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In vitro PROPAGATION OF NON-TRADITIONAL HORTICULTURAL PLANTS (*Actinidia, Chaenomeles, Aronia*)

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Introduction

New, non-traditional horticultural plants are spreading rapidly in home gardens and some of them acquiring commercial value [RUMPUNEN et al. 1998]. To save good qualities of new cultivars they are propagated vegetatively. A rapid and cheap method of vegetative propagation is essential for the successful spread of new cultivars [STANDARDI, CATALANO 1985]. The development of a cost-efficient propagation system consisting of: 1) rapid multiplication of stock-material from new cultivars or forms by micropropagation and 2) propagation by softwood or hardwood cuttings from stock-material for production of commercial plants is very important for spreading of non-traditional horticultural plants. The technique of tissue culture for non-traditional horticultural plants of the genera *Chaenomeles, Aronia* and *Actinidia* has not been investigated sufficiently for commercial needs. Methods of microvegetative propagation have been prepared for *Actinidia chinensis* [STANDARDI, CATALANO 1985; PIAGNANI et al. 1986], but other species of this genus have not been investigated yet. The purpose of this study was to investigate cultivation conditions and to establish efficient and reproducible methods for *in vitro* mass propagation of kolomicta actinidia, bower actinidia, draf Japanese quince and black chokeberry from mature plants to allow commercial production.

Materials and methods

Plant material

Shoot apices and nodal segments (3–7 mm long) taken from plants growing in the field and fruiting of 22 selected *Chaenomeles japonica* (Thunb.) Lindl. ex Spach plants and *Aronia melanocarpa* (Michx.) Elliott and from growing in the field and greenhouse of *Actinidia kolomicta* (Maxim.) Maxim. cultivars Landë

and Paukshtes sharkava and a male plant, *A. arguta* (Siebold et Zucc.) Planch. ex Miq. cultivars Kijevska hibridna, Sentiabrska, Figurna, Purpurova sadova, Kijevska krupnaplodna and a male plant were used as initial explants. Shoot segments were sterilized for 15 minutes in 0.1% dioxide solution and rinsed in sterile water three times.

Culture procedure and conditions

Explants were planted in MS [MURASHIGE, SKOOG 1962] medium supplemented with 0.5; 0.75; 3 mg·dm^{-3} BAP; 0–0.1 mg·dm^{-3} GA; 0.5; 1 mg·dm^{-3} IBA; 0.02; 0.2 mg·dm^{-3} NAA, 30 g sucrose. The pH value of the nutritive medium was adjusted up to 5.8 prior to autoclaving. For shoot rooting, shoots 20–40 mm long of dwarf Japanese quince and black chokeberry were transferred to MS medium with IBA or NAA supplements. After 4 weeks, not-rooted shoots of dwarf Japanese quince were immersed into 30 mg·dm^{-3} IBA solution for 18 hours and after this rooted to the peat substrate and kept in high humidity environment.

Isolated explants were cultured in the cultivation room at 25±20°C, lighting up to 3000 l x over an explant for a 16 h photoperiod. In each treatment 20–40 explants were used. Shoot growth and the state, rhisogenesis rate and multiplication coefficient were assessed every 4 weeks. Alternative parameters were calculated by the method of disperse analysis according to VOLF [1966].

Results

The first stage of a microvegetative propagation is obtaining a stable proliferating culture. It is not always a 100% success ful. A good start of establishment in the culture is when an explant remains viable, axenic and its hypersensitive reactions can be controlled. During this stage rescued organs and tissues are brought under stress: mechanic injury of tissues, expression of internal infection. All this may stimulate a supersensitive reaction of tissues, in which phenol combinations are produced rapidly and, as a result, most often explants are killed. These traits are very dependent on species. In the trials witch explants, dwarf Japanese quince produced phenol combinations most intensively. Therefore, explants had to be often subcultivated, treated with antioxidants and lighting intensity had to be adjusted. Explants of black chokeberry leached out phenol substances on a smaller scale and explants of both species of actinidia did not leach out phenol substances at all. Thus, successful establishment on a culture was determined by inner bacterial infection and plant physiological state.

Actinidia

Explants isolated from field plants for high inner infection. It manifested itself after a few subcultivations. During three subcultivation periods, 89.2% of explants were aborted due to inner infection. Therefore, explants from greenhouses intensively treated with fungicides were utilized in further investigation. In this case, during three subcultivations 36.6% of explants were aborted. Depending on a plant genotype, 62.5–100% of explants developed in *in vitro* conditions (Fig. 1).

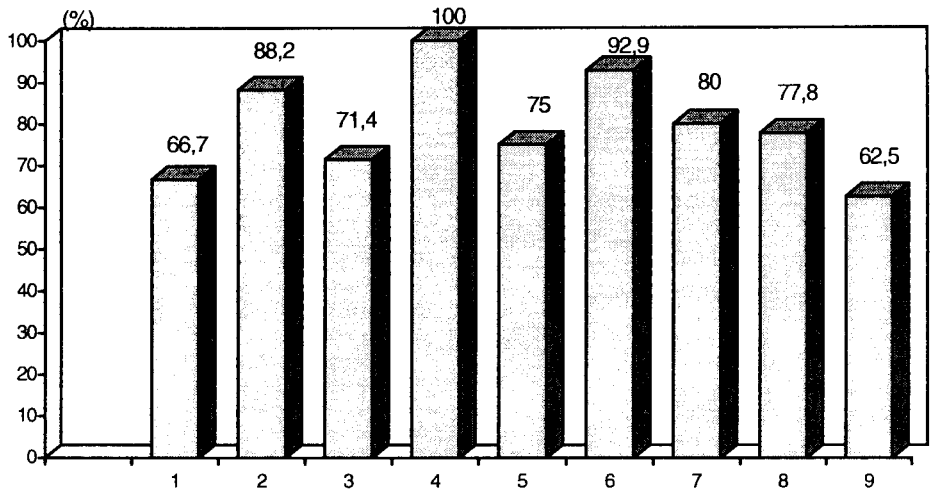

Fig. 1. Rate of development *in vitro* of *actinidia* explants. *Actinidia kolomicta* (Maxim.) Maxim. 1 – a male plant; 2 – Landë; 3 – Paukshtes shakarva; *A. arguta* (Siebold et Zucc.) Planch. ex Miq., 4 – Sentiabrska; 5 – Figurna; 6 – Kijevska hibridna; 7 – Purpurova sadova; 8 – Kijevska krupnaplodna, 9 – a male plant

Rys. 1. Tempo rozwoju przeszczepów *Actinidia* w warunkach *in vitro*. *Actinidia kolomicta* (Maxim.) Maxim. 1 – roślina męska; 2 – Landë, 3 – Paukshtes shakarva; *A. arguta* (Siebold et Zucc.) Planch. ex Miq., 4 – Sentiabrska, 5 – Figurna, 6 – Kijevska hibridna, 7 – Purpurova sadova, 8 – Kijevska krupnaplodna, 9 – roślina męska

Differences were observed between separate cultivars. It should be noted that explants from male plants of both species developed at a reliably lower rate in *in vitro* culture. *In vitro* development of explants depended on the medium composition (Tab. 1). In *in vitro* conditions, actinidia of both species multiplicated by forming shoots from apical meristems rather than adventitious buds and shoot auxiliary buds. Under the investigation conditions we failed to induce formation of adventitious shoots. Thus, shoot length and the number of internodes determined the coefficient of actinidia multiplication. Shoot length was limited by the medium composition. During 4–5 subcultivation weeks, the shoots produced in the medium with cytokinin BAP were significantly smaller than in the medium with BAP and NAA. Explants of male kolomicta actinidia grew more vigorously in the medium with BAP. The correlation coefficient between the multiplication coefficient and the height of a microplant was 0.69, between the multiplication coefficient and the number of internodes – 0.77. The correlation coefficient between the height of a microplant and the number of internodes was 0.87. In both media shoot rhizogenesis was observed. Its rate was higher and reached 100% for all the cultivars in the medium supplemented with auxins. The highest rhizogenesis rate in the medium supplemented with 0.75 mg·dm^{-3} BAP was observed in the variants with male plants. Initial multiplication of both actinidia species was observed after 4 subcultivations. However, multiplication coefficient became stable and the culture fully adapted in *in vitro* conditions after 9 subcultivations the multiplication coefficient was increasing during the culture adaptation period.

Table 1; Tabela 1

Growth of *actinidia* explants on different media
Wzrost przeszczepów *Actinidia* na różnych pożywkach

Cultivars Odmiany	Investigated explants Badane przeszczepy	Medium suplements Pożywka ($mg \cdot dm^{-3}$)	Rhizogenesis percent* Rizogeneza (%)	Multiplication coefficient Współczynnik rozmnożenia		Microplant height** Wysokość mikrorośliny **	Internode number ** Liczba międzywęźli **
				after 4 subcultivations po 4 okresach uprawy	after 9 subcultivations po 9 okresach uprawy		
A. kolomicta ♂	40	MS+0.75 BAP	100 a	1	–	2.07±0.15	–
A. kolomicta ♂	40	MS+0.5 BAP+0.2 NAR	100 a	1.7	1.3	1.0±0.08	2.8±0.13
Landë	40	MS+0.75 BAP	61.1 b	1	–	1.05±0.15	–
Landë	38	MS+0.5 BAP+0.2 NAR	100 a	1.1	2.4	1.4±0.11	3.4±0.16
Paukshtes shakarva	40	MS+0.75 BAP	14.2 c	1	–	1.05±0.21	–
Paukshtes shakarva	40	MS+0.5 BAP+0.2 NAR	100 a	1	2.4	2.7±0.26	4.2±0.25
A. arguta ♂	40	MS+0.75 BAP	89 b	1	–	–	–
A. arguta ♂	34	MS+0.5 BAP+0.2 NAR	100 a	1.9	3.0	2.0±0.28	4.2±0.49
Sentiabrska	38	MS+0.75 BAP	25.0 d	1	–	1.86±0.18	–
Sentiabrska	24	MS+0.5 BAP+0.2 NAR	100 a	2.3	2.5	2.6±0.15	4.7±0.33
Figurna	40	MS+0.75 BAP	0	1	–	1.20±0.31	–
Figurna	40	MS+0.5 BAP+0.2 NAR	100 a	2.0	2.9	3.4±0.19	4.5±0.27
Kijevska hibridna	40	MS+0.75 BAP	61.5 c	1	–	2.06±0.44	–
Kijevska hibridna	40	MS+0.5 BAP+0.2 NAR	100 a	2.6	3.8	4.0±0.32	4.7±0.40
Purpurova sadova	32	MS+0.75 BAP	25 d	1	–	–	–
Purpurova sadova	36	MS+0.5 BAP+0.2 NAR	100 a	2.2	4.5	2.7±0.23	4.6±0.34
Kijevska krupnaplodna	36	MS+0.75 BAP	13 e	1	–	–	–
Kijevska krupnaplodna	38	MS+0.5 BAP+0.2 NAR	100 a	2.4	3.9	3.3±0.27	4.7±0.30

* Means are significantly different at $P \leq 0.01$, applying Duncans multiple range test; Średnie są istotnie różne przy $P \leq 0{,}01$, zastosowano test wielokrotnego wyboru Duncana

** Mean ±SD; Średnia ±SD

Explants of male kolomicta actinidia were an exception. The multiplication coefficicnt decreased in the culture adaptation process. Under non-sterile condi-

tions 69–100% of transplanted plants survived. Plants reached realization condition in 5 months when transplanted to non-sterile environment in spring and in 8 months – when transplanted in autumn. Plants transplanted in autumnentered dormancy in winter, therefore, the production period of plants suitable for realization became longer. The reaction of male plants of both actinidia species to the effect of exogenic growth regulators allows us to suppose that the phytohormone balance in a plant plays an important role in sex formation, as in the case with cucumbers and other plants [LVOVA 1978; KUMAR, JAISWAL 1984]. Thus, when propagating male plants it is necessary to make sure that the plant sex does not change in *in vitro* system, which is oriented to a certain phytohormone regime.

Dwarf Japanese quince

Isolated explants (0.5–1 cm long) were planted on an MS medium supplemented with 3 mg·dm^{-3} BAP. According to the reaction to the cultivation conditions the genotypes of dwarf Japanese quince were subdivided into three groups (Fig. 2).

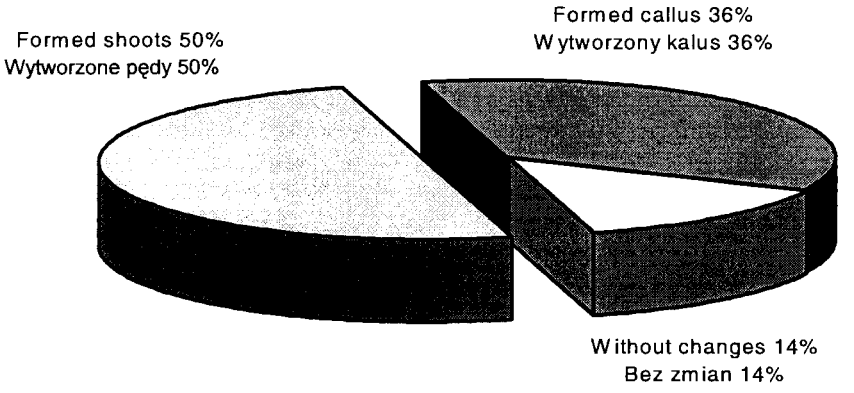

Fig. 2. Distribution of quince genotypes according to the behavior of isolated explants

Rys. 2. Rozkład genotypów pigwy według zachowania wyizolowanych przeszczepów

The first group was formed by quince genotypes, whose explants started formation of new shoots. Such genotypes made up 50% of the investigated plants. The second group consisted of quince genotypes, whose explants formed callus in the medium. They made up 36%. The third group (14%) consisted of genotypes, whose explants did not change or were aborted during the first subcultivation. Multiplication coefficient of dwarf Japanese quince depended on properties of the them clone, level of culture adaptability and medium composition (Tab. 2).

Table 2; Tabela 2

Relationship between multiplication coefficient of dwarf Japanese quince and plant genotype, medium composition and culture adaption level

Związek między współczynnikiem rozmnożenia karłowatej pigwy japońskiej a genotypem rośliny, składem pożywki i stopniem adaptacji kultury

Clone No. Nr klonu	Medium composition Skład pożywki (mg·dm^{-3})	Multiplication coefficient after 3 subcultivations Współczynnik rozmnożenia po 3 okresach uprawy	Medium composition Skład pożywki (mg·dm^{-3})	Multiplication coefficient after 60 subcultivations Współczynnik rozmnożenia po 60 okresach uprawy
43	MS+3 BAP	2.2	MS+0.75 BAP	2.8
			MS+3 BAP; 0.1 GA; 0.5 IBA; 0.02 NAA	4.1
47	MS+3 BAP	1.8	MS+0.75 BAP	2.9
			MS+3 BAP; 0.1 GA; 0.5 IBA; 0.02 NAA	3.3
9365	MS+3 BAP	2.4	MS+0.75 BAP	1.9
			MS+3 BAP; 0.1 GA; 0.5 IBA; 0.02 NAA	2.0

Multiplication coefficient of the adapted culture depending on a plant genotype differed twice as much. At higher BAP concentrations in the medium, multiplication coefficient of clones 43 and 47 increased significantly and of clone 9365 it did not increase or change insignificantly. Auxins IBA and NAA and gibberellin in the medium provided sufficient shoot extension in the background of high cytokinin BAP concentration. Shooting up on MS + (mg·dm^{-3}) 3 BAP; 0.1 GA; 0.5 IBA; 0.02 NAA medium was equal to shoot growth on MS + 0.75 mg·dm^{-3} BAP medium. Multiplication coefficient was increasing during plant adaptation *in vitro*. Only the multiplication coefficient of genotype 9365 was lower in the adapted culture. When shoots were 2–3 cm long they were transferred to ½ MS medium with 0–1 mg·dm^{-3} IBA and NAA that induced rhizogenesis. Higher rhizogenesis was obtained in the medium supplemented with ISA. After 5 weeks on a rhizogenesis medium, 19–34% of shoots regenerated roots in the medium with ISA and 22–25% – in the medium with NAA. The rhizogenesis results are similar to the results obtained by PANAVAS [1994]. The results were mostly dependent on the genotype of an investigated plant. Rhizogenesis of different clones ranged from 0 till 45 per cent. The investigation showed that root regeneration mainly depended on the medium composition utilized for shoot propagation (Tab. 3). Shoots rooted reliably better when they were propagated on MS+0.75 mg·dm^{-3} BAP medium than on MS+(mg·dm^{-3}) 3 BAP; 0.1 GA; 0.5 IBA; 0.02 NAA medium. We suppose that excessive saturation of microshoot tissues with cytokinins (high ratio of cytokinin/auxins) affects rhizogenesis *in vitro* negatively. In the medium without auxins, rhizogenesis did not occur irrespective of whether shoots had been propagated in a medium with auxins or without them. Therefore, in order to increase the output of plants it is necessary to subcultivate shoots in the medium with auxins supplements. More successful rhizo-

genesis proceeded in peat substrate (Tab. 3). The data demonstrate that quince rhizogenesis process *in vitro*, unlike the process of rooting of soft wood cuttings [KVIKLYS, RUMPUNEN 1996], is a complicated phenomenon determined by a number of factors. Plants reach realization condition in one vegetation season.

Table 3; Tabela 3

Rhizogenesis of quince microshoots
Rizogeneza mikropędów

Medium to shoot propagation Pożywka do rozmnażania pędów	*In vitro*		In peat; Na torfie	
	investigated explants badane przeszczepy	rhizogenesis rizogeneza (%)	investigated shoots badane pędy	rhizogenesis rizogeneza (%)
MS+0.75 BAP ($mg \cdot dm^{-3}$)	310	30.0	216	66.5
MS+3 BAP; 0.1 GA; 0.5 ISR; 0.02 NAR ($mg \cdot dm^{-3}$)	388	18.2	249	42.5

Black chokeberry

Already after 4 weeks 20% of isolated explants started to produce new shoots in *in vitro* culture. After three subcultivations on MS + 0.75 $mg \cdot dm^{-3}$ BAP medium the multiplication coefficient was 8.2. During adaptation to *in vitro* conditions in the same medium, multiplication coefficient was increasing and in the 12th subcultivation it reached 14.3. Black chokeberry was the most intensively multiplicating species in *in vitro* conditions of all the non-traditional horticultural plant species tested.

When the medium was supplemented with 0.5 $mg \cdot dm^{-3}$ IAA, NAA or IBA, 90–94% of shoots regenerated roots. After 4 weeks of growth on the rhizogenesis-inducing medium, plants had 5–9 rootless which were 1–2 cm long. Sucha level of root development guaranteed high (100%) plant survival rate under non-sterile conditions. Plants reached realization condition in 5 months when transplanted into non-sterile environment in spring and in 8 months – when in autumn. Plants transplanted in autumn entered dormancy in winter, therefore, production period of plants suitable for realization became longer.

Conclusions

Microvegetative propagation is an efficient method of mass propagation of non-traditional horticultural plants: actinidia, dwarf Japanese quince and black chokeberry. Plant genetic characters and optimization of propagation parameters, that are indispensable for each clone, predetermine successful propagation. It is necessary to investigate further stimulation of quince rhizogenesis and formation of actinidia sex *in vitro*.

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Key words: actinidia, dwarf Japanese quince, black chokcberry, microvegetative propagation

Summary

Succesful propagation of selected clones and cultivars of *Actinidia kolomicta* (Maxim.) Maxim., *A. arguta* (Siebold et Zucc.) Planch. ex Miq., *Chaenomeles japonica* (Thunb.) Lindl. ex Spach and *Aronia melanocarpa* (Michx.) Elliott has been achieved by *in vitro* methods. It has been demonstrated that the intensity and pathway of microvegetative propagation depend on the properties of plant species, genotype and sex. Under *in vitro* conditions, actinidia multiplicated by forming shoots from apical meristems and auxiliary buds of shoots, dwarf Japanese quince and black chokeberry – by new adventitious shoots. For *in vitro* development, male plants of actinidia species demanded opposite ratios of auxins and cytokinins than the female plants. They worse adapted to *in vitro* conditions. Multiplication coefficient in the fourth week was as follows: *Actinidia* – 1.3–4.5; *Chaenomeles* – 1.9–4.1; *Aronia* – 14.3.

ROZMNAŻANIE *in vitro* ALTERNATYWNYCH ROŚLIN OGRODNICZYCH (*Actinidia, Chaenomeles, Aronia*)

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Słowa kluczowe: aktynidia, karłowata pigwa japońska, aronia, rozmnażanie mikrowegetatywne

Streszczenie

Przy pomocy metod *in vitro* udało się rozmnożyć wybrane klony i odmiany *Actinidia kolomicta* (Maxim) Maxim, *A. arguta* (Siebold et Zucc.) Planch. ex Miq., *Chaenomeles japonica* (Thunb.) Lindl. ex Spach oraz *Aronia melanocarpa* (Michx.) Elliott. Wykazano, ze intensywność i przebieg rozmnażania zależały od właściwości gatunku rośliny, genotypu i płci. W warunkach *in vitro* aktynidia rozmnażała się poprzez tworzenie pędów z merystemów wierzchołka i pomocniczych pęków na pędach, karłowata pigwa japońska i aronia – poprzez nowe pędy przybyszowe. W celu rozmnożenia w warunkach *in vitro* rośliny męskie wymagały odwrotnych proporcji auksyn i cytokinin niż rośliny żeńskie. Gorzej dostosowywały się do warunków *in vitro*. Współczynnik rozmnażania w czwartym tygodniu był następujący: *Actinidia* – 1.3–4.5; *Chaenomeles* – 1.9–4.1; *Aronia* – 14.3.

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