CALLUS INDUCTION AND PLANT REGENERATION IN VITRO IN *ACTINIDIA*

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ABSTRACT

The dependence of callus induction and organogenesis on auxins (2,4-D, IAA) and cytokinins (BAP, KIN) content in MS medium in *Actinidia arguta*, *A. deliciosa* and *A. polygama* and explants derived from stems, petioles, leaves and roots was investigated. The ability to form callus was good for stem and petiole explants and weaker for leaf and root explants in all three species. The media with low level of 2,4-D (0.01 and 0.1 mg/l) and higher of cytokinins (1.0 and 5.0 mg/l) promoted callus growth and viability. Higher 2,4-D as well as IAA concentrations caused degeneration of calli. The application of BAP was more advantageous than of KIN in the case of *A. arguta* and *A. polygama*. Organogenesis was quick and abundant after passage to media with 0.1-5.0 mg/l of IAA and 1.0-5.0 mg/l of cytokinins only in callus derived from stems. Calli from the other explants showed weaker ability to regenerate. The reactions of the three investigated species, differed slightly, the similarities were however, more significant. Shoots regenerated from callus were rooted and the plantlets were transferred to soil.

KEY WORDS: *Actinidia*, callus, organogenesis, auxin, cytokinin, regeneration in vitro.

INTRODUCTION

Genus *Actinidia* Lindl. (*Actinidiaceae*) consists of about 50 species divided into four sections, basing on pubescence of leaves, stems and fruits. They are distributed in Eastern Asia, mainly in China, Korea, Japan, and Russian Far East. They are deciduous vines up to 30 m high with simple, alternate leaves, often changing colours during vegetation. Actinidias are generally dioecious, with big flowers (1.5 to 5 cm in diameter) and edible berries of a size depending on species or variety (1.5-10 cm) (Liang 1983, Seneta 1991).

Three ploidy levels were described within the genus: diploid $2n=58$, tetraploid $2n=116$ and hexaploid $2n=170$ (Zhang & Beuzenberg 1983, Pringle 1986). Actinidias are cultured for ornamental leaves or for fruits.

Actinidia deliciosa (A. Chev.) C.-F. Liang et A.R. Farguson var. *deliciosa* known also as Chinese gooseberry, is cultured in some subtropical regions as kiwifruit. The other variety *A. deliciosa* var. *chinensis* represents the wild ancestor of kiwi.

Actinidia arguta (Sieb. et Zucc.) Planch. ex Mig. the vigorous vine is resistant to low temperature, common in parks and orchards. In Russia some attempts were made to introdu-

ce this species together with *A. kolomikta* as crops on industrial scale, but they have failed as yet (Seneta 1991).

Actinidia polygama (Sieb et Zucc.) Maxim. cultured as an ornamental because of its decorative leaves with creamy-yellow spots, is also frost resistant.

Actinidias, as crops introduced to agriculture outside Asia quite recently, are becoming an object of research with the aim of improving their agricultural and consumptional values (Considine et al. 1984).

Unfortunately, classical breeding and selection methods are not efficient because of dioecy and high poliploidy in kiwifruit. The application of in vitro techniques offers more possibilities. Regarding to *A. deliciosa*, these methods have been developed since the seventies, but more reports have been published since 1980. Procedures for regeneration via organogenesis and micropropagation from different parts of plant are reported (Harada 1975, Gui 1979, Fraser & Harvey 1986, Marino & Bertazza 1990, Pedroso et al. 1992). Parthenogenetic haploids of kiwifruit have been also obtained (Przywara et al. 1988, Pandey et al. 1990). However, there is a need to elaborate conditions for in vitro culture of some other species of actinidias as it has already been done for kiwifruit.

Up to date most of research is restricted to an economically important variety: *A. deliciosa* cv. 'Hayward', other varieties are rarely considered. There are only very few data on in vitro culture of other species (Considine et al. 1984). Hence, it seems to be interesting and important to carry out research comparing kiwi to some less popular actinidias under in vitro conditions. In the present work besides *A. deliciosa* two other

Abbreviations:

BAP= 6-benzylaminopurine, GA3= gibberellic acid, IAA= indole acetic acid, KIN= kinetin, NAA= naphthalene acetic acid, ZEA= zeatin, 2,4-D= 2,4-dichlorophenoxyacetic acid, 2iP= isopentenyl adenine.

interesting species were investigated i.e. *A. arguta* and *A. polygama*. Both species are considerably cold resistant, grow vigorously in moderate climate and hybridize with *A. deliciosa*. The three species mentioned represent all ploidy levels known in the genus: diploid (*A. polygama*), tetraploid (*A. arguta*), hexaploid (*A. deliciosa* var. *deliciosa*). The major goal of this work was the comparison of reaction to in vitro conditions of kiwi and the newly elaborated actinidias. Preliminary conditions for callus induction in these species as well as preference to exogenous growth regulators are described. Some dependences of morphogenetic and regenerative processes in callus on culture factors such as concentration of phytohormones and auxin/cytokinin ratio in medium, origin of explants, time of culture are also reported.

MATERIAL AND METHODS

Sterile plants for explants were obtained using the following procedure:

Seeds stratified for 30 days in 4°C were next treated with GA3 solution (1g/l) for 24 h.

Superficially sterilized with 70% ethanol and 6% hypochlorite, then rinsed in sterile water, seeds were placed in Petri dishes on 0.4% agar. They were incubated in alternating temperatures with the cycle 16 h in 25°C and 8 h in 4°C. Germinated after 3-4 weeks seedlings were transferred to 11 jars. Explants were excised from plants 25-30 cm high. Fragments of stems, petioles and roots 1.5-2 mm long as well as pieces of leaves 5 mm of diameter were used as the explants.

Murashige and Skoog (1962) basal salts supplemented with myo-inositol 100 mg/l, glycine 2 mg/l, thiamine 0.1 mg/l, pyridoxine 0.5 mg/l and nicotinic acid 0.5 mg/l were used, with 3% sucrose as organic carbon source. Media were solidified with 0.7% (w/v) agar (BBL-agar, Beckton-Dickinson, USA). The pH was adjusted to 5.8 and hormones were added prior to autoclaving for 20 min at 120°C. Concentration of hormones BAP, KIN, 2,4-D, IAA were prepared according to active range advised by manufacturer (Sigma).

TABLE 1. Concentrations of hormones in media used in the experiments in mg/l (μmol/l).

KIN	2,4-D				IAA		
0.1 (0.46)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)
1.0 (4.60)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)
10.0 (46.0)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)
BAP	2,4-D				IAA		
0.1 (0.44)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)
1.0 (4.40)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)
10.0 (44.0)	0.01 (0.045)	0.1 (0.45)	1.0 (4.52)	5.0 (22.6)	0.1 (0.57)	1.0 (5.71)	5.0 (28.5)

Explants were inoculated in 90 mm Petri dishes containing 20 cm³ of medium each, sealed with parafilm and maintained in 26°C under cool florescent light or, in the case of some root explants, in darkness. Subculture period was 4 weeks.

Seeds of *A. arguta* and *A. polygama* were obtained from the University of Warsaw Botanical Garden and from the Arboretum in Rogów, those of *A. deliciosa* originated from authors' own collection from New Zealand.

RESULTS

Callus was formed on explants from all tested organs, i. e. from stems, petioles, leaves and roots. Swelling, particularly near the cut surface, was the first visible sign of morphogenetic changes in explants, and was observed as early as on the 3 day of culture in stem- and 4-5 day in petiole-explants. Callus induction and growth varied depending on origin of explants and concentration of hormones in medium. The earliest and most efficient was the callus initiation in stem explants (Fig. 1A), and a little weaker in the petiole explants. The hormone combinations promoting callus formation for the three investigated species are shown in Tab. 2.

In the presence of 2,4-D callus grew fast, but its appearance and viability was influenced by the type (BAP or KIN) and concentration of cytokinin. Light-green, quite dense callus arose at lower 2,4-D concentrations and prevailing cytokinin contents (Fig. 1D). At higher 2,4-D concentrations callus became colourless and friable (Fig. 1E). Necrotic changes starting as browning or blackening on tissue surface were also observed (Fig. 1F).

IAA stimulated callusing in all three species only at the lowest concentration (0.1 mg/l) and prevailing cytokinin (1.0 - 5.0 mg/l) (Fig. 1G). At 1 mg/l IAA independently on cytokinin callus was dark green, small and very compact. Relatively tolerant to higher concentrations of IAA were explants of *A. deliciosa* which even at 5 mg/l IAA and 1 mg/l KIN formed little calli, but at 0.1 mg/l KIN did not form any callus and eventually degenerated. On the other hand, most sensitive to higher than 1 mg/l IAA concentrations were explants of *A. polygama* whereas *A. arguta* explants positively responded to IAA up to 1 mg/l especially in the presence of KIN.

In *A. arguta* and *A. polygama* differences in reaction of calli to BAP or KIN were noticed especially on media with 2,4-D. The fading and necrosis of callus at higher 2,4-D concentrations were clearly diminished by the influence of higher concentration of BAP. Callus grew slower, but was more compact and more colourful, with correlation to increasing cytokinin/auxin ratio. KIN showed a distinctly weaker action. In *A. arguta* and *A. polygama* a greater tolerance to unfavourable impact of 2,4-D was observed in the case of petiole derived calli in comparison to those derived from stems.

The percentage of explants which degenerated or formed viable callus is for some media presented in Tab. 3. for stem explants of *A. arguta* as an example. The corresponding data for two other species and for petioles are similar.

On leaf explants callus was formed rarely and irregularly at different combinations of hormones in each species. In *A. arguta* callus produced at 5 mg/l BAP and 0.1-1 mg/l 2,4-D was viable and fast growing (Fig. 1C). In *A. deliciosa* callus was initiated on cut surfaces exclusively on the proximal (nearest to the base) side around the vascular bundles at 5 mg/l KIN and 0.1 mg/l 2,4-D. In *A. polygama* at 5 mg/l BAP and 0.1 mg/l 2,4-D or IAA occurred fast growing callus, which in a short time browned and showed necrotic changes.

TABLE 2. The intensity of callus induction and growth on various hormones concentrations (mg/l) in culture media. Number of crosses is corresponding to growth of calli. f – callus pale and friable, g – green and compact, n – necrotic changes.

auxin	species	cytokinin					
		BAP			KIN		
		0.1	1.0	5.0	0.1	1.0	5.0
2,4-D							
0.01	<i>A. arguta</i>	+	+++g	+++g	++g	+++g	++g
	<i>A. deliciosa</i>	+	+++g	++g	+	+++g	++g
	<i>A. polygama</i>	+	+++g	++g	+	++g	+g
0.1	<i>A. arguta</i>	++f,n	++++f	++++g	+++f,n	++++f,n	+++f,n
	<i>A. deliciosa</i>	+n	++++f,n	++++f,n	+n	++++f,n	++++f,n
	<i>A. polygama</i>	+n	++++f	++++g	+n	++++f,n	++++f,n
1.0	<i>A. arguta</i>	+n	++n	+++f,n	+n	++n	+++f,n
	<i>A. deliciosa</i>	n	+n	++++f,n	n	+++n	++++f,n
	<i>A. polygama</i>	++f,n	++n	++++f,n	n	+++n	++++f,n
5.0	<i>A. arguta</i>	+n	+n	++f,n	n	+n	+++f,n
	<i>A. deliciosa</i>	–	n	n	–	n	+n
	<i>A. polygama</i>	n	–	+n	–	n	++f,n
IAA							
0.1	<i>A. arguta</i>	–	+g	++g	–	++g	+++g
	<i>A. deliciosa</i>	+g	+++g	+++g	+g	+++g	+++g
	<i>A. polygama</i>	g	+g	+g	–	+g	++g
1.0	<i>A. arguta</i>	–	g	+g	–	+g	++g
	<i>A. deliciosa</i>	–	g	+g	–	+g	+g
	<i>A. polygama</i>	–	–	–	–	–	–

Root explants responded differently depending on the species. In *A. arguta* callus was induced easily and grew fast in the presence of 2,4-D and BAP. Dense, green, organogenic callus occurred at combination 1 mg/l BAP and 0.01 mg/l 2,4-D and at others, where BAP considerably prevailed. At higher concentrations of 2,4-D callus was white and had a loose consistency. In *A. deliciosa* only two of 80 inoculated root segments gave rise to callus on medium with 5 mg/l KIN and 0.1 mg/l IAA kept in darkness (Fig. 1B). Other combinations did not induce callusing. Only production and an intensive growth of secondary roots occurred at high levels of auxins (1-5 mg/l). Transfer to light resulted in the degeneration of callus. Roots of *A. polygama* did not produce any callus.

Morphogenetic changes in callus

Organogenesis of shoots and roots was observed. Regeneration was sporadic before the first passage, but occurred abundantly and regularly after passaging of fast growing, undifferentiated callus to appropriate media. The processes of regeneration in individual species were as follows:

A. deliciosa

Undifferentiated callus after passaging to media with 0.1 or 1 mg/l IAA and 1 mg/l KIN or BAP showed quick superficial differentiation (Fig. 1H) followed, in 1-3 weeks, by the appearance of shoot apices (Fig. 2A) with rapidly developing leaf primordia. The buds emerged from under the surface of callus, most often in groups of 2-9. This was preceded by the formation of reddish spots on the surface of green callus. At 1 mg/l IAA an induction of roots (rhizogenesis) was noticed. At auxin content greater than of cytokinin rhizogenesis dominated. Root derived callus kept in dark showed intensive rhizogenesis proportionally to IAA concentration. After transferring to semi-darkness, the developing, single shoot apex occurred, but after a few days showed strong vitrification. Regeneration in this species was satisfactory only in stem derived calli.

A. arguta

Transferring of fast growing calli onto the analogous media as for *A. deliciosa* yielded similar changes within callus, starting on the first day after passage. Organogenesis was prece-

TABLE 3. *A. arguta*. The percentage of stem explants which degenerated before (2) or after (3) callusing or formed viable calli (4). 1 – the total number of explants on each medium. The concentrations of hormones are in mg/l, D=2,4-D, I= IAA, B=BAP, K=KIN. On the media not shown in this table, all explants produced viable calli or, in the case of media with 5.0 mg/l IAA or with other IAA concentration and 0.1 mg/l cytokinin, all of the explants eventually degenerated.

Media						Media					
		1	2	3	4			1	2	3	4
			%	%	%				%	%	%
1.0D	0.1B	18	38.9	44.4	16.7	1.0D	1.0K	12	0	41.7	58.3
1.0D	1.0B	15	26.7	40	33.3	1.0D	5.0K	15	0	26.7	73.3
1.0D	5.0B	18	0	27.8	72.2	5.0D	0.1K	10	0	100	0
5.0D	0.1B	12	25	58.3	16.7	5.0D	1.0K	9	0	66.7	33.3
5.0D	1.0B	12	25	41.7	33.3	5.0D	5.0K	10	0	20	80
5.0D	5.0B	12	0	66.7	33.3	0.1I	1.0B	18	5.6	0	94.4
.01D	0.1K	20	15	0	85	1.0I	1.0B	15	0	33.3	66.7
.01D	5.0K	20	5	0	95	1.0I	5.0B	15	26.7	6.7	66.7
0.1D	1.0K	20	5	0	95	0.1I	5.0K	22	9.1	0	90.9
1.0D	0.1K	15	0	80	20	1.0I	1.0K	20	35	0	65

ded by the appearance of characteristic pale-brown spots, where in turn groups of shoot (Fig. 2E) or root apices emerged. Whether shoots (Fig. 2F) or roots (Fig. 2G) regenerated depended on IAA content and relation of this auxin to cytokinins, as it was in former species. Regeneration of shoots was obtained from callus derived from stems, seldom from petioles. In callus derived from leaves, some leaf primordia were formed, developing into almost normally looking leaves, but not into shoots.

A. polygama

After passing organogenesis occurred mainly on media containing 0.1 mg/l IAA in combination with 1.0 or 5.0 mg/l BAP. Replacing of BAP by KIN caused a weaker and delayed response. Higher concentrations of auxin and increasing auxin/cytokinin ratio stimulated formation of agglomerated, adventitious roots. On regenerative media, fast growing callus showed a similar differentiation as in two other species, but the organogenic centres were more distinct than in *A. deliciosa* or *A. arguta*, because they were formed on pale-coloured protuberances elevated over the bulk of callus (Fig. 2B and 2C). Stem explants were more active organogenically than those from petioles. Rooted plantlets were obtained by placing shoots regenerated from callus (Fig. 2H) on rooting media rich in IAA (5-10 mg/l) with 1 mg/l KIN. The plantlets were subsequently transferred to soil (Fig. 2I).

The efficiency of micropropagation has not been precisely calculated, because the part of fast growing callus had to be removed during passages. Nevertheless, the possibility to obtain on average ten fully formed plants from one piece of regenerating callus should be reported.

DISCUSSION

Results obtained in these experiments demonstrate a high ability of the investigated actinidias to dedifferentiate and regenerate *in vitro*. The conditions suitable for callus induction were for all the three species similar: low or middle concentration of 2,4-D accompanied by prevailing amount of cytokinins. At concentrations of 2,4-D higher than of cytokinins the negative influence of this compound to the viability of the tissue increased. It is interesting that the rate of susceptibility to this phenomenon was different depending on the type of source-organ for explants. In *A. arguta* and *A. polygama* petiole derived callus was more tolerant to degenerating impact of 2,4-D. Differences in the effects of BAP and KIN on callus were also conspicuous. In this work it was noticed only for *A. arguta* and *A. polygama*, not for *A. deliciosa*. However this kind of correlation was reported by other authors who noted that the content of chlorophyll in callus cells of kiwi was higher in the presence of BAP, than in the presence of KIN (Marino & Bertazza 1990). Furthermore, analogous alterations in callus colour and structure were observed in many other plants. Another auxin used in the experiments (IAA), was less efficient in callus induction but, unlike 2,4-D, stimulated morphogenesis in the calli.

Many reports on *A. deliciosa* in *in vitro* culture confirm possibility to induce callusing in explants from different parts of plant, as well as stimulation of growth by various combination of phytohormones (Harada 1975, Gui 1979, Hirsch & Fortune 1984, Fraser & Harvey 1986b, Pais et al. 1987, Tsai 1988, Marino & Battistini 1990, Marino & Bertazza 1990, Mezzetti et al. 1991a, Mezzetti et al. 1991b, Oliveira & Pais 1991). In order to induce callus the following cytokinins were used: KIN, 2iP, BAP, ZEA, and auxins 2,4-D, IAA, NAA. Natural cytokinin zeatin is recognized as the most efficient

Fig 1. Callus initiation and growth.

A. Stem explant on 7th day of culture on 0.1 mg/l 2,4-D and 1 mg/l KIN showing intensive callus growth at both cut surfaces, **B.** Root derived callus in *A. deliciosa* maintained in darkness was cream-yellow, dense and fast growing. **C.** Leaf explant of *A. arguta* on 5 mg/l BAP and 1 mg/l 2,4-D with friable colourless callus. **D.** Callus on 0.01 mg/l 2,4-D and 5 mg/l BAP was green and viable. **E.** On 0.1 mg/l 2,4-D and 1.0 mg/l BAP callus became pale and friable but grew very fast. **F.** At concentrations of 2,4-D 1.0 mg/l and of BAP 1.0 mg/l occurred browning and necrosis. (**D.**, **E.**, **F.** – *A. arguta*) **G.** In the presence of 0.1 mg/l IAA and 5 mg/l KIN callus of *A. arguta* was green and compact. **H.** and **I.** Colour changes on the green surface of callus signed a beginning of regenerative processes.

Fig 2. Organogenesis

A. Shoot apex on the surface of *A. arguta* callus. **B.** Two young shoot apices outgrowing from callus in *A. polygama*. **C.** Group of apices on a specific place differing with colour to the green rest of callus and elevated over the surface in *A. polygama*. **E.** Twin shoot apices of *A. deliciosa*. **F.** Organogenically developed shoot with two leaves in *A. arguta*. **D.** Organogenically developed root of *A. deliciosa* on medium rich in auxin (5 mg/l IAA and 0.1 mg/l KIN) **G.** Young adventitious root on *A. arguta* callus (on auxin rich medium). **H.** Callus of *A. polygama* with abundantly regenerating shoots. **I.** *A. polygama* regenerated via organogenesis.

and beneficial to explants, but due to its relatively high price, is commonly replaced by less expensive, synthetic cytokinins (BAP, KIN, 2iP etc.). 2,4-D in combination with cytokinins was broadly used to induce callusing in *A. deliciosa*. The friable, fast growing on 2,4-D, callus is successfully employed for protoplasts isolation in *A. deliciosa* (Pais et al. 1987, Oliveira & Pais 1991). Such calli could be therefore used in the case of other species of *Actinidia*. Blackening, browning and necrosis might be a result of excessive accumulation of harmful phenolic compounds, what was also described in other objects (Giri et al. 1993). Different reactions to hormones, known in kiwi, may result from many reasons, which have not been exhaustively elucidated so far. There is a large variability of factors acting *in vitro* such as light, gelling agent, concentration of metabolic gases, carbon source and many others (Infante et al. 1989, Mezzetti et al. 1991a, Mezzetti et al. 1991b, Tanimoto & Ishioka 1991). In most reports on *A. deliciosa* morphogenetic reaction observed were either the formation of adventitious buds and subsequent shoot regeneration or development of adventitious roots, described as organogenesis. On the other hand, viable somatic embryos in *A. deliciosa in vitro* were observed only under peculiar circumstances in immature stamen culture or directly on leaves exposed to quite unusual complex of stress factors (Fraser & Harvey 1986a, Oliveira & Pais 1992). These data show that somatic embryogenesis is rather incidental, not the normal way of regeneration in actinidias. This has been confirmed by the experiments performed in this work, since no embryoids were found in any of the investigated here species.

Organogenesis is common in *A. deliciosa* and is used for micropropagation. Stem explants were usually described as the most suitable for regeneration. Less effective were explants derived from roots, petioles and leaves (Harada 1975, Gui 1979, Hirsch & Fortune 1984, Fraser & Harvey 1986b, Marino & Battistini 1990, Marino & Bertazza 1990, Mezzetti et al. 1991a, Mezzetti et al. 1991b, Oliveira & Pais 1991, Pedroso et al. 1992). Media for regeneration proposed by all authors contained more cytokinins (ZEA, 2iP, BAP, KIN) than auxins (IAA, NAA). Rhizogenesis was reported on media rich in auxins. In this work organogenesis was obtained on media similar to those used by other authors, although sometimes containing more auxins. In many reports the variability of regeneration potential depending on plant and explant age, sex of source plants and genotype was emphasized, so that the differences described in this paper, though apparent, do not play an essential role and, furthermore, in the case of so many factors affecting morphogenesis, could not be precisely explained (Gui 1979, Hirsch & Fortune 1984, Marino & Bertazza 1990). Only the probable importance of genetic and physiological properties of individuals and species, like it is noted in *A. deliciosa*, should be suggested. Some research on actinidias and other genera indicate the role of superficial peroxidases, the composition of which changes during callusing and transition to organogenesis (Hirsch & Fortune 1984, McDougall et al. 1992). High regenerative potential, similar for the three species of *Actinidia*, two of which have been studied from this point of view for the first time in this work, can be applied for micropropagation or for any other purpose.

Elaboration *in vitro*, other than kiwi species of *Actinidia*, opens the possibility to introduce them to more advanced experiments like these carried out on *A. deliciosa*. The present work makes also a base and contribution to extend the investigations on numerous and interesting, but so far rarely regarded species of the genus *Actinidia*.

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INDUKCJA KALUSA I REGENERACJA ROŚLIN *IN VITRO* U *ACTINIDIA*

STRESZCZENIE

Zbadano zależność indukcji kalusa i organogenezy od zawartości auksyn (2,4-D i IAA) i cytokinin (BAP, KIN) w pożywce MS u *Actinidia arguta*, *A. deliciosa* var. *deliciosa* oraz *A. polygama*. Eksplantaty pochodziły z łodyżek, ogonków liściowych, blaszek liściowych i korzeni. Wykazano u badanych gatunków dużą zdolność do tworzenia kalusa przez eksplantaty z łodyg i ogonków liściowych oraz znacznie słabszą u eksplantatów z blaszek liściowych i korzeni. Pożywki o niższych stężeniach 2,4-D (0.01 i 0.1 mg/l), a wyższych cytokinin (1.0, 5.0 mg/l) najbardziej sprzyjały wzrostowi i żywotności kalusa. Wyższe stężenia tak 2,4-D jak i IAA powodowały degenerację tkanki. U *A. arguta* i *A. polygama* stwierdzono, że w obecności 2,4-D, BAP korzystniej niż KIN wpływa na żywotność kalusa. Organogeneza zachodziła obficie i regularnie po pasażu na pożywkę z 0.1 do 5.0 mg/l IAA i 0.1 do 5.0 mg/l cytokinin tylko na kalusach pochodzenia łodygowego; pozostałe eksplantaty wykazały zmniejszoną w różnym stopniu zdolność do regeneracji. W reakcji na warunki *in vitro* poszczególnych gatunków stwierdzono wyraźne i znaczne podobieństwa, a zaobserwowane różnice obejmowały szybkość indukcji i wzrostu kalusa, skład pożywek optymalnych dla niektórych eksplantatów, wygląd organogenicznych kalusów.

Pędy zregenerowane z kalusa ukorzeniono i uzyskano rośliny przesadzone następnie do gleby.

SŁOWA KLUCZOWE: *Actinidia*, kalus, organogeneza, auksyna, cytokinina, regeneracja *in vitro*.