

## **BENZYLADENINE EFFECT ON ROOTING AND AXILLARY SHOOT OUTGROWTH OF *Gaura lindheimeri* Engelm. A. Gray CUTTINGS**

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**Abstract.** Cytokinins determine plant shape via regulation of meristem activity. Stimulatory effect of cytokinins on lateral shoot outgrowth is used in plant production yet they usually impair rhizogenesis. This excludes their usage in propagation from cuttings, although in some cases cytokinin positively affect rooting. The aim of the research was to determine the influence of benzyladenine in a wide range of concentrations on rooting and development of *Gaura lindheimeri* cuttings and their subsequent growth in order to obtain well branched plants in the shortest time of cultivation. Our study demonstrated high efficiency of some BA treatments in shaping of gaura cuttings without inhibition of rooting. The only combination favouring root formation (by 48%) was BA in  $0.02 \text{ g dm}^{-3}$ . Cuttings treated with BA in concentration  $0.1 \text{ g dm}^{-3}$  and higher formed axillary shoots of considerably increased number and length. The most numerous shoots in both, cuttings and young plants, were observed after application of BA in concentration  $2.0 \text{ g dm}^{-3}$ . Considering rooting and branching into account the same combination occurred to be the most suitable for development of gaura plants.

**Key words:** cytokinin, adventitious roots, branching

### **INTRODUCTION**

Cytokinins are hormones comprising a class of purine derivatives, influencing a wide range of plant developmental processes. Among them root and shoot meristem development, shoot branching, chlorophyll synthesis, leaf senescence, flowering, stress tolerance and nutrient signal transduction are mentioned [Dodd 2005, Sakakibara 2006, Müller and Sheen 2007]. Via regulation of meristem activity, with opposite role in root and shoot morphogenesis [Werner et al. 2001], cytokinins determine plant shape, which allows plant to adjust to site conditions in order to take advantage of the environment. The influence of cytokinins on plant morphogenesis can not be consider without their

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antagonistic interaction with auxins. Studies with *Arabidopsis* callus *in vitro* indicated that either auxins or cytokinins are required for organogenesis; auxin act as a triggering factor, while cytokinins modulate this process [Pernisová et al. 2009]. These two classes of hormones are also responsible for maintenance of meristem activity, based on the balance between cell divisions and cell differentiation [Dello Ioio et al. 2007]. Due to antagonistic effect of auxins and cytokinins on shoot and root meristem development, the direction of plant morphogenesis depends on auxin/cytokinin ratio. Effect of auxin domination, favouring lateral and adventitious root formation has been thoroughly documented, similarly to the effect of cytokinins releasing shoots from apical dominance [Gaspar et al. 2003]. Following these observations exogenous growth regulators are used in plant production to obtain plants of well-developed root system and required shoot architecture. Stimulatory effect of cytokinins in lateral and adventitious shoot induction and outgrowth is used in plant propagation *in vitro* as well as *in vivo* in order to obtain well branched plants, yet their usage during rooting of cuttings, especially in high concentrations, strongly impairs rhizogenesis even in the presence of auxins [Rani Debi et al. 2005]. Contrary to this general knowledge there are also reports indicating positive effect of cytokinin treatment on root formation [Van Staden and Harty 1988, Van Staden et al. 2008].

Much has been done to reveal all aspects of cytokinin influence on meristem induction and development. Yet the role of cytokinins in commercial propagation of ornamental plants *in vivo* seems to be underestimated, although some research give evidence that positive influence of cytokinin on shoot branching together with their equivocal role in root system development may have beneficial effect on production of flower bed plants from the stage of propagation by cuttings [Svenson 1990, Wróblewska and Bąbelewski 2010]. The aim of undertaken research was to determine the influence of benzyladenine (BA) on rooting and development of aerial parts of *Gaura lindheimeri* Engelm. A. Gray cuttings as well as on subsequent growth of plants. A wide range of BA concentrations was used to find balanced solution considering optimal branching and root development. From practical point of view the most interesting purpose of the study was the possibility of obtaining well branched plants in the shortest time of cultivation. *Gaura lindheimeri*, which expresses moderately strong apical dominance at early stages of cultivation, could be considered as a pertinent species for the studies.

## MATERIAL AND METHODS

The experiment with propagating of *Gaura lindheimeri* Engelm. A. Gray 'Corinas Choice' by stem cuttings was carried out in the greenhouse of Wrocław University of Environmental and Life Sciences, Poland. It was established in February 2011 and 2012. Apical stem cuttings, 4 cm in length were treated with benzyladenine (BA), in the following combinations (in  $\text{g dm}^{-3}$ ): 0.01; 0.02; 0.05; 0.1; 0.2; 0.5; 1.0 and 2.0 (respectively:  $4.4 \times 10^{-5}$ ;  $8.9 \times 10^{-5}$ ;  $2.2 \times 10^{-4}$ ;  $4.4 \times 10^{-4}$ ;  $8.9 \times 10^{-4}$ ;  $2.2 \times 10^{-3}$ ;  $4.4 \times 10^{-3}$ ;  $8.9 \times 10^{-3} \text{ mol dm}^{-3}$ ).

BA was applied by spraying on leaves after placing the cuttings in soil. Control cuttings were treated with none of the growth regulators. The soil consisted of white peat,

pine bark and perlite 3:1:1; V:V:V, pH 6.4. It was heated to the temperature of 21°C. Plastic tunnels were installed over the cuttings. The experiment was established in a one factorial design in 6 replications, per 10 cuttings in each replication. The measurements, including percentage of rooting, number of roots per cutting, height of cuttings, as well as number and length of axillary shoots were taken after 4 weeks of rooting. The measurements were done for every cutting in 3 replications, then intact cuttings, not exposed to measurement, were planted into pots, in peat substrate of pH 6.47 containing (in mg dm<sup>-3</sup>): N-NO<sub>3</sub><sup>-</sup> 145, P 119, K 263, Mg 90, Ca 1120 and placed in glasshouse heated to the temperature of 20°C. The experiment was established in 4 replications, per 5 plants in each. After 4 weeks of cultivation the height of plants as well as the number and length of axillary shoots were measured.

Data of the study were subjected to analysis of variance, and least significant differences between means were calculated by the Tukey test at  $p = 0.05$ . Data concerning the percentage of rooted cuttings were formerly transformed according to Bliss function.

## RESULTS AND DISCUSSION

Plant growth is continued as a result of meristem activity in response to environmental stimuli transduced by inner signals. Among them metabolites, RNA and hormones are mentioned. As results from the definition of hormones, they act in distant sites from place of their synthesis therefore transport from the place of origin to the place of action is one of the factors determining their action. Polar auxin transport plays the key role in meristem activity. IAA is produced in apical parts of shoots and transported basipetally through phloem to root system or to the base of cuttings. On the way of IAA downstream throughout the plant it evokes numerous plant responses, such as maintenance of apical dominance and stimulation of root development. Numerous studies underline critical role of cytokinins in root and shoot meristem development, based on auxin-cytokinin interplay. Cytokinins appear to be involved in regulation of polar auxin transport as well as induction of auxin gradient and the subsequent coordination of cell division and differentiation [Pijut et al. 2011]. In fact, cytokinins and auxins mutually regulate each other signaling and metabolism: IAA controls cytokinin content via regulation of their biosynthesis and deactivation [Nördstrom et al. 2004, Moubayidin et al. 2009].

Adventitious and lateral root formation can be divided in three interdependent phases. During the earliest stage of rooting, when none of anatomical changes can be seen, hormone levels and tissue sensitivity to hormone signals are the most significant indicators which allow to distinguish the beginning of rhizogenesis [De Klerk et al. 1995, Li et al. 2009, Pop et al. 2011]. This phase, comprising of differentiation and induction, characterizes with high level of IAA at the base of a cutting and high sensitivity to exogenous auxins [Gaspar et al. 2003]. During this stage of rooting the concentration of natural cytokinins in cuttings remains at relatively constant, low level [Taylor and van Staden 1997]. Treatment with exogenous cytokinins at that time increases the level of endogenous zeatin and, as a result, inhibits rhizogenesis, especially when they are administered in high concentrations [Wightmann et al. 1980, Laplaze et al. 2007].

The mechanism of such inhibition lies in disrupted primordia initiation and regular pattern of cell divisions. This leads to reduced root meristem size due to progressive decrease in the number of meristematic cells [Werner et al. 2003, Doerner 2007, Laplaze et al. 2007, Werner and Schumling 2009]. In details, cytokinins stimulate cell differentiation into vascular tissues through spatially selective expression of cytokinin dehydrogenase gene in the transition zone [Dello Ioio et al. 2007]. Contrary to this there are also reports indicating positive effect of cytokinin treatment on root formation. Such response usually takes place after treatment with cytokinins in low concentration, ranging from  $3 \times 10^{-10}$  mol dm<sup>-3</sup> to  $10^{-7}$  mol dm<sup>-3</sup> in case of zeatin and equal or lower than  $10^{-5}$  mol dm<sup>-3</sup> in case of isopentenyladenine (iP) and kinetin [Wightman et al. 1980, Fabijan et al. 1981, Biddington and Dearman 1982, Van Staden and Harty 1988].

Table 1. The influence of BA application on rooting of *Gaura lindheimeri* Engelm. et A. Gray cuttings

Feature	Year	BA concentration									
		0.00	0.01	0.02	0.05	0.1	0.2	0.5	1.0	2.0	
Percentage of rooting*	2011	83.9	90.0	90.0	90.0	83.9	83.9	90.0	90.0	71.6	
	2012	83.9	90.0	90.0	90.0	83.9	90.0	83.9	90.0	81.1	
	mean	83.9	90.0	90.0	90.0	83.9	87.0	87.0	90.0	76.4	
	LSD for concentration			8.2							
	LSD for concentration × year			11.6							
Number of roots	2011	8.0	7.5	15.4	4.5	10.2	11.2	9.3	10.1	6.4	
	2012	13.6	7.5	16.6	4.6	6.5	6.9	9.6	6.7	17.3	
	mean	10.8	7.5	16.0	4.6	8.4	9.1	9.5	8.4	11.9	
	LSD for concentration			1.9							
	LSD for concentration × year			2.7							

\* Data modified according to Bliss' function

The effect of BA treatment on rhizogenesis in *Gaura lindheimerii* cuttings depended on its concentration. Decreased number of adventitious roots was a consequence of application of BA in 0.01; 0.05; 0.1 and 1.0 g dm<sup>-3</sup> concentrations, whereas in 0.2; 0.5 and 2.0 g dm<sup>-3</sup> BA did not affect root establishment. Under the influence of BA in 0.02 g dm<sup>-3</sup> the cuttings developed significantly increased number of roots. Independently on the year of experiment it rose by 48% in comparison to control cuttings. However, the highest number of roots was recorded in cuttings treated with BA 2.0 g dm<sup>-3</sup> in the second year of research (tab. 1). The influence of BA treatment on the number of rooted gaura cuttings was less expressed. Regardless the year of research only application of BA in the highest concentration (2.0 g dm<sup>-3</sup>) caused slightly decreased percentage of rooting, whereas in none of the other concentrations BA evolved any response (tab. 1). Our experiment indicated that BA treatment may lead to increased number of newly formed primordia, even when it is administered in high concentrations. The rea-

son for that may lie in autoinductive cytokinin regulation. Increasing cytokinin pool promotes further accumulation of cytokinin, and, subsequently their degradation pathways [Kamínek et al. 1997]. Decreased level of endogenous cytokinins after their exogenous application detected in *Arabidopsis* hypocotyl explants corroborates such thesis [Pernisová et al. 2009]. According to the authors up-regulation of endogenous cytokinins due to expression of cytokinin oxidase/dehydrogenase genes from *AtCKX* family is involved in this phenomenon. The pathway of degradation appears to be dependent on the type of applied cytokinins. For example, in derooted *Raphanus sativus* L. seedlings application of exogenous zeatin is associated with stimulation of *N*-glucosylation, whereas BA induces activity of both, conjugation to glucose and oxidase/dehydrogenase pathways [Blagoeva et al. 2004].

Studies of cytokinin-deficient *Arabidopsis* mutants give evidence that cytokinin level is a limiting factor in shoot apical meristem activity and leaf development [Werner et al. 2001]. Apical shoot meristem formation and maintenance of its functional identity requires high cytokinin content [Doerner 2007]. Their role is to repress differentiation and maintain proliferative properties of cells [Kyojuka 2007]. They also allow to couple meristem activity with environmental factors promoting shoot growth, like high nitrogen supply. Axillary shoot meristems, located in leaf axils, form from the primary apical meristem and can stay dormant or activate to produce a branch. The activation involves integration of diverse environmental, physiological and developmental factors, including a network of interacting hormonal signals. High auxin content in the main stem, exported from already active meristems, prevents the activation of further meristems [Ferguson and Beveridge 2008, Shimizu-Sato et al. 2009]. IAA, transported basipetally is not possible to move laterally or acropetally and it does not enter the lateral bud [Ongoro and Leyser 2008, Yaish et al. 2010]. This indicates that IAA acts indirectly and other hormonal signals can influence branching by modulating the auxin transport network. Recent studies give evidence that strigolactones, a novel class of hormones, are involved in auxin-regulated inhibition of axillary bud outgrowth [Ferguson and Beveridge 2009, Xie et al. 2010]. Another group of hormones involved in this process are cytokinins which regulate distribution of auxins via control of gene expression of auxin efflux carriers from *PIN* family [Moubayidin et al. 2009, Pernisová et al. 2009]. They are the only known compounds releasing buds from dormancy [Shimizu-Sato et al. 2009]. In contrast to auxins cytokinins act directly to promote axillary bud outgrowth [Yaish et al. 2010]. Their biosynthesis is dually suppressed by IAA derived from shoot apical meristem [Sakakibara 2006]. Removing apical bud leads to rapid increase of cytokinin level [Turnbull et al. 1997] due to enhanced synthesis in the nodal stem rather than their transport from roots [Tanaka et al. 2006]. Similar results are obtained after application of exogenous cytokinins leading to enhanced axillary bud outgrowth, showing significant impact on their quality.

Our study demonstrated high efficiency of BA treatment in shaping of gaura plants from the stage of rooting of cuttings. Cuttings treated with BA in concentration  $0.05 \text{ g dm}^{-3}$  and higher formed considerably increased number of axillary shoots comparing to control cuttings. The most numerous shoots, 5-folded as compared to control cuttings, developed cuttings treated with BA in the highest concentration (tab. 2). Increased number of the shoots resulted also in increased sum of lateral shoot length after

treatment with BA in the same concentrations, although only the application of BA in concentrations higher than 0.1 stimulated elongation of single stems. The longest shoots were observed after application of BA in concentration 2.0 g dm<sup>-3</sup>.

Table 2. The influence of BA on shoot development on *Gaura lindheimeri* Engelm. et A. Gray cuttings

Feature	Year	BA concentration								
		0.00	0.01	0.02	0.05	0.1	0.2	0.5	1.0	2.0
Lenght of main shoots	2011	98.0	89.1	84.8	83.4	71.4	167.1	130.1	111.1	89.9
	2012	104.1	86.8	81.3	74.9	148.1	123.4	110.5	107.2	88.4
	mean	101.1	88.0	83.1	79.2	109.8	145.3	120.3	109.2	89.2
	LSD for concentration				15.0					
	LSD for concentration × year				21.3					
Number of axillary shootsh	2011	2.0	1.8	2.4	5.0	6.0	5.4	7.8	4.7	7.8
	2012	1.8	1.8	2.4	5.4	2.5	2.7	4.0	4.8	12.3
	mean	1.9	1.8	2.4	5.2	4.3	4.1	5.9	4.8	10.1
	LSD for concentration				1.0					
	LSD for concentration × year				1.4					
Lenght of axillary shoots	2011	2.8	6.3	4.0	3.1	4.4	16.7	13.8	6.4	10.4
	2012	4.8	6.4	3.9	3.9	6.2	4.2	6.4	12.0	16.7
	Mean	3.8	6.4	3.9	3.5	5.3	10.5	10.1	9.2	13.5
	LSD for concentration				2.4					
	LSD for concentration × year				3.0					
Sum of axillary shoot length	2011	5.6	11.2	9.6	15.1	26.5	88.4	108.7	30.7	80.0
	2012	8.7	11.3	9.1	21.3	14.4	10.0	25.7	56.2	202.3
	mean	7.2	11.3	9.4	18.2	20.5	49.2	67.2	43.5	141.2
	LSD for concentration				10.1					
	LSD for concentration × year				14.2					

Positive effect of the same BA treatments on shoot number persisted also during subsequent growth of plants received from examined gaura cuttings, apart from the combination with BA in concentration 0.2 g dm<sup>-3</sup>. Significantly longer axillary shoots, as well as the sum of shoot length were developed after treatment with BA in concentration 0.2 g dm<sup>-3</sup> and higher (tab. 3). These results pertained to studies with *Petunia* cultivars, showing that single BA application appears to be beneficial for poor branching plant taxa, whereas plants with stronger tendency for tillering require repeated BA application to respond by improved branching [Carey et al. 2007]. Apart from mentioned gaura and petunia, cytokinin treatment occurred to have stimulatory effect on axillary shoot outgrowth in various ornamental plant taxa *in vivo* and *in vitro*, for example *Chamelaucium* [Dawson and King 1993], *Dianthus* [Pobudkiewicz 2005], *Clematis* [Parzymies and Dąbski 2012], leading to more attractive plant shape, more abundant flowering or increased propagation ratio. On the other hand there are also reports indi-

cating no branching response of *Rhododendron* [Bell et al. 1997] and *Magnolia* [Pope-noe 2006] after cytokinin application. Such species-depended responses may be associated with the fact, that interaction between cytokinins and auxin efflux carriers may depend on the tissue and specific *PIN* gene [Pijut et al. 2011].

Table 3. The influence of BA applied on cuttings on subsequent growth of young plants of *Gaura lindheimeri* Engelm. et A. Gray

Feature	Year	BA concentration								
		0.00	0.01	0.02	0.05	0.1	0.2	0.5	1.0	2.0
Lenght of main shoots	2011	214.5	219.2	238.4	208.5	168.2	244.2	265.0	262.9	162.7
	2012	230.5	219.9	220.3	180.9	204.6	215.9	233.0	258.3	197.3
	Mean	222.5	219.6	229.4	194.7	186.4	230.1	249.0	260.6	180.0
	LSD for concentration				20.6					
	LSD for concentration $\times$ year				29.1					
Number of axillary shoots	2011	9.7	8.4	8.7	11.6	14.5	8.0	16.7	7.3	17.7
	2012	9.9	11.1	11.1	13.4	11.1	11.0	7.2	16.0	23.4
	mean	9.8	9.8	9.9	12.5	12.8	9.5	12.0	11.7	20.6
	LSD for concentration				1.6					
	LSD for concentration $\times$ year				2.3					
Lenght of axillary shoots	2011	8.4	12.9	11.6	12.0	15.4	51.5	7.5	74.5	40.6
	2012	12.2	10.4	11.5	13.7	4.3	42.1	94.3	43.9	43.5
	mean	10.3	11.7	11.6	12.9	9.9	46.8	50.9	59.2	42.1
	LSD for concentration				11.6					
	LSD for concentration $\times$ year				16.0					
Sum of axillary shoot length	2011	79.8	104.8	100.8	137.0	222.0	410.4	121.3	511.2	703.6
	2012	117.6	116.3	121.9	182.9	48.4	447.8	670.9	694.4	1016.9
	mean	98.7	110.6	111.4	160.0	135.2	429.1	396.1	602.8	860.3
	LSD for concentration				77.7					
	LSD for concentration $\times$ year				110.0					

Hormonal regulation depends not only on hormone concentration in plant tissues, but also on tissue susceptibility to growth regulators, which changes depending on stage of plant, organ or tissue development. Thus, the responses evoked by hormones are rarely proportional to their pool [Gaspar et al. 2003]. Such phenomenon was observed in case of BA influence on the height of gaura cuttings. Suppressed growth of main shoot was observed after treatment of BA in 0.05 and 0.1 g dm<sup>-3</sup> as well as in the highest concentration 2.0 g dm<sup>-3</sup>. Inhibited growth of main shoot accompanied by stimulated development of axillary shoots resulted in attractive, compact shape of plants received from such treated cuttings. In combinations dealing with BA in 0.2 and 0.5 g dm<sup>-3</sup> the growth of main shoot of gaura cuttings was enhanced, whereas BA 0.5 and 1.0 g dm<sup>-3</sup> occurred to stimulate it during subsequent growth of plants.

## CONCLUSIONS

Treatment with BA, depending on concentration, decreased or did not influence the number of roots in *Gaura linheimerii* cuttings. Significantly (by 48%) increased number of roots developed only under the influence of BA in 0.02 g dm<sup>-3</sup>. The influence of BA treatment on the number of rooted gaura cuttings was less expressed. Cuttings treated with BA in concentration 0.1 g dm<sup>-3</sup> and higher formed axillary shoots of considerably increased number and length. It led to better branched plants also during their subsequent growth. The longest and the most numerous shoots on both, cuttings and plants, were observed after application of BA in concentration 2.0 g dm<sup>-3</sup>.

Our study demonstrated high efficiency of BA treatment in shaping of gaura plants from the stage of rooting of cuttings. Taking rooting and branching into account, treatment with BA in concentration 2.0 g dm<sup>-3</sup> occurred to be the most suitable for development of Gaura cuttings.

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## WPLYW BENZYLOADENINY NA UKORZENIANIE I WZROST PĘDÓW BOCZNYCH NA SADZONKACH GAURY LINDHEIMERA

*Gaura lindheimeri* Engelm. A. Gray

**Streszczenie.** Poprzez regulację aktywności merystemów cytokininy warunkują pokrój roślin. Stymulujący wpływ cytokinin na wzrost pędów bocznych jest wykorzystywany w produkcji roślin ozdobnych. Z drugiej strony jednak najczęściej wpływają one ujemnie na rizogenezę, co wyklucza ich stosowanie przy rozmnażaniu przez sadzonki, mimo iż zdarza się, że cytokininy poprawiają ukorzenianie. Celem badań było określenie wpływu benzyloadeniny w szerokim zakresie stężeń na ukorzenianie i rozwój sadzonek gaury *Lindheimera Gaura lindheimeri* oraz wzrost następczy roślin. Wyniki wykazały wysoką skuteczność stosowania BA w niektórych stężeniach w kształtowaniu pokroju sadzonek gaury bez negatywnego wpływu na ukorzenianie. Jediną kombinacją stymulującą tworzenie korzeni (o 48%) była BA w stężeniu 0,02 g dm<sup>-3</sup>. Sadzonki traktowane BA w stężeniu 0,1 g dm<sup>-3</sup> i wyższych wytworzyły znacznie dłuższe i liczniejsze pędy boczne. Najdłuższe i w największej liczbie pędy stwierdzono na sadzonkach traktowanych BA w stężeniu 2,0 g dm<sup>-3</sup>. Biorąc pod uwagę ukorzenianie oraz rozkrzewianie się roślin, ta sama kombinacja okazała się najbardziej korzystna dla rozwoju roślin gaury *Lindheimera*.

**Słowa kluczowe:** cytokinina, korzenie przybyszowe, rozkrzewianie

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