

In vitro multiplication and cultivation of *Actinidia arguta* in the Republic of Moldova

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Actinidia arguta is a perennial fruit vine, not characteristic for natural flora of Moldova. This species is of interest to gardeners due to its high ornamental value, as well as exotic fruits (hardy kiwifruits). The research of this species has been carried out at the Experimental station of the Institute of Genetics and Plant Physiology of the Academy of Science of Moldova throughout in the period of eight years. Particular attention was paid to optimization of the methods of vegetative propagation and conditions of cultivation of a perspective and highly productive clone named Tatiana, previously selected from the generative propagated plants. The apical meristems of adult plants served as source for elaborating the method of *in vitro* micropropagation. As a result of testing different nutrients, the cultural medium for *in vitro* propagation (with the multiplication factor equal to seven) and organogenesis was elaborated. Plants adaptation to *ex vitro* conditions was assured by gradually increasing the light intensity, reducing the relative humidity from 95% to that of natural. In conditions of Central Moldova the crop yield from one 5–7-year plant of Tatiana clone was varying between 2 and 10 kg. The hardy kiwifruits were characterized by valuable organoleptic and biological properties. The antioxidant activity of kiwifruits juice determined by the potentiometric method and expressed in equivalent of vitamin C (VCEAC) was equal to $9.26 \pm 0.28 \mu\text{Mol/g}$ (per dry residue). Due to high content of phenols in hardy kiwifruits of Tatiana clone that remains stable during storage they can become a valuable source of nutrient substances.

Key words: *Actinidia arguta*, *in vitro* micropropagation, hardy kiwifruits, antioxidant activity, phenolics

INTRODUCTION

The relict plant of *Actinidia arguta* is a woody vine that in natural conditions grows on stony

soils in southern Siberia, Manchuria, northern China, Korea and Japan (Zuo et al., 2012). The climbing vines of *Actinidia arguta* can reach up to 25 m in length and 15–18 cm in thickness. This fact gives the possibilities to use these plants for ornamental decoration of gardens.

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The fast-growing, climbing vine is very hardy and capable of surviving gradual temperature drops up to $-34\text{ }^{\circ}\text{C}$, although young shoots can be vulnerable to frost in the spring. Late spring frost is very common in Moldova and other European countries (Marosz, 2009). The attention of many European scientists goes towards *Actinidia arguta* because of the possibilities to cultivate it as edible fruits (hardy kiwifruits). Searching for new plants that can be grown commercially and that are valuable for human health is an important task for horticultural science. Actually, in the international market *Actinidia* fruit sales are dominated by a single kiwifruit cultivar *Actinidia deliciosa* "Hayward". There are a considerable number of cultivars and selections in the genus that have a widely diverse shape, size, and hairiness (Nishiyama et al., 2004). In fruits of *Actinidia arguta* and their interspecies hybrids, the contents of chlorophyll, lutein, and beta-carotene were much higher than in "Hayward". In particular, these fruits were found to be the richest dietary source of lutein among commonly consumed fruits (Nishiyama et al., 2005). Recent studies have found the medicinal efficacy such as antioxidant activity of kiwifruit extracts (Zuo et al., 2012; Motohashi et al., 2001; Scalzo et al., 2005; Ivanova et al., 2010; Latocha et al., 2010; Hunter et al., 2011). In the last decade many authors have reported their results of successful introduction and adaptation of *Actinidia* genus plants in Ukraine (Skriptchenko, 2001), Poland (Marosz, 2009; Bieniek, 2012) and Lithuania (Česonienė et al., 2010). In Moldova there are no orchards of *Actinidia*, but during last eight years at the Experimental station of the Institute of Genetics & Plant Physiology of the Academy of Science of Moldova (IGPP, ASM) *Actinidia arguta* is propagated and investigated. The first goal of this research was to elaborate the methods of multiplication *in vitro*, followed by plant adaptation to *ex vitro* conditions. The second goal was to evaluate the total phenolic content and antioxidant activity of *Actinidia arguta* fruits, harvested from our experimental station, during storage.

MATERIALS AND METHODS

Plant material

Primarily, *Actinidia arguta* plants were obtained from seeds. Genotypes characterized by rapid growth and strong root systems were evaluated and finally a vigorous and highly productive Tatiana clone was selected (Fig. 1). In environmental conditions of IGPP experimental station (lat. $47^{\circ}01'$, long. $28^{\circ}52'$, alt. 173 m above sea level) *Actinidia arguta* buds appear in May and remain closely associated with sepals until early June. It is noteworthy that during this period late spring frosts are excluded and there is no danger for plants to be damaged by frost. The flowers, mostly hidden in foliage, persist for several weeks. Flowers of male exemplars are assembled in inflorescence with numerous flowers (Fig. 2a), but female plants are separated or aggregated in clusters with 2–3 flowers (Fig. 2b). The leaves are long (up to 15 cm), oval with rounded apices. Their dark green colour keeps almost unchanged throughout the growing season, only in fall the leaves turn yellow (Fig. 3). The apical shoots from adult plants were used for elaborating the method of *in vitro* micropropagation. They were sterilized by immersing during 15 min



Fig. 1. Tatiana clone of *Actinidia arguta*

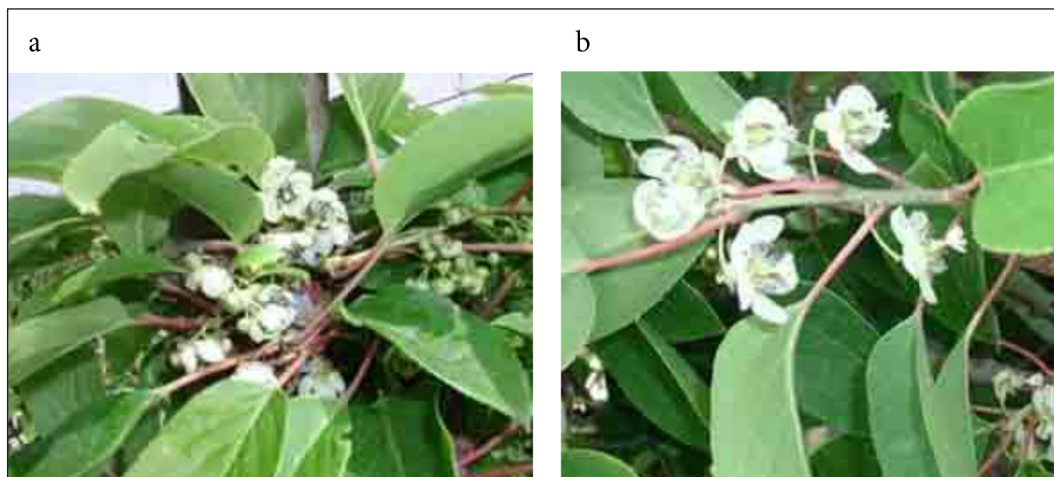


Fig. 2. Male (a) and female (b) plants of *Actinidia arguta* (Tatiana clone)

in solution containing chlorine (20% ACE), followed by washing with sterile water.

Plant multiplication and cultivation

The medium based on classical mineral salt composition (Murashige et al., 1962) was used for shoots cultivation *in vitro*. Explants were grown at temperature 26 °C under light (1 000 lux) with photoperiod about 16 hrs at light and 8 hrs in darkness. Plants adaptation to *ex vitro* conditions was assured by increasing gradually the light intensity from 1 000 lux up to 7 000–9 000 lux and reducing the

relative humidity from 95% to natural. After adaptation to *ex vitro* conditions, during the first year, plants were grown in the nursery and then transferred to a constant location, maintaining the relationship between feminine and masculine plants 5:1.

Characteristics of the Tatiana clone fruits

Ovate or oblong hardy kiwifruits (Fig. 4) were collected from mature plants of Tatiana clone (5–6 years old). The crop yield from one 5–7-year plant of Tatiana clone was varying between 2 and 10 kg. Fruits were 2–3 cm in length

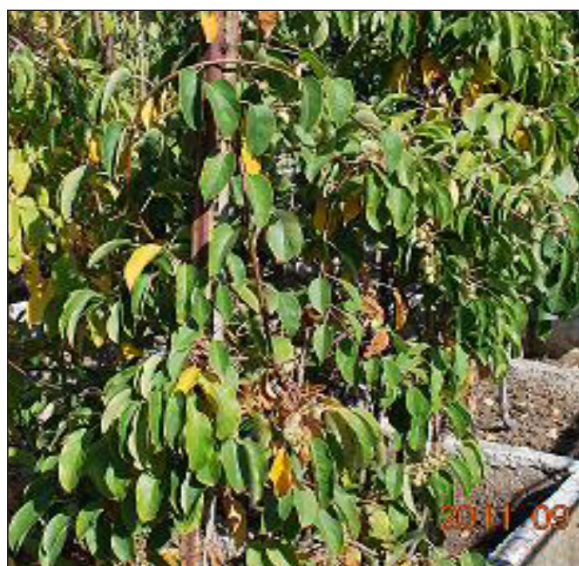


Fig. 3. Fall image of *Actinidia arguta* (Tatiana clone)



Fig. 4. Hardy kiwifruits of Tatiana clone

and weighed 4.5 g, depending on conditions and load of plants with fruits. The fruits were considered ripe when they became brown-violet and the content of soluble sugars reached 6–7%. Collection of fruits at the brown-green stage is also possible, due to their capacity of after-ripening during storage at temperatures above 4 °C. In conditions of our experimental station the fruits of Tatiana clone ripen in September and October. For determining their biochemical characteristics, the ripened fruits were frozen and until analysis were stored at –18 C. Ordinarily, the antioxidant activity and phenolic content was provided in kiwifruits juice obtained by direct press operation using Hydraulic Plant Sap Press (Spectrum Technologies Inc., USA).

Determination of total phenolic content

The total phenolic content of juice was assessed by using the Folin-Ciocalteu phenol reagent method (Singleton et al., 1999). To 0.1 ml of the cell sap, previously diluted 1:2.5 (vol/vol) with distilled water, 2.3 ml of distilled water and 0.2 ml Folin-Ciocalteu reagent were added. The mixture was kept at room temperature for 3–5 min and after that 1.4 ml of sodium carbonate (29% wt/vol) was added, vigorously mixed, and before measuring the absorbance on a spectrophotometer UV/VIS (Agilent 8453) at 760 nm were incubated for 40 min at 18–20 °C. A mixture of water and reagents was used as a blank. The total phenolic content was expressed as gallic acid equivalents (GAE) in g/l of juice. Each sample was measured in triplicate.

Evaluation of antioxidant activity

Antioxidant activity was evaluated by measuring peroxy radical scavenging activity (RSA). The assay is based on the degree of inhibition of potassium iodide oxidation by antioxidants that scavenge peroxy radicals generated from thermal degradation of 2,2'-azobis(2-amidinopropane)-dihydrochloride (Sano et al., 2003; Ivanova, 2007). The amount of iodine, released during the oxidation, was determined by potentiomet-

ric titration using the automatic titrator "Titroline easy" (Germany) with minimum titration amount of sodium thiosulfate 0.1ml. RSA of tested juice was expressed as the inhibition ratio for iodine release in comparison with control sample (without juice). The reproducibility of RSA assay is good. The coefficients of variance are less than 4.78% and mean relative errors are within $\pm 7.01\%$. The linear relationship between RSA and concentration of standard antioxidant (gallic, elagic, chlorogenic, ascorbic acids and rutin) had high Pearson's correlation coefficients ($r^2 = 0.848-0.997$) (Ivanova et al., 2004). Antioxidant potential of kiwifruits juice was compared with equivalent activity of ascorbic acid pure substance and calculated in the vitamin C equivalent antioxidant capacity (VCEAC).

RESULTS AND DISCUSSION

Multiplication *in vitro*

For *in vitro* propagation of clone Tatiana plants different cultivation mediums were tested. The best results were obtained with modified Murashige-Stoog methods. The comparative results are included in Table.

As a result, the optimal cultural medium and conditions of cultivation, allowing the propagation of plants with the multiplication factor equal to seven, were determined (Fig. 5).

Antioxidant activity

During the biochemical analysis we evaluated that 100 g of fresh kiwifruits contain 85.50 ± 0.45 mg of ascorbic acid; 127.64 ± 6.09 mg of total phenolics, including 7.30 ± 0.36 mg of flavonols and 1.80 ± 0.09 mg of oxcinnamic acids. Antioxidant activity of kiwifruits was compared with the same index of edible fruits and berries growing in Moldova (Fig. 6). VCEAC of kiwifruits was equal to 9.26 ± 0.28 $\mu\text{Mol/g}$ (per dry residue) that was higher than antioxidant capacities of apricot and Moldova grape, but significantly lower than those of strawberries and red currant. Our results are supported by the data of other

Table. The results of Tatiana clone plants propagation *in vitro* obtained by utilizing the classical Murashige-Skoog medium and its two modifications

Index	Method		
	I	II	III
Reagent content in cultivation medium	Classical Murashige-Skoog (Murashige et al., 1962)	Classical Murashige-Skoog + 6-benzil-aminopurin (Vysotskii et al., 1991)	½ from classical Murashige-Skoog + activated carbon (Calugaru-Spataru et al., 2013)
pH medium after sterilization	5.8	5.8	5.8
Growing dynamics, days			
Growing start	16–19	12–15	7–10
1.5–2.0 cm	38–42	28–30	15–20
3–5 cm	48–54	38–42	28–30
Full cycle, weeks	18–19	14–16	10–12
Cycles for the year	3	3.5	4.5
Coefficient of propagation	5.2	6.4	7.2

researchers utilizing other procedures of antioxidant activity determination (Scalzo et al., 2005).

During the storage of frozen fruits a decrease of the content of phenolic compounds and antioxidant activity of fruit juice was observed (Fig. 7). The major drop of phenolic compounds content occurred in the first six weeks of storage (23%). Later their content decreased very slowly during one year of storage (from 23% to 29%). This implies that some individual components degrade more quickly than others. On the contrary, during the first months of storage the index of antioxidant activity of fruit juice remained practically unchanged, while

starting from 4–5 months of storage its value decreased very rapidly. During one year storage the values of this index were decreased by approximately 40%. Given the fact that in this period the reduction of phenolic compounds in juice of hardy kiwifruits was insignificant, it can be assumed that the reduction of radical scavenging activity was mainly because of ascorbic acid oxidation. It is known that the content of ascorbic acid in fruits decreases during long-term storage. Also, there is some information that the carotenoid and phenolic components in *Actinidia* fruits are more resistant to light, low temperature and storage time than ascorbic acid (Gil et al., 2006). Our

**Fig. 5.** Dynamics of *Actinidia arguta* *in vitro* growing

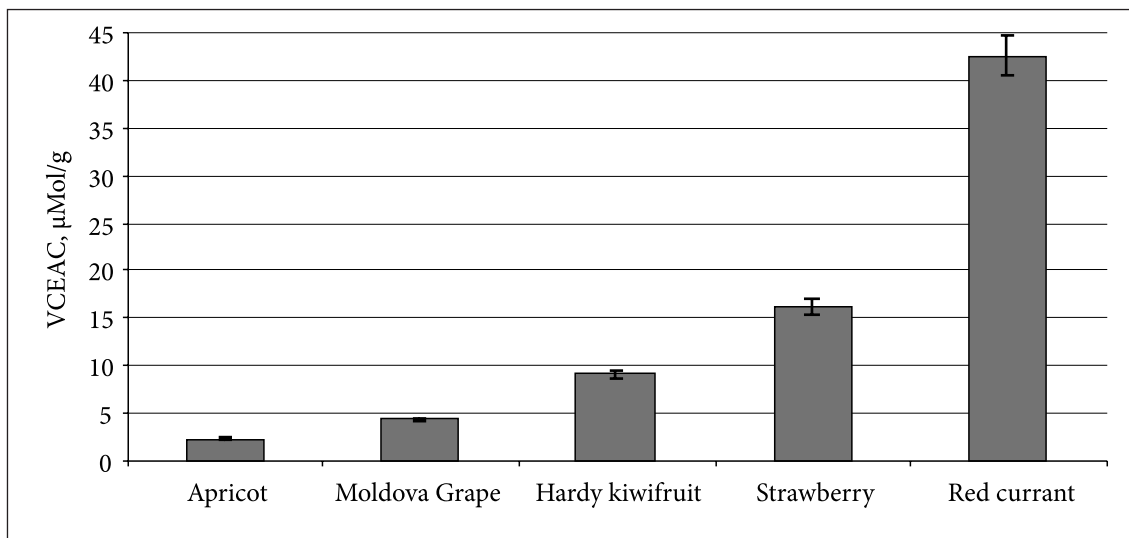


Fig. 6. Antioxidant activity (VCEAC) of edible fruits and berries growing in Moldova

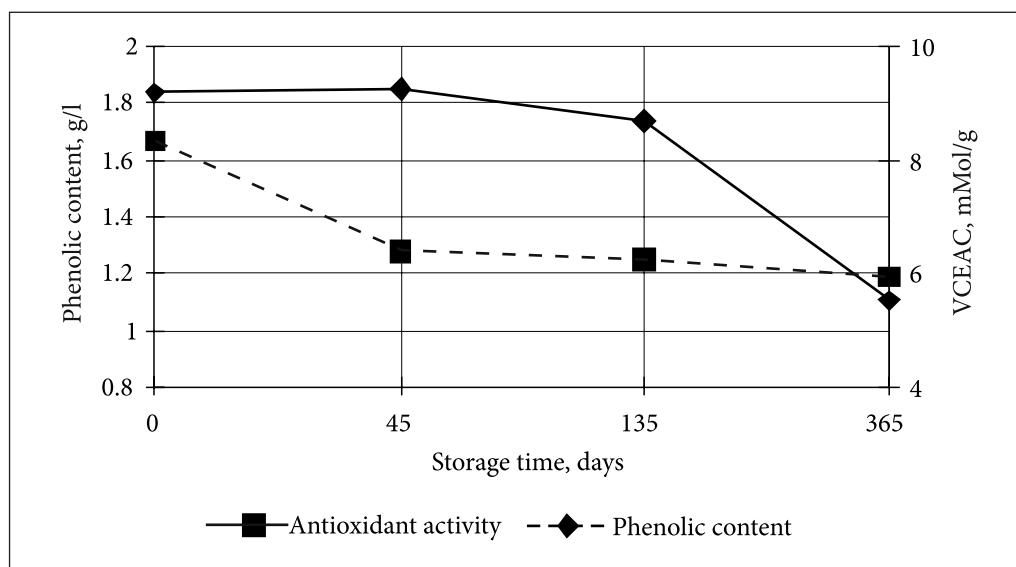


Fig. 7. Changes in phenolic content and antioxidant activity (VCEAC) of hardy kiwifruits juice during storage

data show that approximately 71% of phenolic compounds are stable in frozen fruits during one year of their storage.

CONCLUSIONS

1. Tatiana is a valuable genotype clone of *Actinidia arguta* which can be recommended for cultivation in Moldova ensuring artificial watering during drought.

2. The developed methods of *in vitro* micropropagation and cultivation support the idea about the possibility of achieving highly productive plantations of *Actinidia arguta* in a short time.

3. The hardy kiwifruits of Tatiana clone contain biochemical substances with the significant antioxidant potential and nutritional value which are preserved during one year storage of frozen fruits.

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- ex vitro* sąlygomis buvo patikrinta palaipsniui didinant šviesos intensyvumą ir mažinant santykinį oro drėgnumą nuo 95 % link natūralaus. Vidurio Moldovos sąlygomis vieno 'Tatiana' klonu derlius 5–7 metų laikotarpiu svyravo tarp 2 ir 10 kilogramų. Buvo įvertintos vaisių biologinės ir juslinės savybės. Vaisių sulčių antioksidacinis aktyvumas buvo nustatytas potenciometriniais metodais; vitamino C kiekis buvo $9,26 \pm 0,28 \mu\text{Mol/g}$ (sausosiose dalyje). Dėl didelio fenolinių junginių kiekio 'Tatiana' klonų vaisiai išlieka stabilūs visą laikymo laikotarpį ir gali tapti vertingu maistingos medžiagos šaltiniu.

Raktažodžiai: *Actinidia arguta*, mikrodauginimas *in vitro*, antioksidacinis aktyvumas, fenoliai

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ACTINIDIA ARGUTA DAUGINIMAS IR AUGINIMAS IN VITRO MOLDOVOS RESPUBLIKOJE

Santrauka

Actinidia arguta yra vaisius vedantis vynmedis, nebūdingas natūraliai Moldovos augmenijai. Dėl aukštos dekoratyvinės vertės ir egzotinių vaisių ši rūšis labai domina sodininkus. Moldovos mokslų akademijos Genetikos ir augalų fiziologijos instituto Bandomajame sektoriuje ši rūšis tyrinėta aštuonerius metus. Ypatingas dėmesys buvo skirtas aukšto produktyvumo klonu 'Tatiana', pasirinkto iš anksčiau atliktų vegetatyvinių dauginimų, auginimo sąlygoms ir vegetatyvinių dauginimo metodams tobulinti. Šių suaugusių augalų viršūninės meristemos buvo naudojamos kaip tyrimų *in vitro* medžiaga. Augalo dauginimas *in vitro* (kai dauginimo veiksnys lygus 7) ir organogenezė buvo detalizuota išbandant skirtingas maistines medžiagas. Augalų adaptacija