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MICROPROPAGATION OF *AJANIA PACIFICA* (NAKAI) BREMER ET HUMPHRIES WITH SINGLE-NODE METHOD

MIKROROZMNAŻANIE GATUNKU *AJANIA PACIFICA* (NAKAI) BREMER ET HUMPHRIES METODĄ JEDNOWĘZŁOWYCH FRAGMENTÓW PĘDU

Summary. Ajania is closely related to the chrysanthemum, and may become an interesting alternative for it, the more that the cultivation of both species can be carried out simultaneously. The present study investigated the efficiency of micropropagation of three cultivars of *Ajania pacifica* (Nakai) Bremer et Humphries: 'Bea', 'Bess', 'Silver and Gold'. The plants were propagated *in vitro* with single-node method on the modified MS medium without growth regulators. In all the three cultivars there was observed a similar propagation coefficient. After eight weeks of culture there were produced about 10 microcuttings from a single shoot. 'Silver and Gold' produced definitely shorter internodes than 'Bea' and 'Bess'.

Key words: *Ajania pacifica* (Nakai) Bremer et Humphries, *in vitro,* single-node method

Introduction

The *Ajania* genus includes a few dozen or so species. *Ajania pacifica* (Nakai) Bremer et Humphries (synonym *Pacific chrysanthemum*) used to be referred to as *Dendranthema pacificum* and *Chrysanthemum pacificum* as it represents the same family as chrysanthemums – Asteraceae. At the moment the species is attracting more and more interest, becoming their alternative. Chrysanthemums have been successfully propagated in cultures *in vitro* for as much as 30 years, while ajania has been still a novelty in that respect and it is propagated *in vivo* from shoot cuttings, obtained from maternal plants.

The single-node method involves a transverse division of microcuttings into fragments containing the single node; it is applicable in micropropagation of plants of

strong apical dominance. In practice this is the method used to propagate *in vitro* e.g. araucaria, ivy, chrysanthemum, carnation, gypsophila, hydrangea, garden balsam, geranium. The value of the coefficient of multiplication is, on average, four-five microcuttings per shoot (JERZY and KRZYMIŃSKA 2005). The plants obtained from axillary buds develop from the already existing meristems and thus they are genetically homogeneous (HODOWLA... 1984).

The aim of the present paper was to evaluate the efficiency of micropropagation of three ajania cultivars propagated using the single-node method.

Material and methods

The research was performed from June 24 to August 19, 2010. To sample the explants, microcuttings of three cultivars of *Ajania pacifica* (Nakai) Bremer et Humphries were applied: 'Bea', 'Bess' as well as 'Silver and Gold'. The plants were propagated *in vitro* applying the single-node method on the modified MS medium (MURASHIGE and SKOOG 1962) without growth regulators. The medium modification involved increasing the content of calcium and iron by half. The medium contained 30 $g \cdot dm^{-3}$ of saccharose, it was solidified with 8 $g \cdot dm^{-3}$ of agar, and its pH was determined prior to autoclaving at the level of 5.8. The single-node explants were polarly inoculated, five in each 350 cm^3 jar, containing 40 cm^3 of the medium. The experiment was set up in completely randomised design for a single factor (the cultivar). Each combination involved nine replications, with five single-node explants each.

Cultures *in vitro* were performed in the growth room at the temperature of 24 ±2°C exposed to daylight emitted by fluorescent lamps Philips TLD 36W/54, for 16 h per day. The photon flux density was 30-35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For eight weeks there were made observations into the dynamics of leaf development on growing shoots. There was calculated the number of nodes to calculate the coefficient of multiplication (being the number of single-node microcuttings which can be produced from the division of a single regenerated shoot) and the length of microcuttings grown from single-node explants. The results were statistically verified with a single-factor analysis of variance and the treatment means were compared with the Tukey's test at the significance level of $\alpha = 0.05$.

Results and discussion

In the first week of the culture the bigger number of leaves was produced by 'Silver and Gold' – on average 3.8, and the lowest number by 'Bea' – an average of 2.4 (Fig. 1). Starting from the second week, in 'Bea' there was observed, however, the highest rate of leaf development (microcuttings formed, on average, more than two leaves a week, while 'Bess' and 'Silver and Gold' – less). In each cultivar, in the sixth and seventh week there were fewest of them, while in the last one in 'Bea' and 'Silver and Gold' – most.

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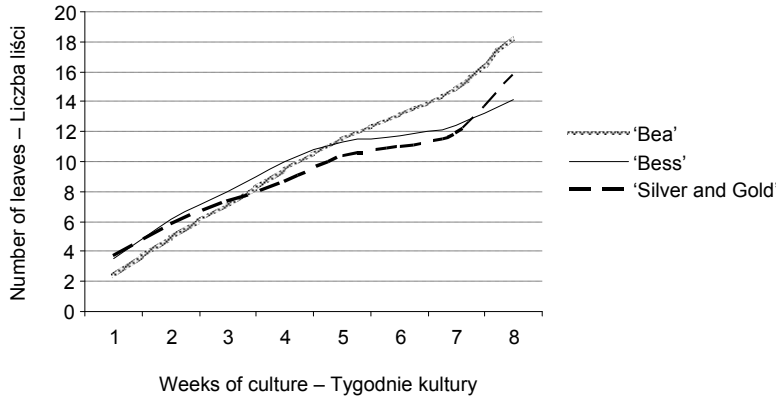

Fig. 1. Leaf development dynamics in respective ajania cultivars
Rys. 1. Dynamika rozwoju liści poszczególnych odmian ajanii

After eight weeks of culture, 'Bea' and 'Silver and Gold' produced a similar number of leaves, despite a considerable difference in the shoot length (Table 1). 'Bea' produced over four leaves more than 'Bess', while the average shoot length was similar. The shoots formed in 'Silver and Gold' were shortest; on average they were half-shorter than the other two cultivars (Fig. 2). According to MILER et AL. (2005) the advantage of the microcuttings with short internodes is their better acclimatization. The reports by ZALEWSKA and ANTKOWIAK (2011) under conditions *in vivo* confirm that 'Silver and Gold' demonstrates poor growth.

Table 1. Quality of microcuttings of ajania after eight weeks of culture
Tabela 1. Jakość mikrosadzonek ajanii po ośmiu tygodniach kultury

Cultivar Odmiana	Number – Liczba		Shoot length Długość pędu (cm)
	of leaves liści	of nodes on shoot węzłów na pędzie	
'Bea'	18.31 a	10.40 a	4.46 a
'Bess'	14.00 b	10.31 a	4.10 a
'Silver and Gold'	15.73 ab	9.27 a	2.08 b

Means marked in columns with the same letters do not differ significantly at $\alpha = 0.05$.
Średnie oznaczone w kolumnach tymi samymi literami nie różnią się istotnie przy $\alpha = 0{,}05$.

Of all the factors affecting the growth of cuttings *in vitro*, light, plant hormones and topophysis are among those worth considering. Light can affect growth regulators contained in the plant, thus affecting morphogenetic processes (KOPCEWICZ et AL. 1992). LATKOWSKA and CHMIEL (1996) found that red light stimulates the elongation growth of shoots in 'Escort' chrysanthemum, while the blue light – quite the opposite. The shoot elongation was enhanced by the IAA content in the medium and a lack of growth regulators,

Fig. 2. Microcuttings of ajania of 'Bea', 'Bess' and 'Silver and Gold' cultivars (from the left) produced from single-node explants after eight weeks of culture
Rys. 2. Mikrosadzonki ajanii odmian 'Bea', 'Bess' oraz 'Silver and Gold' (od lewej) uzyskane z jednowęzłowych fragmentów pędu po ośmiu tygodniach kultury

however it was counteracted by the presence of BA. MILER et AL. (2005) report on the highest microcuttings of 'Richmond' chrysanthemum produced when exposed to red and yellow and to daylight on the IAA-containing medium, whereas the shortest – to the daylight on the medium without growth regulators. In 'Lilac Wonder' the longest microcuttings were observed when exposed to green, yellow and blue light, elongation growth inhibition was recorded. Red light coincided with the lowest number of leaves (MILER and ZALEWSKA 2006). The light of a varied colour also stimulates the shoot elongation in other species. Reports by WITOMSKA and KOSZEWSKA (2002) demonstrated that the light quality affects the organogenesis of *Petunia hybrida* Vilm., representing the Ursynia group. When exposed to a low photon flux density, 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$, red light on the medium without cytokinins stimulated shoot elongation growth, while blue in the presence of BA inhibited it. When blue light of the photon flux density of 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and white light of the photon flux density of 68 $\mu mol \cdot m^{-2} \cdot s^{-1}$ without cytokinins were applied, the shoot growth was most intensive. The shoot elongation, irrespective of the light colour, was stimulated by a lack of cytokinins in the medium. The reaction of plants to the type of light applied depends on many factors, including the level of endo- and exogenous growth regulators (LATKOWSKA and CHMIEL 1996).

Topophysis is a varied cutting growth and development depending on their location on the maternal plant (MOLISH 1916). It could be the case that in ajania sampling explants from different plant levels will affect the growth of microcuttings, which should be considered when you want to obtain high quality and high coefficient of multiplication. However, one shall remember that one cultivar can be topophysis-dependent, while another one – not. Reports by ZALEWSKA et AL. (2010) show that out of five chrysanthemum cultivars, three appeared to be dependent on the topophysis, while two – not. In 'Lady Amber', 'Lady Orange' and 'Lady Vitroflora' the best results were recorded by sampling single-node explants from the central and proximal plant part.

The index of propagation with the single-node method depends on the number of buds we can use. If their number is low, also the index is inconsiderable and the propagation – very slow. The more the leaves, the more the buds and the faster the propagation (PIERIK 1987). In the present experiment there were observed considerable differences in the length of the microcuttings produced and the number of leaves formed, however, the number of nodes per shoot was similar in all the cultivars (Table 1). LATKOWSKA and CHMIEL (1996) found that the blue light application enhances the number of nodes. The shoots formed when exposed to such light, on the IAA-containing medium showed the greatest number of nods, with very short internodes, whereas those growing in red light, on the medium without growth regulators formed their lowest number. In the present experiment the coefficient of multiplication in each cultivar was recorded in each cultivar to be twice higher than in chrysanthemum. In 'Lilac Wonder' the highest coefficient of multiplication (over six) was recorded in green light, while the lowest one – in blue, yellow and red lights. Although 'Silver and Gold' reached the half the height of the other two cultivars, its number of nodes was similar, which is connected to shorter internodes. Longer microcuttings of ajania do not stand for a higher number of nodes, and hence a higher coefficient of multiplication. The three cultivars involved in the present research turned out to be same efficient. Each bud found in the corner of the leaf can be isolated onto the medium and develop further under conditions *in vitro* (PIERIK 1987). The presence of the meristem in explants which serve to initiate culture *in vitro* and plant propagation is indispensable since the explants which do not contain it do not ensure the repeatability of characters and as such they do not ensure the genetic cultivar stability (JERZY 2000).

Conclusions

1. For 'Bea', 'Bess' and 'Silver and Gold' *Ajania pacifica* cultivars a similar value of the coefficient of multiplication is reported, however, in 'Silver and Gold' there were noted shorter internodes than in 'Bea' and 'Bess'.

2. An eight-week 'Bea', 'Bess' and 'Silver and Gold' microcutting can produce, on average, ten single-node explants.

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Streszczenie. Ajania jest spokrewniona z chryzantemą i może stać się dla niej interesującą konkurencją, tym bardziej, że uprawę obu gatunków można prowadzić jednocześnie. W pracy przedstawiono wyniki badań dotyczące wydajności mikrorozmnażania trzech odmian ajanii spokojnej (*Ajania pacifica* (Nakai) Bremer et Humphries): 'Bea', 'Bess' oraz 'Silver and Gold'. Rośliny rozmnażano metodą jednowęzłowych fragmentów pędu na zmodyfikowanej pożywce MS bez regulatorów wzrostu. U wszystkich trzech odmian zaobserwowano podobny współczynnik namnażania. Po ośmiu tygodniach kultury uzyskano średnio 10 mikrosadzonek z jednego pędu. Odmiana 'Silver and Gold' wytworzyła zdecydowanie krótsze międzywęźla niż 'Bess' i 'Bea'.

Słowa kluczowe: *Ajania pacifica* (Nakai) Bremer et Humphries, *in vitro*, metoda jednowęzłowych fragmentów pędu

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