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MICROPROPAGATION OF BISEXUAL *Actinidia kolomikta*

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Abstract. A rare bisexual plant of *Actinidia kolomikta* (variety 'Iisaku') was introduced into Estonia from the Far East (from the vicinity of the Botanical Garden of Vladivostok), and it was micropropagated in order to preserve its genetic potential. The culture was initiated from 1 cm long single node stem cuttings on Murashige & Skoog basal medium (MS) + 6-benzyladenine (BA) (1.0 mg/l) + indolebutyric acid (IBA) (0.01 mg/l) and multiplicated on WMS (Woody Plant Medium macroelements + MS microelements and vitamins) + BA (1.0 mg/l) + IBA (0.1 mg/l). On this medium shoot multiplication occurred at a rate of 7 folds after 4 weeks. Shoots were elongated on 1/2 MS + kinetin (0.1 mg/l) + indoleacetic acid (IAA) (1.0 mg/l) and rooted on the medium 1/2 MS + IAA (0.2 mg/l). The shoots could be directly rooted in vivo with up to 89.3% survival.

Key words: *Actinidia kolomikta*, tissue culture, micropropagation.

INTRODUCTION

The natural distribution area of *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. is the Far East (Russian Primor'e krai, North Korea, Sakhalin, China) (Rehter, 1949). In Estonia it has been cultivated as a decorative plant, but it deserves attention as a berry plant as well (Elliku, 1985). However, its use for the latter purpose is hindered by its unisexuality. In 1972 more than 200 two to three year old plants of *A. kolomikta* were brought to Estonia from the Far East (from the vicinity of the Botanical Garden of Vladivostok). One of the plants turned out to be bisexual. According to the present localization of the plant in north-eastern Estonia it has got the varietal name 'Iisaku'. Beginning from 1976 the plant has fructified richly every year including the summers after the very cold winters of 1978/79 and 1986/87 when the temperature in the north-east of

Estonia fell below −43 °C. This plant was described by Elliku (1985) and Roht (1986).

In the 1970s another bisexual plant of *A. kolomikta* was found in Finland among the material brought by an expedition from the Amur district (Harlahti, 1993).

Species of *Actinidia* have been widely propagated in tissue culture. However, attention has been focused mostly on *A. chinensis* as it is commercially the most important species (Harada, 1975; Monette, 1986; Pedroso et al., 1992; Shen et al., 1996). Other species of *Actinidia* have also been propagated by tissue culture methods (Nayak & Beyl, 1987).

The objectives of this work were to establish an aseptic culture of *A. kolomikta* in order to avoid an accidental destruction of the genetic potential of this plant and to develop a method for its mass propagation.

MATERIAL AND METHODS

A. kolomikta shoots were cut out from an outdoor-grown plant in March and thereafter young nonlignified shoots with three to four buds were forced to develop in a phytotrone (for about 2 weeks). These shoots were cut into approximately 2 cm long sections. They were surface-sterilized by rinsing for 30 s in 70% ethanol, followed by vigorous stirring for 20 min in a filtered solution of calcium hypochlorite (6% w/v) supplemented with a few drops of Tween 80. The shoots were then rinsed three times in sterile distilled water, and approximately 1 cm long nodal sections, each with one bud, were placed horizontally on the initiation medium MS (Murashige & Skoog, 1962) + 1 mg/l 6-benzyladenine (BA) + 0.01 mg/l indolebutyric acid (IBA).

MS basal medium with full (1/1 MS) or half concentration (1/2 MS) of macronutrients, or a modified Woody Plant Medium (WPM) (Lloyd & McCown, 1981) was used. The plantlets were cultured in glass jars capped with aluminium foil.

The cultures were maintained in phytotron at 23±2 °C with a 16 h photoperiod under cool white lamps. The light intensity was about 1000 lx during the multiplication stage and 3000 lx at the end of the elongation and rooting stage.

RESULTS

After 5 weeks on the initiation medium three to five new 0.5–0.7 cm shoots had developed. Addition of zeatin (1 mg/l) instead of MS + BA to the initiation medium did not influence the number of the shoots, but in its presence leaves on the shoots were larger and their tips were turned downwards resulting sometimes in lifting the mother tissue from the medium.

These microshoots were transferred to different shoot multiplication media, viz. MBA (MS + 1.0 mg/l BA + 0.1 mg/l IBA), MZ (MS + 1.0 mg/l zeatin + 0.1 mg/l IBA), and WMS (WPM macronutrients + MS micronutrients and vitamins + 1.0 mg/l BA + 0.1 mg/l IBA). After 4 weeks on the MZ medium single unbranched shoots with approximately four nodes were formed, which had remarkably larger leaves than the shoots growing on the other media. On the MBA medium approximately two shoots with three nodes and two buds were formed. On the WMS medium about four short shoots with two nodes and three to six buds were formed (Fig. 1).

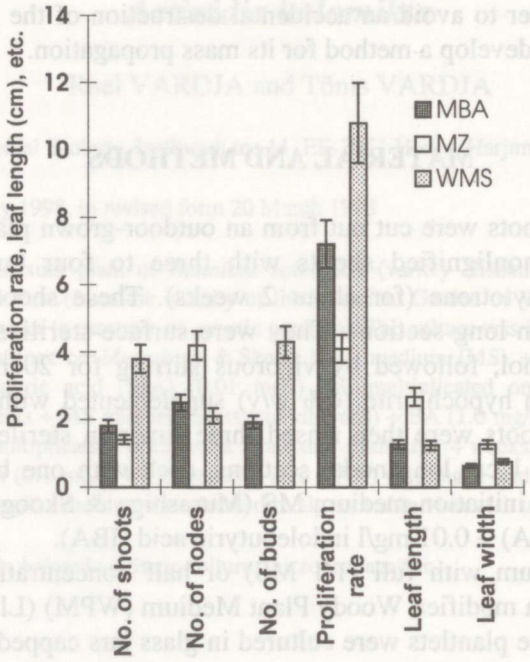

Fig. 1. Effect of nutrient medium on the growth and shoot proliferation of *Actinidia kolomikta* (growth period 6 weeks; MBA = MS + 1.0 mg/l BA + 0.1 mg/l IBA; MZ = MS + 1.0 mg/l zeatin + 0.1 mg/l IBA; WMS = WPM macroelements + MS microelements and vitamins + 1.0 mg/l BA + 0.1 mg/l IBA.

The multiplication rate was considerably higher on the WMS medium than on the WLS medium (WPM macroelements + LS (Linsmaier & Skoog, 1965) microelements and vitamins) (Fig. 2). The LS medium lacks B_6 and PP vitamins and glycine. On the WMS medium *A. kolomikta* leaves are dark green, while on the WLS medium they are light green.

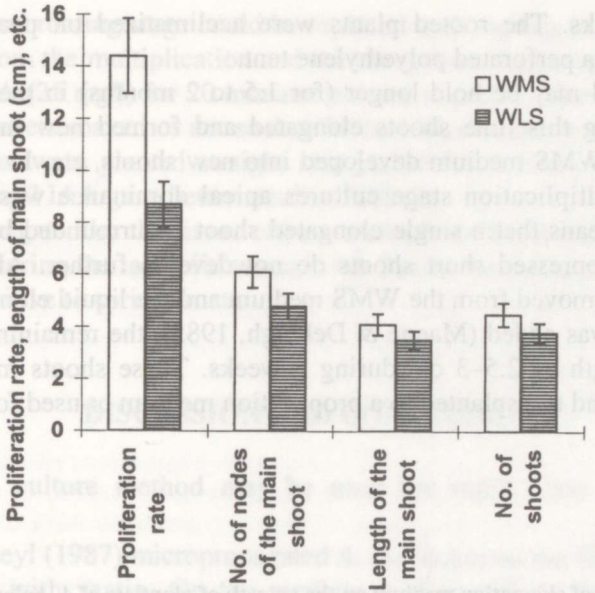

Fig. 2. Influence of glycine and vitamins on in vitro growth and proliferation of *Actinidia kolomikta* (6 weeks; WMS = WPM macroelements + MS microelements and MS vitamins + 1.0 mg/l BA + 0.1 mg/l IBA; WLS = WPM macroelements + MS microelements + LS vitamins + 1.0 mg/l BA + 0.1 mg/l IBA).

The later strategy of the propagation was different as well. On the MZ medium the shoots were further propagated by single node cuttings, whereas on the WMS and MBA media further multiplication was achieved by subculturing individual shoots by single nodal cuttings when the shoots had two or more nodes and were with buds.

After 4 weeks the multiplication rate was maximum in case of the WMS medium as there was the greatest number of shoots (although they were too short) and buds.

The shoots that had grown for 4 weeks on the WMS propagation medium were transferred to the elongation media (1/2 MS + 0.1 mg/l kinetin + 1.0 mg/l indoleacetic acid (IAA) and 1/2 MS + 0.1 mg/l BA + 0.1 mg/l IBA). After 3 weeks on both elongation media approximately 3 cm long plantlets with 0.5–1 cm long roots had developed. (On the IBA medium the roots were thicker.) After 6 weeks these plantlets were about 5 cm long. On the elongation medium with kinetin and IAA more nodes developed. The length and the number of roots were not significantly different (Table 1).

The shoots obtained on the MZ medium were transferred directly to the rooting medium (1/2 LS + 0.2 mg/l IAA) where they formed up to 1.5 cm long

roots in 3 weeks. The rooted plants were acclimatized on peat–perlite (1:1) substrate under a perforated polyethylene tent.

The material may be hold longer (for 1.5 to 2 months) in the multiplication medium. During this time shoots elongated and formed new internodes; buds formed on the WMS medium developed into new shoots, etc. In old (more than 6–8 weeks) multiplication stage cultures apical dominance was visible in the cluster. This means that a single elongated shoot is surrounded by several short shoots. The suppressed short shoots do not develop further. After the largest shoot tip was removed from the WMS medium and the liquid elongation medium with charcoal was added (Maene & Debergh, 1985), the remaining small shoots obtained a length of 2.5–3 cm during 3 weeks. These shoots may be cut into microcuttings and transplanted to a propagation medium or used for rooting.

Table 1

Effect of elongation medium on the growth of plantlets of *A. kolomikta* (growth period 6 weeks)

Medium 1/2 MS +	Length of shoot, cm	No. of nodes	Length of roots, cm	No. of roots
0.1 mg/l kinetin + 1.0 mg/l IAA	5.1 ± 0.6	8.3 ± 1.2[a]	0.5–4.5	7.1 ± 1.3
0.1 mg/l BA + 0.1 mg/l IBA	4.8 ± 0.7	6.2 ± 0.8[a]	0.5–3.0	6.8 ± 0.9

[a] differ significantly from each other at 0.1% level.

Table 2

Effect of various treatments on rooting and transplantation survival

Treatment	Total number of shoots	Number of rooted and acclimatized plants	Survival, %
In vitro rooted shoots	120	104	86.7
Elongated shoots directly transplanted (control)	50	31	62
Elongated shoots soaked in 25 mg/l IBA before transplantation	50	34	68
Elongated shoots transplanted and irrigated with 1/2 MS + 10 mg/l IAA	125	101	89.3

The aseptical rooting stage could be replaced with rooting in vivo. Part of the long shoots from the multiplication and the elongation media were soaked in the solution of IBA (25 mg/l) for 20 min and planted in perlite. The difference in the rooting of plantlets soaked or not soaked in the IBA solution was not significant. The shoots that were planted straight into perlite followed by moistening with 1/5 MS + 10 mg/l IAA gave better results (Table 2).

Plants propagated by the tissue culture method remained resistant to the low temperatures during the last mild winters, but due to their short winter dormancy they were sensitive to spring frosts.

DISCUSSION AND CONCLUSION

The tissue culture method may be used for rapid mass propagation of *A. kolomikta.*

Nayak & Beyl (1987) micropropagated *A. kolomikta* on the MS basal medium supplemented with zeatin. On the medium with zeatin *A. kolomikta* formed one shoot with large leaves. This was observed in case of *Pyrus* and *Malus* (Hutchinson, 1984; Shen & Mullins, 1984) too. Our work shows that zeatin may be replaced by BA. However, the shoots multiplied in BA containing medium require an elongation stage. For propagation it is better to use shoots with smaller leaves like these formed in the presence of BA.

Frequently, adventitious shoots arise indirectly from callus at the base of subcultured shoot mass. Shoots arising from callus are not desirable, because there is a high probability to obtain genetically deviant plants (George, 1993). In order to avoid deviations we did not use for subculture shoots that had arisen indirectly from callus.

Shen with coworkers (1996) showed in their experiments that both axillary and adventitious bud plants of Chinese gooseberry (*Actinidia deliciosa*) were indistinctive in morphology from each other and their originating clone, but significant differences were found in flowering between the two types of test tube plants (the flowering of adventitious bud plants was poor). In our experiment no significant difference was found in growth and flowering between plants obtained from adventitious and axillary buds.

High frequency rooting of the shoots can be achieved in vivo simultaneously with acclimatization.

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KAHESUGULISE *Actinidia kolomikta* MIKROPALJUNDUS

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Kahesugulise *Actinidia kolomikta* (Maxim. et Rupr.) Maxim. taim on saadud juhuleiuna Vladivostoki botaanikaaia lähedalt. Taime praeguse asukoha järgi Kirde-Eestis Iisakus on ta nimetatud var. 'Iisaku'. Taime genofondi säilitamiseks viidi ta koekultuuri.

Koekultuuri saamiseks kasutati umbes 1 cm pikkusi ühe pungaga võrselõike, mis asetati Murashige–Skoogi (MS) söötmele, kuhu oli lisatud 1,0 mg/l bensüüladeniini (BA) ja 0,01 mg/l indolüülvõihapet (IBA). Parim paljunemine saadi söötmel, mis koosnes Lloydi–McCowni söötme makroelementidest ja MS söötme mikroelementidest, lisandiks 1,0 mg/l BA ja 0,1 mg/l IBA. Võrsete pikendamiseks kasutati söödet, milles oli 1/2 MS, 0,1 mg/l kinetiini ja 1,0 mg/l indolüüläädikhapet (IAA). Samal söötmel võrsed ka juurdusid. Zeatiiniga MS paljundussöötmel moodustus üks võrse, mis juurutati 1/2 MS söötmel, millele oli lisatud 0,2 mg/l IAA. Võrseid võib edukalt juurutada ka *in vivo*. Juurdumisprotsent on kuni 89,3.