



In vitro adventitious organogenesis in *Ajania pacifica* (Nakai) Bremer et Humphries

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Abstract

This study tested the effects of explant type, medium composition, and cultivar on *in vitro* regeneration of callus, shoots, and roots in *Ajania pacifica* (Nakai) Bremer et Humphries. In Experiment 1, “Bea”, “Bess” and “Bengo” *ex vitro* internodes and *ex vitro* leaf explants were inoculated on a modified MS (Murashige and Skoog, 1962) medium with 3 mg · dm⁻³ benzylaminopurine (BAP) and 0.5 mg · dm⁻³ indole-3-acetic acid (IAA). The share of internodes and leaf explants that proliferated callus were 92–96% and 56–100%, respectively. Adventitious shoot regeneration was observed on “Bengo” and “Bea” internodes (1.12 and 0.32 shoots, respectively, per explant inoculated) and on “Bea” leaf explants (0.2 shoots per explant inoculated). In Experiment 2, “Silver and Gold” *ex vitro* leaf explants were placed on a modified MS medium with the addition of auxin and cytokinin: 0, 0.5, and 1 mg · dm⁻³ 1-naphthaleneacetic acid (NAA) or thidiazuron (TDZ) or BAP. Callogenesis was not reported only in the medium without plant growth regulators. Adventitious roots were seen to regenerate on media without plant growth regulators and with 0.5 or 1 mg · dm⁻³ NAA (0.47, 1.2, and 2.93 roots per explant inoculated, respectively). In Experiment 3, “Bea”, “Bess” and “Bengo” *ex vivo* internodes were inoculated on a modified MS medium with 0.6 mg · dm⁻³ BAP and 2 mg · dm⁻³ IAA. All explants produced calli. Shoots regenerated only on “Bengo” internodes (1.3 shoots per explant inoculated). These results confirmed the possibility of *in vitro* callogenesis and adventitious organogenesis in *Ajania pacifica*.

Key words: adventitious root, adventitious shoot, callus, internode, leaf explant, micropropagation

Introduction

Ajania pacifica (Nakai) Bremer et Humphries is becoming increasingly popular worldwide as an alternative to chrysanthemum. The *Ajania* genus belongs to the Asteraceae family and includes more than 30 species (Bremer and Humphries, 1993) that are valuable as ornamental (*Ajania pacifica* [Nakai] Bremer et Humphries, synonym Pacific chrysanthemum, formerly *Dendranthema pacificum* and *Chrysanthemum pacificum*) or medicinal plants (*Ajania fruticulosa* [Ledeb.] Polj.) (Hao et al., 1999; Meng et al., 2001; Zhao et al., 2008). *Ajania pacifica* (Nakai) Bremer et Humphries is a perennial that originated from Central Asia and Eastern Europe. The species forms many auxiliary shoots with dense green and silver-colored leaves that remaining decora-

tive beyond the flowering period in autumn (Bremer and Humphries, 1993).

Ajania is propagated mainly *in vivo* from shoot cuttings and cultivated as a pot plant or for cut flowers. With regard to *in vitro* propagation of *Ajania pacifica*, to date, only the simple and cost-efficient single-node method has been used (Zalewska et al., 2012). Important research results concerning synthetic seeds and cryopreservation of this species for medium- and long-term storage have been recently published (Kulus and Aburatowska 2017). In the scientific literature, there are no reports of research concerning callogenesis and adventitious organogenesis in this species; however, reports of such results are potentially important not only from the scientific point of view, but also for plant production and

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breeding programs. It is worth mentioning that mutation induction that is combined with *in vitro* regeneration of adventitious shoots is a very common breeding technique in *Chrysanthemum × grandiflorum*/Ramat./Kitam, which is a species closely related to *Ajania pacifica* (Zalewska et al., 2010; Zalewska et al., 2011b). *Ajania* may become an interesting alternative for chrysanthemum, especially because the cultivation of both species can be carried out simultaneously. Furthermore, the technique of *in vitro* adventitious bud regeneration is a well-known source of somaclonal variation in chrysanthemum (Zalewska et al., 2007; Teixeira da Silva and Kulus, 2014) and, possibly, also in *Ajania*. This variation could have positive outcomes in terms of providing new cultivars.

Micropropagation is a valuable tool for mass production of plants under laboratory conditions. Over time, several micropropagation techniques have been developed (Kulus, 2015a), with adventitious organogenesis being one of the most effective and efficient methods (Lema-Rumińska and Kulus, 2014). In plants, *in vitro* adventitious organogenesis is affected by many factors, such as plant growth regulators, the genotype (Park et al., 2005; Park et al., 2007; Tymoszuik and Zalewska, 2014a), the explant type (Lu et al., 1990), the division and inoculation position (Gahan and George, 2008; Tymoszuik and Zalewska, 2014b), as well as levels of endogenous growth regulators (Kumar and Kanwar, 2006). The explants excised from young organs regenerate the callus faster (Gahan and George, 2008) and produce more shoots than the explants from older parts (Lu et al., 1990; Nakano et al., 1994). *In vitro* adventitious organogenesis strongly depends on the type and the concentration of plant growth regulators, especially auxins and cytokinins, added to the medium (Kulus, 2014). Most often, a high concentration of cytokinins and a low concentration of auxins induce the formation of shoots, and a high concentration of auxins and a low concentration of cytokinins induce rhizogenesis (Park et al., 2005; Park et al., 2007; Tymoszuik and Zalewska, 2014). Thus far, none of mentioned factors have been analyzed in *Ajania pacifica*.

The aim of the present research was to determine the effect of plant growth regulators, the explant type, and cultivars on callogenesis and the regeneration of adventitious shoots and roots in *in vitro* cultures of *Ajania pacifica*.

Materials and methods

Ajania pacifica (Nakai) Bremer et Humphries “Silver and Gold”, “Bess”, as well as “Bea” and “Bengo” (representing the Bellania group) were used in the experiments. These four cultivars were bred by Kientzler Jungpflanzen (Kientzler GmbH & Co. KG, Germany).

In all of the experiments, the MS (Murashige and Skoog, 1962) medium was used, albeit in a modified form obtained by increasing the content of calcium and iron by 50% ($660 \text{ mg} \cdot \text{dm}^{-3} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$, $41.7 \text{ mg} \cdot \text{dm}^{-3} \text{ FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $55.8 \text{ mg} \cdot \text{dm}^{-3} \text{ Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$). The media were solidified with 0.8% (w/v) Plant Propagation LAB-AGAR™ (BIOCORP, Poland) with all the nutrients added prior to autoclaving. The medium pH was adjusted to 5.8. Tissue cultures were kept in 350 ml jars with 40 ml of the medium.

In vitro cultures were maintained at $24 \pm 2^\circ\text{C}$ in a growth chamber that was exposed to a 16-hour photoperiod by using Philips TLD 36W/54 fluorescent lamps that emitted cool daylight. The quantum irradiation intensity was set at approximately $35 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Experiment 1. Effect of *ex vitro* explant type and cultivar on the regeneration of callus and adventitious shoots

The MS medium was used, as described earlier, after being modified with an increase in the concentration of thiamine to $0.4 \text{ mg} \cdot \text{dm}^{-3}$ and decrease in the content of sucrose to $10 \text{ g} \cdot \text{dm}^{-3}$. In addition, plant growth regulators, that is, $3 \text{ mg} \cdot \text{dm}^{-3}$ benzylaminopurine (BAP; Sigma-Aldrich) and $0.5 \text{ mg} \cdot \text{dm}^{-3}$ indole-3-acetic acid (IAA; Sigma-Aldrich) were added (Jerzy and Lubomski, 1992). Whole leaf (blade with petiole) and internode explants were derived from “Bengo”, “Bea” and “Bess” microcuttings propagated *in vitro* using a single-node method on the MS medium. Leaf explants (2 cm-in-length) were polarly inoculated, 0.5-cm fragments of internodes were horizontally inoculated. For each experimental treatment, five replications were applied; five explants in each.

Experiment 2. Effect of plant growth regulators on the regeneration of callus and adventitious roots on *ex vitro* leaf explants in *Ajania* “Silver” and “Gold”

The “Silver and Gold” cultivar has very short internodes, and its usage in Experiment 1 was impossible. We used the MS medium supplemented with: $30 \text{ g} \cdot \text{dm}^{-3}$ sucrose, auxin 0, 0.5, or $1 \text{ mg} \cdot \text{dm}^{-3}$ 1-naphthaleneacetic acid (NAA; Sigma-Aldrich), and cytokinin 0, 0.5, or

1 mg · dm⁻³ thidiazuron (TDZ; Sigma-Aldrich) or BAP. Whole leaves (2-cm-in-length) were polarly inoculated as explants. For each experimental treatment, three replications were applied, and there were five explants each.

Experiment 3. Effect of cultivar on the regeneration of callus and adventitious shoots on *ex vivo* internodes

The MS medium supplemented with 30 g · dm⁻³ sucrose and 0.6 mg · dm⁻³ BAP and 2 mg · dm⁻³ IAA was used. *Ex vivo* internodes (1 cm in length) were collected from “Bengo”, “Bea” and “Bess” plants cultivated in the greenhouse. First, the internodes were rinsed under running tap water, placed in 5% detergent solution for 5 min, and, then, transferred into a 70% ethanol solution for 5 s. Explants were then incubated in 20 ppm Ag and Cu nanocolloid for 10 min. Prior to horizontal inoculation on the medium, the explants were dried on sterile paper. For each experimental treatment, 20 replications were applied, with two explants in each replication.

Parameters analyzed and statistical analysis

For 10 successive weeks, the dynamics of the shoots and roots regeneration were observed. The regenerated shoots and roots were counted at a magnification of between 0.7 × 10 and 4.5 × 10 under a stereoscopic microscope MS-Z TRI provided by PRECOPTIC Co. (Poland). The share of the explants regenerating calli, shoots, or roots and the mean number of shoots or roots per explant inoculated were estimated.

The experiments were set up in a completely randomized design. To obtain the normal distribution of the features investigated, the real numerical data (*x*) for the mean number of shoots and roots were transformed by $\sqrt{x + 0.5}$ whereas, for the data expressed as a percentage, the Freeman–Tukey transformation was used. The transformed data were statistically verified using the analysis of variance (ANOVA), and means for the treatment were evaluated with the Tukey test at a significance level of *P* = 0.05. Tables with results of experiments 1–3 provide real untransformed numerical data, with alphabets indicating the homogeneous groups.

Results and discussion

Experiment 1. Effect of *ex vitro* explant type and cultivar on the regeneration of callus and adventitious shoots

In the first week of culture, all of the explants grew visually bigger as a result of cytokinin and water intake

from the medium. The internodes grew longer and swollen, especially in the region where they were cut. In leaf explants, parts of petioles immersed in the medium increased their volume more than the blades. In the second week, callus regeneration began, initially around the cut region and, later, on the entire surface of internodes and leaf petioles. In the third week, the callus in “Bess” and “Bengo” started to turn yellow and, in the fourth week, they began to go brown. “Bea” proliferated callus more intensively, thereafter yellowing and browning appeared in the fourth and in the fifth week, respectively. Adventitious shoot regeneration occurred via calli and was observed on internodes in “Bengo” and “Bea” (Fig. 1). The first shoots in “Bengo” and in “Bea” appeared in the third and fourth weeks of culture, respectively. Successive formation of shoots in “Bengo” continued until the end of culture. In “Bea”, in the seventh and eighth weeks, the number of shoots remained at the same level; however, in subsequent weeks, the regeneration of new shoots progressed, although not as intensively as in “Bengo”.

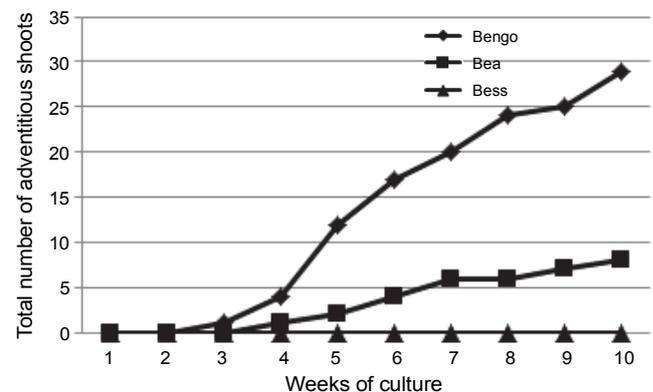


Fig. 1. Dynamics of adventitious shoots regeneration on *Ajania* “Bengo”, “Bea” and “Bess” *ex vitro* internodes on the modified MS (1962) medium with 3 mg · dm⁻³ BAP and 0.5 mg · dm⁻³ IAA

The regeneration of shoots on leaf explants occurred only in “Bea” (Fig. 2 and Fig. 3). The first shoot appeared in the seventh week of culture, and the regeneration progressed until the ninth week.

There was callus formation on all inoculated leaf explants in “Bea” (Table 1). Internodes proliferating callus accounted for 96% of the cultures in “Bea” and “Bess”, and 92% in “Bengo”. All of these values did not differ significantly. With regard to “Bengo”, the callus formed more often on the internodes (92%) than on the

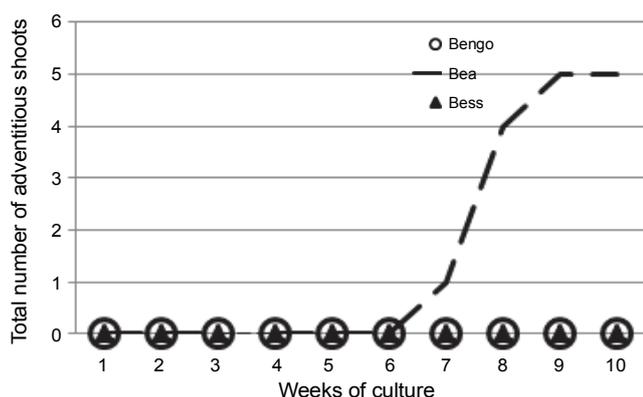


Fig. 2. Dynamics of adventitious shoots regeneration on *ex vitro* leaf explants of *Ajania* “Bengo”, “Bea” and “Bess” on the modified MS (1962) medium with $3 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $0.5 \text{ mg} \cdot \text{dm}^{-3}$ IAA

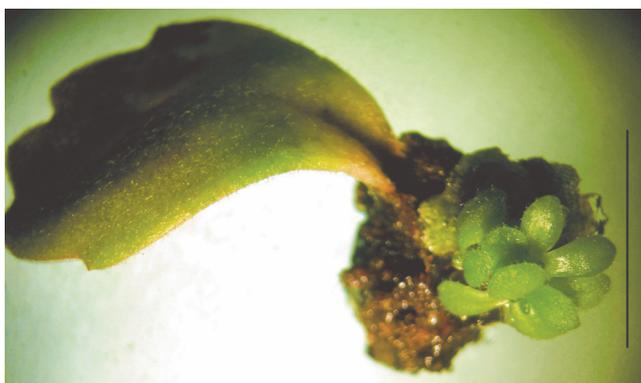


Fig. 3. Callus and adventitious shoots on the *ex vitro* leaf explant of *Ajania* “Bea” on the modified MS (1962) medium with $3 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $0.5 \text{ mg} \cdot \text{dm}^{-3}$ IAA after 10 weeks of culture; 1 bar = 1 cm

leaf explants (56%). In comparison, the percentage values between the explant type did not differ significantly in “Bea” and “Bess”. Approximately 16% of the inoculated “Bengo” internodes formed adventitious shoots, but the leaves did not form shoots. In “Bea” the percentage of internodes and leaves regenerating shoots was the same (4%). In “Bess” no regeneration of shoots was observed. Most shoots (1.12 per explant) formed on the internodes in “Bengo”. The mean number of shoots per internode and leaf explant inoculated in “Bea” was 0.32 and 0.2, respectively, and did not differ statistically. In comparison, the explants of “Breeze White” and “Capitola” chrysanthemum ligulate florets inoculated on the same medium formed two shoots on average (Jerzy et al., 2015). The regeneration of adventitious shoots

strongly depends on the type of explant used and the cultivar (Park et al., 2005; Park et al., 2007), which was also confirmed in the present experiment. Similarly as with *Ajania* “Bengo”, Zalewska and coworkers (2011a) as well as Miler and Zalewska (2014) observed that the regeneration of adventitious shoots in chrysanthemum cultivars is more effective on internodes than on leaf explants. Explants from different organs and tissues are often unevenly affected by the same plant growth regulators. This may be caused by different levels of endogenous growth regulators as well as the age and the number of cells undergoing regeneration.

Experiment 2. Effect of plant growth regulators on the regeneration of callus and adventitious roots on *ex vitro* leaf explants in *Ajania* “Silver” and “Gold”

After the first week of culture, parts of petioles put directly onto the medium increased their volume. In the second week, leaf blades started yellowing, and callus regeneration was noted on the media with the addition of growth regulators. Explants on the MS medium without plant growth regulators did not display callus formation. Direct regeneration of adventitious roots occurred on the modified MS medium without plant growth regulators. Indirect regeneration of adventitious roots was observed on the MS media with auxin only (Fig. 4 and Fig. 5). The first roots were observed in the second week of culture on the MS medium without plant growth regulators and on the medium with $1 \text{ mg} \cdot \text{dm}^{-3}$ NAA. At the specified auxin concentration, root formation was most intensive and proceeded until the end of the experiment. In the MS medium without NAA, the subsequent formation of roots was observed until the fifth week. Between the fifth and sixth weeks, no new root regeneration was recorded. In the seventh week, the number of roots reached the maximum number. Rhizogenesis started in the third week on explants inoculated on the MS medium with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ NAA, and new roots continued regenerating until Week 5.

Only on the MS medium without plant growth regulators did we observe non-proliferation of the callus (Table 2). The share of explants regenerating calli on media with cytokinin ranged from 66.67 to 86.67%, and 93.33% on the medium with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ NAA only. On media with both auxin and cytokinin, all inoculated leaf explants formed calli. Moreover, the positive effect of a simultaneous addition of auxin and cytokinin to the media on callus formation has been recorded in gerbera

Table 1. Share of *ex vitro* internodes and *ex vitro* leaf explants regenerating callus or adventitious shoots and the mean number of shoots in *Ajania* “Bengo”, “Bea” and “Bess” on the modified MS (1962) medium with $3 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $0.5 \text{ mg} \cdot \text{dm}^{-3}$ IAA

Cultivar	<i>Ex vitro</i> explant type	Callus regeneration [%]	Adventitious shoots regeneration [%]	Mean number of adventitious shoots per one explant inoculated
Bengo	internode	92.00 ^a	16.00 ^a	1.12 ^a
	leaf	56.00 ^b	0.00 ^b	0.00 ^b
Bea	internode	96.00 ^a	4.00 ^{ab}	0.32 ^{ab}
	leaf	100.00 ^a	4.00 ^{ab}	0.20 ^{ab}
Bess	internode	96.00 ^a	0.00 ^b	0.00 ^b
	leaf	96.00 ^a	0.00 ^b	0.00 ^b

a, b – means in columns followed by the same letter do not differ significantly at $P = 0.05$ (Tukey test)

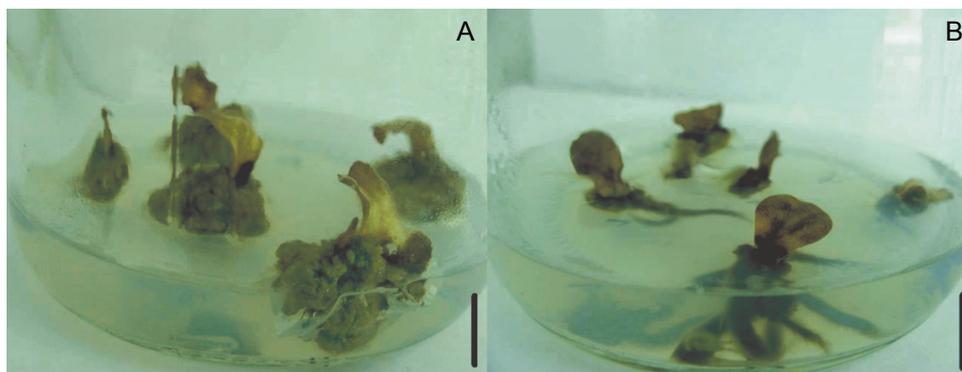


Fig. 4. Intensive regeneration of callus on the modified MS (1962) medium with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ NAA and $1 \text{ mg} \cdot \text{dm}^{-3}$ TDZ (A) and rhizogenesis on the modified MS (1962) medium with $1 \text{ mg} \cdot \text{dm}^{-3}$ NAA (B) in the sixth week of culture on the *ex vitro* leaf explants in *Ajania* “Silver and Gold” (1 bar = 1 cm)

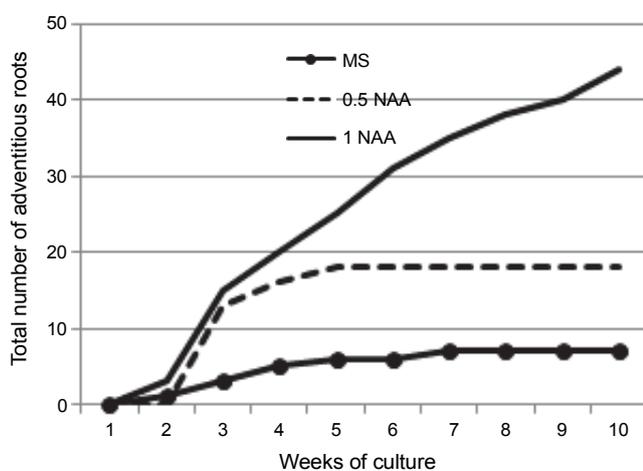


Fig. 5. Dynamics of adventitious roots regeneration on the modified MS (1962) media on the *ex vitro* leaf explants in *Ajania* “Silver and Gold”

(Kumar and Kanwar, 2005) or chrysanthemum (Tymoszuk and Zalewska, 2014a). However, in the present experiment, no regeneration of adventitious shoots was observed. The statistical analysis demonstrated that the regeneration of adventitious roots strongly depends on the qualitative and quantitative composition of the growth regulators added to the medium. Rhizogenesis occurred only on the explants inoculated on the medium without growth regulators and on the medium with 0.5 or $1 \text{ mg} \cdot \text{dm}^{-3}$ NAA. In these cases, the share of regenerating explants was 40, 33.33 and 53.33%, respectively. Most roots (2.93 per explant) formed on explants inoculated on the medium with the highest NAA concentration. Significantly fewer roots regenerated on the medium with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ NAA (1.20) and on the medium

Table 2. Share of *ex vitro* leaf explants regenerating callus or adventitious roots and the mean number of roots in *Ajania* “Silver and Gold” depending on the plant growth regulators added to the modified MS (1962) medium

Concentration of plant growth regulators [mg · dm ⁻³]			Callus regeneration [%]	Adventitious roots regeneration [%]	Mean number of adventitious roots per one explant inoculated
NAA	TDZ	BAP			
0	0	0	0.00 ^c	40.00 ^a	0.47 ^{bc}
0	0.5	0	86.67 ^{ab}	0.00 ^b	0.00 ^c
0	1.0	0	80.00 ^{ab}	0.00 ^b	0.00 ^c
0	0	0.5	86.67 ^{ab}	0.00 ^b	0.00 ^c
0	0	1.0	66.67 ^b	0.00 ^b	0.00 ^c
0.5	0	0	93.33 ^{ab}	33.33 ^a	1.20 ^b
0.5	0.5	0	100.00 ^a	0.00 ^b	0.00 ^c
0.5	1.0	0	100.00 ^a	0.00 ^b	0.00 ^c
0.5	0	0.5	100.00 ^a	0.00 ^b	0.00 ^c
0.5	0	1.0	100.00 ^a	0.00 ^b	0.00 ^c
1	0	0	100.00 ^a	53.33 ^a	2.93 ^a
1	0.5	0	100.00 ^a	0.00 ^b	0.00 ^c
1	1.0	0	100.00 ^a	0.00 ^b	0.00 ^c
1	0	0.5	100.00 ^a	0.00 ^b	0.00 ^c
1	0	1	100.00 ^a	0.00 ^b	0.00 ^c

a, b, c – means in columns followed by the same letter do not differ significantly at $P = 0.05$ (Tukey test)

Table 3. Effect of *Ajania* cultivar on the share of *ex vivo* internodes regenerating callus or adventitious shoots and the mean number of shoots on the modified MS (1962) medium with 0.6 mg · dm⁻³ BAP and 2 mg · dm⁻³ IAA

Cultivar	Callus regeneration [%]	Adventitious shoots regeneration [%]	Mean number of adventitious shoots per one explant inoculated
Bengo	100.00 ^a	50.00 ^a	1.30 ^a
Bea	100.00 ^a	0.00 ^a	0.00 ^b
Bess	100.00 ^a	0.00 ^a	0.00 ^b

a, b – means in columns followed by the same letter do not differ significantly at $P = 0.05$ (Tukey test)

without plant growth regulators (0.47). A similar tendency was observed in “Brigitte” chrysanthemum. Single-node explants of this cultivar regenerated most roots on the medium without plant growth regulators as well as on the media with 1 or 2 mg · dm⁻³ IAA (Kulpa et al., 2002). Kulus and Zalewska (2014) recorded a high rooting efficiency after transferring the synthetic seeds with shoot tips of four chrysanthemum cultivars to MS

medium supplemented with 2 mg · dm⁻³ IAA. However, synthetic seeds cultured earlier for 60 days on the recovery MS medium without plant growth regulators and on the medium with 0.1 mg · dm⁻³ KIN produced more roots (5.4 and 4.1 roots per explant, respectively) than the synthetic seeds on the medium with 0.1 mg · dm⁻³ BAP (three roots per explant). Moreover, roots from the BAP-supplemented medium were shorter. On the other

hand, no plant growth regulators were required to produce 100% rhizogenesis in *Kalanchoe tubiflora* (Harvey) Hamet. adventitious shoots (Kulus, 2015b). Cytokinins such as BAP or TDZ act as inhibitors of rhizogenesis, which was also confirmed in our experiment. This influence depends on the cultivar as well as the type and concentration of cytokinin added to the medium. Possibly, the application of KIN, instead of BAP and TDZ, would not inhibit the rhizogenesis on *Ajania* leaf explants.

It is worth mentioning that roots regenerated *in vitro* can be used subsequently as a good source of explants for the regeneration of calli and somatic embryos, which was reported in chrysanthemum by Lema-Rumińska and coworkers (2015) and, maybe, in *Ajania*.

Experiment 3. Effect of cultivar on the regeneration of callus and adventitious shoots on *ex vivo* internodes

In the first days of culture, swelling of the whole surface of all the explants was observed, most intensively in the region of cutting. In the second week, callus regeneration began around the cutting regions. In “Bea”, callus proliferation was very intensive and occurred on the whole surface of the internodes. Starting in the fourth week, there was yellowing of the callus tissue in “Bess” and “Bengo”. In the third week, regeneration of adventitious shoots via callus occurred in “Bengo”. In “Bea” and “Bess”, no regeneration of adventitious shoots was observed (Fig. 6).

In each of the cultivars tested, all the *ex vivo* inoculated explants formed calli (Table 3); however, regeneration of adventitious shoots was only reported in *Ajania* “Bengo” (on 50% of inoculated *ex vivo* internodes). The cultivar affected the number of shoots regenerated from a single explant. One inoculated *ex vivo* internode in “Bengo” regenerated approximately 1.3 shoots, similarly to *ex vitro* internodes (1.12; Experiment 1). In Miler and Zalewska’s (2014) experiment with different chrysanthemum cultivars, the efficiency of adventitious shoot regeneration per *ex vitro* internode on the MS medium with $0.6 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2 \text{ mg} \cdot \text{dm}^{-3}$ IAA ranged from 1.15 to 3.11. In the case of “Bea” cultivar, no regeneration of shoots was reported on *ex vivo*, compared to *ex vitro*, internodes. It can be assumed that *ex vitro* internodes are better explants for shoot regeneration than *ex vivo* internodes. Beck and coworkers (1998) found that the percentage of *Acacia mearnsii* De Wild. nodal explants producing shoots was higher when

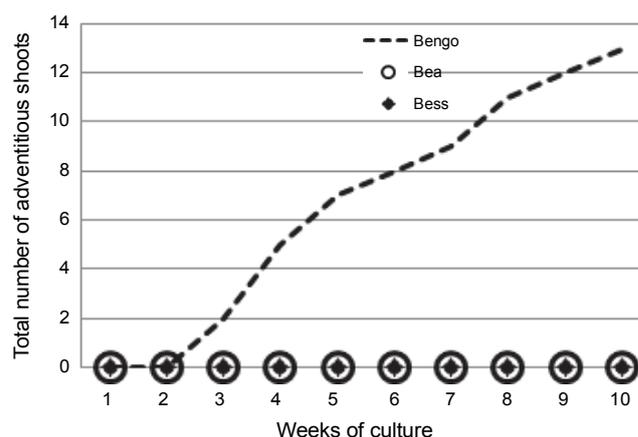


Fig. 6. Dynamics of adventitious shoots regeneration on the *ex vivo* internodes of *Ajania* “Bengo”, “Bea” and “Bess” on the modified MS (1962) medium with $0.6 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2 \text{ mg} \cdot \text{dm}^{-3}$ IAA

ex vitro explants were used. Furthermore, Chakradhar and Pullaiah (2006) reported better results in *Wattakaka volubilis* (L.f.) Stapf axillary shoot multiplication using *ex vitro* nodal explants.

Conclusions

In *Ajania* “Bengo”, “Bea” and “Bess”, a high share of callus proliferation was observed on *ex vitro* internodes (92–96%) and *ex vitro* leaf explants (56–100%) inoculated on the modified MS medium with $3 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $0.5 \text{ mg} \cdot \text{dm}^{-3}$ IAA. Adventitious shoot formation was reported on “Bengo” and “Bea” internodes (16 and 4% explants producing shoots; 1.12 and 0.32 shoots per explant inoculated, respectively) and on “Bea” leaf explants (4% explants producing shoots; 0.2 shoots per explant inoculated). The cultivar and explant type strongly influenced adventitious organogenesis.

Simultaneous addition of NAA auxin and TZD or BAP cytokinin at a concentration 0.5 or $1 \text{ mg} \cdot \text{dm}^{-3}$ to the modified MS medium had a positive effect on the callus formation of *ex vitro* leaf explants in *Ajania* “Silver and Gold”. The best efficiency of adventitious roots formation was reported on the modified MS medium with $1 \text{ mg} \cdot \text{dm}^{-3}$ NAA (53.33% explants regenerating roots and 2.93 roots per explant inoculated).

After inoculation of *Ajania* “Bengo”, “Bea” and “Bess” *ex vivo* internodes on the modified MS medium supplemented with $0.6 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2 \text{ mg} \cdot \text{dm}^{-3}$ NAA, only the “Bengo” cultivar showed regeneration of

adventitious shoots (50% explants regenerating shoots and 1.3 shoots per explant inoculated). A strong influence of cultivar type on adventitious shoot formation was demonstrated. Moreover, *ex vitro* internodes are a better type of explants than *ex vivo* internodes for regeneration of adventitious shoots.

To improve the efficiency of adventitious organogenesis in *Ajania*, further research should be conducted on 1) the influence of the qualitative and quantitative composition of the growth regulators added to the medium, 2) other explant types (e.g. fragments of leaf blades, petals), and 3) physical factors that contribute to the maintenance of the *in vitro* culture (e.g. light color and intensity).

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