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Agrobacterium rhizogenes-mediated transformation and regeneration of bisexual *Actinidia kolomikta*

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Abstract

The aim of the work was to produce *Actinidia kolomikta* plants with modified growth and root capacity. Four highly branched root clones were obtained from leaf explants of bisexual *A. kolomikta* plants by *Agrobacterium rhizogenes* transformation. Two transformed shoot clones were regenerated from hairy roots on solid half-strength Woody Plant Medium with varying concentrations of benzyladenine (0.5 to 5.0 mg l^{-1}). Shoot cultures of transformed plants required culture media for proliferation and rooting different from those suitable for the control. The response to exogenous hormones was modified in the transformants. Shoot proliferation required benzyladenine as the only hormone. Microcuttings from transformed in vitro plants formed dense root systems on auxin-free Murashige and Skoog medium. Leaf explants from transformed plants produced roots on hormone-free Murashige and Skoog medium, a typical response of plants regenerated from hairy roots. No rooting was observed on untransformed actinidia leaf explants under the same conditions. Transformants that had grown in the unheated greenhouse suffered from pests, and they tended to be sensitive to frost in winter.

Key words: *Agrobacterium rhizogenes*, bisexual *Actinidia kolomikta*, hairy roots, plant regeneration.

Introduction

In Estonia *Actinidia kolomikta* has been cultivated mainly as an ornamnetal plant. *A. kolomikta* deserves attention as a berry plant as well. Its fruits are tasty and contain a large amount of vitamin C (Bojarkova 1949). *A. kolomikt*a is usually unisexual (Kachalov 1970). A bisexual plant of *A. kolomikta* was found among the plants introduced to Estonia from the Far East (vicinity of Vladivostok). This plant was micpropagated in order to preserve its genetic potential (Vardja, Vardja 1998). The growth of *A. kolomikta* vines is about one meter during the growing season. The *Agrobacterium rhizogenes* transformation system has been used previously to modify plant growth and development (van der Salm et al. 1996). Plants regenerated from transformed roots had a "hairy root" phenotype – altered growth and morphology, a very dense root system, reduced apical dominance, short internodes, the ability to profusely regenerate roots from leaf explants, and some other features. In this work, we report on the genetic transformation of bisexual *A. kolomikta* using *A. rhizogenes* as a vector.

Materials and methods

The plant material for *A. rhizogenes*-mediated transformation was derived from intensively growing *in vitro* plants (Vardja, Vardja 1998).

The Agropine strain of *A. rhizogenes* LBA 9402 (gift from Norwich Laboratory, England) was used in the present study. For transformation experiments, a 24-h-old bacterial suspension grown in liquid yeast-mannitol (0.5 g l^{-1} K_2HPO_4, 0.2 g l^{-1} $MgSO_4$, 0.1 g l^{-1} NaCl, 10 g l^{-1} mannitol, 0.4 g l^{-1} yeast extract, 50 mg l^{-1} kanamycin, pH 7.0) medium at 25 °C on a rotatory shaker (90 rpm) was used.

Plant explants (whole leaves with petioles, leaf petioles and pieces of leaves) were co-cultured with *A. rhizogenes* suspension diluted with Murashige, Skoog (1962; MS) liquid medium. The explants were immersed in the bacterial suspension for 50 min. After two days of co-culture on MS solid medium, the explants were rinsed in liquid MS medium containing cefotaxime (0.5 g l^{-1}). The explants were then transferred to solid MS medium without growth regulators and supplemented with both cefotaxime (500 mg l^{-1}) and kanamycin (50 mg l^{-1}) to eliminate bacteria and to select kanamycin-resistant primary hairy roots. To confirm the absence of bacteria in root cultures, root pieces were cultured on solid yeast-mannitol medium (with 10 g l^{-1} agar at 25 °C in the dark conditions). After purification from bacteria the root culture was maintained and propagated for several subcultures in liquid hormone-free ½ Woody Plant Medium (Lloyd, McCown 1981; WPM) with 20 g l^{-1} sucrose.

To induce shoot regeneration, segments of each root clone with a length of about 2 cm were cultured on solid ½ WPM media with varying benzyladenine (BA) concentration (0.5 to 5.0 mg l^{-1}). This experiment was repeated three times with 30 root explants per treatment. The shoots regenerated from hairy roots were micropropagated and maintained on standard MS medium with BA as the only hormone or without any hormone.

Results and discussion

Hairy root formation on inoculated whole leaf explants was low (6.6 %). The other explants, both non-inoculated and inoculated, did not produce any roots.

Four of ten kanamycin-resistant root clones were highly branched and grew rapidly in hormone-free liquid medium. In three months shoot regeneration from roots had occurred only in the case of two hairy root clones on solid half-strength WPM medium. The clone 4 regenerated with 0.5 mg l^{-1} BA and the clone 3 only with 5 mg l^{-1} BA. No shoots from the other two clones developed, although calli were formed on root explants (data not shown).

The transformants grew and proliferated poorly on the medium used for propagation of wild-type actinidia (Vardja, Vardja 1998). Therefore, MS media supplemented with different hormones were tested for propagation (Table 1). The final medium adopted for propagation was the standard MS with BA as the only hormone. The observation that shoot cultures of transformed plants require proliferation and rooting media different from those suitable for the control has been previously reported (Lambert 1992).

Abundant callus (to 1.5 cm diameter) formation was observed at the base of microcuttings (C3, C4) when cultured on MS medium with indolebutyric acid (0.1 to 1.0 mg l^{-1}). Numerous roots and also buds developed from this callus (data not shown).

Table 1. Proliferation, growth and root regeneration of transformed clone 4 of *A. kolomikta* (growth period 6 weeks) on Murashige and Skoog medium without growth regulators or supplemented with different growth regulators (BA, benzyladenine; GA, gibberellic acid; Z, zeatin, and ABA, abscisic acid) or their combinations. Means within columns with different letters are significantly different ($P < 0.05$) according to the Duncan's test. [1], new buds from callus; [2], most roots derived from callus; [3], most shoots were vitrified

Growth regulator (mg l^{-1})	Type of explant	Diameter of callus (cm)	Roots per microcutting	Axillary shoots per explant	Length of the longest shoot (cm)	Number of nodes of the longest shoot
Control	Shoot tip	0.3 - 0.5^{d}	20 - 30^{2}	1.4^{b}	3.6^{a}	3.1^{b}
	One-node	0.1 - 0.2^{e}	20 - 25^{2}	1.2^{c}	3.2^{b}	3.8^{a}
BA 0.5	Shoot-tip	0.3 - 0.5^{d}	20 - 30^{2}	1.5^{b}	3.5^{a}	3.3^{b}
	Two-node	0.3 - 0.5^{d}	20 - 30^{d}	1.6^{b}	3.5^{a}	3.0^{b}
BA 1.0	Two-node	0.1 - 0.2^{e}	4.1 ± 1.3	2.0^{a}	2.9^{b}	3.0^{b}
BA 1.0 + GA 1.0	Shoot tip	0.6 - 0.8^{1c}	3.0 ± 0.8	1.1^{d}	2.0^{c}	2.0
	One-node	0.1 - 0.2^{e}	10 - 15	1.0^{d}	3.2^{b}	3.0^{b}
	Two-node	0.1 - 0.2^{e}	5.0 ± 1.4	2.0^{a}	2.1^{c}	3.1^{b}
Z 1.0	Shoot-tip	0.1 - 0.2^{1e}	4.1^{2}	2.2^{a}	1.8^{c}	2.8^{b}
	One-node	0.3 - 0.51^{d}	0	1.0^{d}	1.6^{c}	2.8^{b}
	Two-node	0.3 - 0.5^{d}	1.5 ± 0.5	2.0^{a}	1.8^{c}	2.8^{b}
Z 1.0 + GA 1.0	Shoot tip	1.5 - 1.7^{a}	2.0 ± 1.2	1.1^{d}	1.5^{c}	2.0^{c}
	One-node	1.0 - 1.3^{b}	2.0 ± 1.3	1.1^{d}	1.5^{5c}	2.2^{c}
	Two-node	1.0 - 1.3^{b}	2.0 ± 1.1	1.1^{d}	1.6^{5c}	2.0^{c}
ABA 2.6	One-node	1.5 - 1.7^{a}	5.1 ± 1.5	1.0^{d}	1.1^{d}	2.0^{c}

Microcuttings from transgenic plants showed an increased ability to produse roots on auxin-free MS medium (Table 2). Leaf explants from transformants (C4 and C3) produced roots on hormone-free MS medium, a typical response of plants regenerated from hairy roots. No rooting was observed on untransformed *A. kolomikta* leaf explants under the same conditions. Transgenic plants of clone 4 exhibited enhanced growth in vitro and in greenhouse conditions as compared to clone 3 and control plants. An increased growth

Table 1. The growth and rooting of shoot explants of bisexual *A. kolomikta* subcultured on hormone-free MS medium (four weeks). Transformed clones (C3; C4) and wild type. Means within columns with different letters are significantly different ($P < 0.05$) according to the Duncan's test

Plant	Axillary shoots per explant	Length of shoots (cm)	Number of nodes	Roots per microcuttings	Length of leaves (cm)
Wild type	1.0^{b}	1.7^{b}	3.6^{b}	1.0	2.1^{a}
Clone 3	1.0^{b}	1.2^{c}	2.7^{c}	>30	1.7^{b}
Clone 4	1.4^{a}	3.2^{a}	4.3^{c}	>20	1.3^{c}

rate of transgenic plants was previously reported in work on aspen (Tzfira et al. 1999).

Transformants that had grown in the unheated greenhouse suffered from pests, and they tended to be sensitive to frost in winter.

The results from this study demonstrate that *A. rhizogenes*-mediated transformation is an effective method for producing bisexual *A. kolomikta* plants with altered growth and increased rooting capacity.

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