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MICROPROPAGATION OF BABY KIWI (ACTINIDIA ARGUTA) USING MATURE STEM SEGMENTS

Adrian PETICILA[1], Florin STANICA[1], Roxana MADJAR[2], Oana VENAT-DUMITRIU[1]

[1]University of Agronomical Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Horticulture Bioengineering Resources Department, 59 Marasti Blvd., 011464 Bucharest, Romania; apeticila@yahoo.com;
[2]University of Agronomical Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, Soil Sciences Department, 59 Marasti Blvd., 011464 Bucharest, Romania; rmadjar@yahoo.com;

Corresponding author email: apeticila@yahoo.com

Abstract

An effective in vitro culture system for mature stem segments of Actinidia arguta was established. The 1.2 cm nodal segments, cut from young shoots, was sterilized and established in vitro. The successfully induced culture was achieved on three types of growing media standing for three experimental variants were prepared according to the reference bibliography. The first culture medium variant is the classic MS with unchanged components, the second is the modified MS medium with a double quantity of ammonium nitrate (2N) and the third variant is the modified MS medium with a triple quantity of ammonium nitrate (3N) in the composition of macroelement salt solutions. These changes result from the study of reference literature where the ammonium nitrate was modified in order to boost the vegetative growth of explants. This experimental scheme was applied for the male and female genitor of Actinidia arguta. The shoots of micro cuttings were rooted in ½ MS supplemented with 0.5mg/l IBA, 12.5g/l sucrose. Regenerated shoots successfully acclimatized to vegetation house.

Key words*: Actinidia arguta, baby kiwi, micropropagation, European funds*

INTRODUCTION

Actinidia arguta is native to Northern China, Korea, Russian Siberia and possibly Japan. In forests from which it originates, it grows as a vine exceeding at times 50 cm height when climbing in tree crowns. It is most cultivated in European countries such as Italy, Greece, Portugal and Spain, in Asian countries – Turkey, South Korea and Japan - and in the U.S. as well, namely in California.

Actinidia family includes 55 species and about 76 taxa native to Central China and geographically-spread all over China and South-East Asia. Paleobiological research studies roughly date this genus back to some 20-26 million years ago.

As any other vine, the stem has no support tissue, is voluble and wraps around the supporting system of any other branches it may come in contact with. *A. arguta* looks more delicate than *A. deliciosa*: it has hairless oval or ovoid leaves 5 to 8 cm long, showing the specific leaf green glaze in its upper part, and red leafstalk with serrated border. Odor-feint flowers are about 3 cm in diameter, their color varies from white to cream and grow at leaf axils, no more than three in a bunch. The plant is dioceous and needs cultivated male plants to produce fruit. *Actinidia arguta* cv. Issai ist he unique cultivar known to have the perfect bloom. The fruit is a green smooth berry, 2 to 3 cm long and of oval, ovoid or oblong shape according to type. Its fruits are generally sweeter than those of *A. deliciosa* and their sugar content ranges between 14 and 29%. The *Actinidia arguta* species needs a longer vegetation time (about 150 frost-free days). While dormant, this species may face temperatures up to -30^{o}C. However, it needs gradual climate adaptation as any dramatic temperature fall may cause the splitting of the stem and further damages. It tolerates half-light, but it grows better on sunny fields. The support system is mandatory on commercial plantations.

A. arguta loves water and needs plenty of it throughout the vegetation period. However, it

shall be necessarily watered during summer. Dry cutback is needed if higher output is sought for. Cutback systems rely on the replacement of bearing canes. As far as diseases and vermin are concerned, the species poses no problem, possible due to the fact that it has not been intensively cultivated on large areas.

Although the genetic diversity of the genus *Actinidia* provides an outstanding potential for cultivar improvement, it shows features (including the genuine greenness of the vine, the juvenile period of up to 5 years, the dioceous nature and the poliploid netlike structure) that make this genus to be less flexible for particular reproduction targets over other agronomic cultures. Tissue culture, *in vitro* handling of cells, tissues and organs of the plant, is a major bio-technological technique from which may methods have been used to overcome some of the limitations shown by *Actinidia* in relation to traditional reproduction.

The first micropropagation protocol for *Actinidia* was suggested by Harada (1975) [1] and las afterwards improved. Standardi, 1983[2]; Wessels *et al,* 1984[3]; Monette, 1986[4]. The Murashige & Skoog (MS) method is the most used to prepare culture medium and is beneficial to regeneration and callusing. Other media like Gamborg B_5 and N_6 have been successful as well.

The stimulation of axillary buds is subject to a relatively high concentration of cytokinin which inhibits apical dominance and allows buds to grow (Pierik, 1987)[5]. A large number of even shoots may be obtained from a single explant in short time because this method is simpler than other micropropagation methods ad genetic stability is usually maintained. For *Actinidia* sp., this technique is the most viable propagation method for large-scale plant growth (Zuccherelli, 1994)[6] due to use of nodal segments (Velayandom et al., 1985)[7] and/or shoot ends (Monette, 1986a, b)[4]. However, much attention must be paid to prevent the propagation of shoots regenerated from basal callus because *Actinidia* sp. regenerates easily from undifferentiated cells and show high appetite for somatic variation.

The root induction methods vary; most of them include IBA as potent root-inducer: the immersion of the basal part of the shoot in solutions with high content of IBA (50mg/l) for a few seconds Standardi, 1981[8], 1983[2]; Wessels *et al.,* 1983 [3]. One alternative includes overnight immersion of shoot's basal part in solutions with low content of IBA (0.3, 1, 2 mg/l), which results in high rooting output.

OBJECTIVES

The research studies aim at defining the work protocol to obtain plants by micropropagation or *in vitro* growth from plants already adapted to existing conditions in the fruit garden of the Faculty of Horticulture within USAMV Bucharest.

MATERIAL AND METHOD

Micropropagation is the quickest way to obtain kiwi plants. In vitro proliferation studies aim to adapt abovementioned technologies to micropropagation conditions in the laboratory of the Faculty of Horticulture, USAMV Bucharest, where experiments are conducted under the research scheme below: din two plants, one female plant and one male plant that turned in time to be fruitful and well-adapted to climate conditions of our country, have been selected from the collection of *Actinidia arguta.*

The vegetal material required to set up the culture consists in shoots grown from the vegetative buds.

The vegetal material used consisted in plant R10P14 belonging to the species *Actinidia arguta* (male) and plant R10P2 of *Actinidia arguta* (female).

Three types of growing media standing for three experimental variants were prepared according to the reference bibliography. The first culture medium variant is the classic MS with unchanged components, the second is the modified MS medium with a double quantity of ammonium nitrate (2N) and the third variant is the modified MS medium with a triple quantity of ammonium nitrate (3N) in the composition of macroelement salt solutions. These changes result from the study of reference literature where the ammonium nitrate was modified in order to boost the vegetative growth of explants.

We therefore have for *Actinidia arguta* male plant the V 1- classic induction MS variant, V2- the MS 2N induction variant and V3 – the MS 3N induction variant. After 2-3 months of induction phase monitoring, the shift is made to V4- classic multiplication MS, V5- the multiplication MS 2N variant and V6- the multiplication MS3N variant for a period of 6 months of observation and measurements. These are followed by the V7 MS classic rooting variant, the V8 MS 2N rooting variant and the V9 MS 3N rooting variant for 2 months and afterwards by the acclimatization period of in vitro plants. This experimental scheme shall also be applied for *Actinidia arguta* female and male plants. The experiment started in January 2012 with the induced growth of section of plant shoots according to the research scheme above.

RESULTS AND DISCUSSIONS

Results obtained from the induction of *in vitro Actinidia arguta* cultures

	CUL-TURE MEDIUM	21.03	26.03		3.04		17.04		1.05	
		INITIAL NUMBER	No.	%	No.	%	No.	%	No.	%
R10P14	V1	15	15	100	12	80	9	60	9	60
	V2	15	14	93.3	13	86.6	13	86.6	13	86.6
	V3	15	10	66.6	9	60	7	46.6	7	46.6
R10P2	V1	12	10	83.3	8	66.6	8	66.6	8	66.6
	V2	12	10	83.3	8	66.6	8	66.6	8	66.6
	V3	12	10	83.3	10	83.3	4	33.6	4	33.6

The review of tabular data above shows that the best medium to initiate the *Actinidia arguta* culture for the male plant (86.6%) and for the female plant (66.6%) was the variant V2 (V2- MS 2N induction variant) and variants V1 (V 1- classic MS) and V2 (V2- MS 2N induction variant) respectively.

As far as the multiplication phase for the two baby kiwi varieties, the results are given in the table below with explanations for the impact of the growing medium on the multiplication rate, for average growth and differences recorded between male plants and female plants.

Table 1. Two-factor experimental scheme, a = variety, b = growing medium

Factor a = variety	Factor b = fertilization type
a1 = R10P14-Male a2 = R10P2-Female	b1 = MS+ b2= MS+2N b3= MS+3N

Table 2. Impact of variety (factor a) and of growing medium (factor b) over Actinidia arguta growth

a/b	b1 = MS+	b2= MS+2N	b3= MS+3N
a1 = R10P14-Male	a1.07a	b0.52b	b0.30b
a2 = R10P2-Female	a1.46b	a1.89a	a1.13b
B constant A variable: Dl 5%=0.51*cm; Dl 1%=1.01 cm; Dl 0.1%=2.73cm A constant B variable: Dl 5%=0.37* cm; Dl 1%=0.55 cm; Dl 0.1%=0.82 cm			

The analysis of the variant highlights major differences given by the growing medium for MS+2N and MS+3N, the highest values being recorded in centimeters for R10P2 female of 1.89 cm on the culture medium MS+2N. No major differences given by the growing medium have been found for the growing medium MS+.

The analysis of the variant shows major differences for the same R10P14 male variety between the growing medium MS+ and the other two media MS+2N used, namely MS+3N, with the highest values reached at MS+ of 1.07cm. As for the R10P2 female variety, the highest growth rate was noticed on the growing medium MS+2N of 1.89cm, with significant differences over MS+ and MS3+3N.

Conclusion: The best experimental variant for the growth of *Actinidia arguta* explants on various multiplication media is the variety R10P2-female on the MS+2N medium of 1.89 cm.

Table 3. Impact of variety (factor a) and of growing medium (factor b) over the multiplication rate of Actinidia arguta

a/b	b1 = MS+	b2= MS+2N	b3= MS+3N
a1 = R10P14-Male	a1.69a	a1.78a	a1.53a
a2 = R10P2-Female	a1.65a	a2.47a	a1.77a
B constant A variable: Dl 5%=1.11*; Dl 1%=2.17; Dl 0.1%=5.76 A constant B variable: Dl 5%=0.85*; Dl 1%=1.24; Dl 0.1%=1.86			

The variant analysis shows no major differences generated by the variety and the growing medium with impact on the multiplication rate of *Actinidia arguta.* The highest multiplication rate, i.e. of 2.74, was obtained for the variety R10P2- female on the growing medium MS+2N.

Table 4. Impact of the culture medium on the spring of roots and on their size

	CULTURE MEDIUM	30.04	3.08		3.08	
		INITIAL NUMBER EXPLANTS	No. of rooted explants	%	Average number of roots	Average length of roots in cm
R10 P14	V7	12	2	16.6	1.2	0.56
	V8	12	8	66.6	2.4	2.86
	V9	12	3	25	1.9	1.2
R10 P2	V7	12	12	100	3.2	2.92
	V8	12	4	33.3	2.1	1.4
	V9	12	6	50	1.9	2.5

The review of tab.4 data shows that the v8 culture medium for r10p14 influenced the growth of many rooted explants (66.6%) with the optimum average length, while the v7 culture medium for r 10p2 resulted in 100% rooted explants of 2.92cm average root **LENGTH.**

CONCLUSIONS

To initiate the culture of *Actinidia arguta,* the most successful medium for the male plant (86.6%) was V2 (V2- MS 2N induction variant) and the media in variants V1 (V 1- classic MS) and V2 (V2- MS 2N induction variant) for the female plant (66.6%).

The best experimental variant for growth of *Actinidia arguta* explants on different multiplication media is the variety R10P2- female on the medium MS+2N, with 1.89 cm.

The best rooting was achieved in medium MS2N+ (V8) for R10P14 and in
MS+ (V7) for R10P2.

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