach and Reby 1994] and bromelia [Kromer et al. 1996; Kukulczanka and Kromer 2000]. In the research on *Prunus serotina*, Fuenkranz et al. [1990] observed the highest number of roots in yellow light. The quality of light used had a significant influence on root length in *Gardenia* (tab. 3). Root elongation growth was stimulated most strongly by yellow light (33.1 mm), whereas it was inhibited by blue light (5.8 mm). Roots formed in yellow and white light were characterized by the highest fresh weight (27.8 and 24.4 mg; tab. 3). Light had a similar effect on the growth of roots in *Tibouchina urvilleana* [Kozak et al. 2010].

## CONCLUSIONS

1. The influence of light quality and BA on the growth and development of *Gardenia* was shown in the present study.
2. The elongation growth of the main shoot was the best in yellow light, with no BA in the medium.
3. Light quality was found to have no effect on the formation of axillary shoots cultured on growth regulator-free medium.
4. Yellow light stimulated the formation of axillary shoots in the presence of 25  $\mu\text{M}$  BA in the medium as well as on shoot elongation growth with 5  $\mu\text{M}$  BA in the medium.
5. Shoots formed the roots only on the medium without BA, the most in red and yellow light.
6. Yellow light stimulated the elongation growth of roots, while yellow and white light had the most beneficial effect on their fresh weight.

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## WPLYW BARWY ŚWIATŁA I BA NA WZROST I ROZWÓJ GARDENII JAŚMINOWATEJ (*Gardenia jasminoides* Ellis) *in vitro*

**Streszczenie.** Gardenia jest ozdobnym krzewem lub małym drzewem o grubych, ciemnozielonych liściach i białych, pachnących kwiatach. Ma zastosowanie jako roślina do ogrodów (w łagodnym klimacie) lub jako kwitnąca roślina doniczkowa. Podejmowano wiele badań nad rozmnażaniem *in vitro* gardenii, głównie w celu szybkiego wprowadzenia do uprawy nowych odmian. W niniejszej pracy badano wpływ światła białego, niebieskiego, żółtego i czerwonego na regenerację pędów i korzeni gardenii jaśminowatej (*Gardenia jasminoides* Ellis). Wierzchołkowe fragmenty pędów pochodzące z ustabilizowanej kultury *in vitro* wykładano na pożywkę Murashige i Skooga z dodatkiem BA 5 lub 25  $\mu\text{M}$  oraz na pożywkę bez regulatorów wzrostu. Doświadczenie prowadzono 6 tygodni i powtórzono 2-krotnie. Stwierdzono korzystny wpływ światła żółtego na długość pędu głównego na pożywce kontrolnej. Nie stwierdzono wpływu barwy światła na liczbę pędów kątowych regenerujących z wierzchołków pędów rosnących na pożywce bez BA. Światło żółte wywierało najkorzystniejszy wpływ na regenerację pędów kątowych w obecności 25  $\mu\text{M}$  BA i stymulowało najsilniej wzrost wydłużeniowy pędów na pożywce z dodatkiem 5  $\mu\text{M}$  BA. Światło czerwone i żółte wpływało najkorzystniej na liczbę korzeni na pożywce kontrolnej, zaś światło żółte stymulowało wzrost wydłużeniowy korzeni. Przy świetle żółtym i białym uzyskano korzenie o największej świeżej masie.

**Słowa kluczowe:** *Gardenia jasminoides*, cytokinina, rozkrzewianie, ukorzenianie, jakość światła, mikrorozmnażanie

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