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# IN VITRO VEGETATIVE PROPAGATION OF ACTINIDIA CHINENSIS PLANCH. FROM JUVENILE AND ADULT PLANT SEGMENTS

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The fruit tree Chinese gooseberry (*Actinidia chinensis* P l a n c h.) can be vegetatively propagated *in vitro*, when either the juvenile or the mature tissues were used as initial explants. When stem tips (1–2 mm) were excised and grown in culture, bud multiplication clearly occurred by lateral branching. *De novo* bud formation was induced in callus tissue, developed on hypocotyl, cotyledon, or root segments of young seedlings. Those buds also gave rise to vegetative clones, further propagated by lateral branching. Benzylaminopurine (1 mg $l^{-1}$) was essential for bud multiplication Adventitious roots were readily induced at a wide range of indolyl–3–butyric acid concentrations. Rooted plantlets supported well the transfer to non–sterile conditions. When planted outdoors, seedlings continued growing and in the second year developed about 2 m high vines. It is concluded that the procedure described in this paper is suitable for large–scale *A. chinensis* propagation.

Key words: *Actinidia chinensis* Planch., Chinese gooseberry, vegetative propagation *in vitro*, propagation of male and female plants.

Ključne reči: *Actinidia chinensis* Planch., kineska ribizla, vegetativno razmnožavanje *in vitro*, razmnožavanje muških i ženskih biljaka.

## INTRODUCTION

The Chinese gooseberry *(Actinidia chinensis* P l a n c h.) is a fruit crop, recently introduced in Macedonia and cultured in several agricultural centers. It is well–known

that certain difficulties exist in the propagation of this species by conventional means. *A. chinensis* is a dioecious plant; in order to ensure good pollination, the ratio of male to female plants should be approximately 1:7. Plants raised from seeds flower after 7 years and are not, therefore, suitable for building up a plantation. Propagation by cuttings, or grafting is a rather slow process, considerably hampering the rapid large–scale production of uniform plants. This situation already prompted several authors to attempt the application of *in vitro* techniques to *A. chinensis* propagation. Tissue cultures, derived from stem segments, flower buds and immature fruits were obtained (Hirsch *et al.*, 1977) and their organogenic properties studied (Hirsch and Bligny–Fortune, 1979). Vegetative propagation was attempted from staminal filaments, which produced an organogenic callus tissue (Tripathi and Saussay, 1980). Harada (1975) succeeded in inducing direct bud formation in stem and root segments, as well as callus tissue and globular embryos; the explants had a long lasting potency for bud formation and complete plant regeneration. Gui (1979) demonstrated for two *A. chinensis* varieties that callus produced in stem segments of male and female plants had the capacity of producing buds and plantlets, the frequency of regeneration being higher in female than in male plants. Standardi (1981, 1983) elaborated methods for micropropagation starting from resting apical buds, and demonstrated a high genetic stability of *A. chinensis* buds in culture (Standardi, 1982).

Vegetative propagation in culture can be started from lateral branching of apical buds, or from buds regenerated in callus tissues by appropriate hormone treatment. The former method has certain advantage, since the risque of inducing variability in regenerated plants is lower. The main purpose of the present work was to study the capacity of stem tips and other explants for plant regeneration in culture. The results might be useful for the production of large number of plants in shorter time, than by conventional methods.

## MATERIAL AND METHODS

Initial explants for cultures were taken: a) from various parts of germinating seedlings, and b) from apical and axillary buds of adult plants.

*A. chinensis* seeds were soaked for 24 h in water containing 2500 mg $l^{-1}$ gibberellic acid (Lawes and Anderson, 1980), then sterilized for 30 min in 5% sodium hypochlorite solution, containing 0.1% benomyl and 0.1% captan, and washed 3 times with sterile water. The sterile seeds were germinated on 1/4 strength of mineral MS (Murashige and Skoog, 1962) medium, in light, at 25 °C. After 20 days, apical buds 1–2 mm long, and parts of hypocotyls, cotyledons, petioles and roots were excised and put in culture.

Adult annual shoots from field grown male and female plants were harvested, stored at 4 °C for a short time, then washed and sterilized with 70% alcohol + Tween 20 for 15 s, followed by 5% calcium hypochlorite + 0.1% benomyl + 0.1% captan for 1 h. After three rinsings with sterile water, bud apices were isolated and put onto the nutrient media.

The basal culture medium contained mineral solution MS, 0.7% agar, 3% sucrose and (in mg $l^{-1}$): thiamin 0.4, pyridoxin 1.0, nicotinic acid 0.5, m–inositol 100. Indolyl–3–butyric acid (IBA), gibberellic acid ($GA_3$) and benzylaminopurine (BAP) were added in different concentrations, as indicated in the text.

The cultures weere maintained in white fluorescent light („Tesla", 4500 °K). Light intensity was 2000–3000 lx, day length 16 h and temperature 25 ± 1 °C.

## RESULTS

### Initiation of cultures

All initial explants were cultured on the basal medium, supplemented with 1 mg $l^{-1}$ BAP, 0.1 mg $l^{-1}$ $GA_3$ and 0.1 mg $l^{-1}$ IBA.

Cultures of juvenile tissue were obtained from seedling parts, 20 days after seed germination. In several experiments, more than 300 apical buds were isolated. The survival was good, since about 85% of explants was able to grow in culture. A small callus developed within 15 days in about 80% of viable explants, while the stems started elongating after 30 days. Lateral branching began within 8 weeks, the number of laterals usually being 3 per explant (Tab. 1).

From the same seedlings, segments of hypocotyls, cotyledons, petioles and roots were cultured. Most segments produced a callus tissue in about 4 weeks. After 8 weeks buds started regenerating, a single bud usually developing in the middle of the callus, or at the cut surface of the explant. Root–derived tissue had the lowest capacity for bud formation, as compared to the other explants (Tab. 1).

*Tab. 1. – Initiation of cultures from seedling explants; results after 8 weeks.*

| Origin of cultures | No. | % explants with callus | buds | none | No. of buds per explant |
|---|---|---|---|---|---|
| Apical buds | 89 | 11.3 | 88.7 | 0 | 3 |
| Hypocotyls | 83 | 56.5 | 40.4 | 0 | 1 |
| Cotyledons | 13 | 46.1 | 38.4 | 15.5 | 1 |
| Petioles | 25 | 48.0 | 40.0 | 12.0 | 2 |
| Roots | 85 | 87.0 | 3.5 | 9.5 | 1 |

Several hundreds of stem apices were isolated from apical and axillary buds taken from the branches of male and female plants. Their development in culture was rather slow. During the first 30 days, about 70% of explants developed a voluminous callus. The explants were transferred to a fresh medium and, following the next 30 days, in about 40% of explants a single bud developed. Only in the third subculture the buds started branching and after 90 days each explant consisted of the main stem and 2 axillary buds, emerging from the basal callus tissue. It was visible in most explants that the buds originated from the stem nodes and not from the callus tissue.

### Multiplication and elongation of shoots

Once the bud cultures of different origin were established, their further growth in culture was similar. The composition of the medium for primary explants was chosen according to the results with some other species, but it proved suitable for both the initiation and multiplication in *A. chinensis* cultures. The presence of BAP was essential for bud multiplication. Optimal dose was 1 mg $l^{-1}$. In 0.1 mg $l^{-1}$ bud production was significantly lower, while a ten times higher dose (10.0 mg $l^{-1}$) did not increase the number of buds, but decreased their length. $GA_3$ and IBA were not essential for

multiplication, but their presence improved the bud growth and, consequently, they were retained (Tab. 2).

*Tab. 2. – Shoot multiplication in cultures derived from seedling apical buds in different media.*

| Hormones, mg $l^{-1}$ | No. of cultures | No. of buds per culture | Stem length, cm | Callus |
|---|---|---|---|---|
| BAP 1.0<br>IBA 0.1<br>$GA_3$ 0.1 | 51 | 2.25 | 3.4 | + |
| BAP 1.0 | 41 | 1.9 | 2.3 | + |
| BAP 1.0<br>$GA_3$ 0.1 | 52 | 2.4 | 2.8 | + |
| BAP 1.0<br>IBA 0.1 | 50 | 2.3 | 2.3 | ++ |
| IBA 0.1<br>$GA_3$ 0.1 | 43 | 0 | – | – |
| $GA_3$ 0.1 | 16 | 0 | – | – |

In cultures derived from cotyledons, hypocotyls, petioles and roots, a constant rate of multiplication was attained after 6–8 subcultures. The average multiplication rate in 4 weeks was 1:4. New buds usually developed from the axillaries at the stem base. Shoots of about 1 cm were the best material for transplantation and the number of buds was still increased by laying them horizontally.

*Tab. 3. – Shoot multiplication in cultures derived from male and female stem tips in different media.*

| Hormones, mg $l^{-1}$ | MALE PLANTS | | | FEMALE PLANTS | | |
|---|---|---|---|---|---|---|
| | No. of cultures | No. of buds | Stem length, cm | No. of cultures | No. of buds | Stem length, cm |
| BAP 1.0<br>$GA_3$ 0.1<br>IBA 0.1 | 18 | 3.0 ± 0.2 | 1.9 | 19 | 2.2 ± 0.5 | 1.9 |
| BAP 1.0 | 18 | 3.2± 0.5 | 2.0 | 16 | 1.4± 0.5 | 1.8 |
| BAP 1.0<br>$GA_3$ 0.1 | 21 | 2.3± 0.3 | 2.2 | 16 | 1.6± 0.2 | 1.8 |
| BAP 1.0<br>IBA 0.1 | 18 | 3.0± 0.2 | 2.2 | 16 | 1.9± 0.3 | 1.6 |
| $GA_3$ 0.1<br>IBA 0.1 | 18 | 2.0± 0.1 | 1.8 | 16 | 1.2± 0.1 | 1.3 |
| $GA_3$ 0.1 | 7 | 2.0± 0.3 | 2.0 | 16 | 1.5± 0.3 | 1.5 |

Cultures derived from male and female plants differ slightly in their multiplication rate, male plants producing more buds in all media tested (Tab. 3).

As can be seen from the Tables 2 and 3, buds which arise in the multiplication medium reach a length of 2–3 cm in a period of 4–6 weeks. They usually have thick and firm stems and well developed, dark green leaves. Therefore, it was not necessary to change the hormone content in the medium, in order to induce bud elongation.

## Induction of adventitious roots

Rooting was induced in stems 1–2 cm long, by planting them on agar with IBA for 18 h and then on a medium with 1/2 strength of mineral solution, without hormones (Tab. 4).

*Tab. 4. – IBA effect on rooting of shoots derived from juvenile apical buds; IBA applied for 18 h; rooting observed after 30 days.*

| IBA, mg $l^{-1}$ | No. of shoots | % rooted | No. of roots per shoot | Length of longest root, cm | Callus |
|---|---|---|---|---|---|
| 0.0 | 37 | 27 | 2– 3 | 2 | – |
| 0.5 | 19 | 63 | 2– 3 | 2 | – |
| 1.0 | 18 | 88 | 4– 6 | 3 | – |
| 8.0 | 16 | 100 | 6–12 | 4 | + |
| 10.0 | 29 | 100 | 15–20 | 4 | + |
| 20.0 | 22 | 100 | 18–20 | 4 | + |
| 30.0 | 22 | 100 | 20–22 | 4 | + |
| 40.0 | 28 | 100 | 15–17 | 3 | ++ |
| 50.0 | 47 | 100 | 13–15 | 3 | ++ |

Considering all the parameters in Table 4, it is clear that 10.0 mg $l^{-1}$ IBA can be taken as quite satisfactory for rooting. It should be noted that rooting can also be induced by keeping the shoots for 4 weeks in a lower IBA concentration.

Cultures of male and female plants differ in their response to IBA. In male plants, 100% rooting was attained at a lower IBA concentration, and the number of roots per stem was higher (Tab. 5).

*Tab. 5. – IBA effect on rooting of male and female shoots; IBA applied for 18 h; roots observed after 30 days.*

| | Male shoots | | | Female shoots | | |
|---|---|---|---|---|---|---|
| IBA, mg $l^{-1}$ | No. of shoots | % rooted | No. of roots per shoot | No. of shoots | % rooted | No. of roots per shoot |
| 0.0 | 11 | 45 | 2 | 12 | 33 | 2 |
| 10.0 | 12 | 100 | 13 | 12 | 91 | 3 |
| 20.0 | 11 | 100 | 23 | 12 | 100 | 5 |
| 30.0 | 11 | 100 | 11 | 12 | 100 | 4 |
| 40.0 | 11 | 90 | 10 | 12 | 100 | 3 |
| 50.0 | 14 | 85 | 5 | 12 | 100 | 4 |

### Acclimatization of plants

The rooted plantlets were left in agar until the roots attained 2–4 cm in length. They were then transferred into plastic pots, in sterile peat, sand and perlite mixture (1:1:1), moistened with 1/2 strength MS solution. They were covered with glass beakers and kept for 30 days in an air–conditionned room in weak light, at 25 °C and relative air humidity of 80–90%. About 90% of plants were able to survive the transfer. For the next 30 days the beakers were removed and the plants partly covered with transparent plastic. Under these conditions most plants started growing vigorously, so that they could be transferred into soil and grown outdoors. In 4–5 months they reached the height of 50 cm, and in 2 years they were over 2 m high.

## DISCUSSION AND CONCLUSIONS

The attempts to elaborate methods for vegetative propagation of *A. chinensis in vitro* proved successful. It has been demonstrated that vegetative clones can be established from juvenile tissues and from adult stem apices. When stem tips were used as primary explants, multiplication clearly occurred by lateral branching. In hypocotyl, cotyledon, petiole and root segments, first buds that appeared must have been regenerated *de novo*, but their later multiplication occurred most frequently by lateral branching again. Therefore, the chance of inducing malformations and genetic changes by culture conditions were minimal.

A relatively simple nutrient medium was suitable for initiation of cultures, bud multiplication and their elongation. The only change of the medium was necessary for the induction of rooting. Acclimatization of plants presented no great problems, provided the plants were protected in humid atmosphere during the first few weeks. Therefore, the entire procedure permitted the production of a large number of plants in a relatively short time and can be recommended for the large scale vegetative propagation of *A. chinensis*. The *in vitro* propagation technique, starting from adult plants of known sex, could enable the growers to produce adequate number of male and female plants and to use the available field more economically.

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## Rezime

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### VEGETATIVNO RAZMNOŽAVANJE ACTINIDIA CHINENSIS PLANCH. U KULTURI IN VITRO POČEV OD ISEČAKA JUVENILNIH I ADULTNIH BILJAKA

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Kineska ribizla *(Actinidia chinensis* Planch.) može da se vegetativno razmnožava u kulturi *in vitro,* kada se kao početni materijal koriste delovi bilo juvenilnih, bilo adultnih biljaka. Kada su vršni delovi stabla (1–2 mm) otsecani i gajeni u kulturi, razmnožavanje pupoljaka se očevidno dešava putem bočnog grananja. U kalusnom tkivu, koje se razvija na otsečcima hipokotila, kotiledona, lisnih drški, ili korena, pupoljci se formiraju regeneracijom *de novo.* Ovi pupoljci kasnije takođe proizvode vegetativne klonove putem bočnog grananja. Benzilaminopurin (1 mg $l^{-1}$) je neophodan za razmnožavanje pupoljaka. Adventivni korenovi se bez teškoća obrazuju pomoću indolil–3–buterne kiseline u širokom opsegu koncentracija. Ukorenjene biljke dobro podnose prenos u nesterilnu sredinu. Kada se presade u polje, biljke nastavljaju da rastu i u drugoj godini dostignu visinu od oko 2 m. Zaključeno je da je postupak opisan u ovom radu pogodan za masovnu proizvodnju sadnica *A. chinensis.*