Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

10.2478/v10067-012-0030-z

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXVIII, 2 SECTIO C 2013

EWA DZIADCZYK*[1], MARCIN DOMACIUK[1], PIOTR DZIADCZYK[2], IWONA PAWELEC[1], EWA SZCZUKA[1], JÓZEF BEDNARA[1]

[1]Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University
Akademicka 19, 20-033 Lublin, Poland
[2]Rzeszów University of Technology, Department of Biochemistry and Biotechnology
Aleja Powstańców Warszawy 6, 35-959 Rzeszów, Poland

Optimization of *in vitro* culture conditions influencing the initiation of raspberry (*Rubus idaeus* L. cv. Nawojka) cell suspension culture

Optymalizacja warunków kultury *in vitro* umożliwiających uzyskanie zawiesiny komórkowej maliny (*Rubus idaeus* L. cv Nawojka)

ABSTRACT

The purpose of our investigation was to determine appropriate conditions for induction of raspberry (*Rubus idaeus* cv. Nawojka) cell suspension culture. The established callus culture obtained from leaf explants was used as an inoculum for cell culture initiation. Five combinations of plant growth regulators: 1) 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP; 2) 0.25 mg l^{-1} 2,4-D; 3) 0.5 mg l^{-1} 2,4-D; 4) 2.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP; 5) 4.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP, added into modified Murashige and Skoog (1962) medium, were tested in order to get the callus culture suitable for initiation of a cell suspension. The best callus (vigorously growing, healthy and friable) was obtained on the medium supplemented with 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP. To find the appropriate culture conditions for dispersing callus tissue in liquid medium into single cells and small aggregates, four combinations of plant hormones (auxins and cytokinins) were tested. The best culture medium for induction of raspberry cv. Nawojka cell suspension appeared to be the one supplemented with 1.0 mg l^{-1} 2,4-D. Also the medium with 8.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP was similarly efficient.

Keywords: cell suspension culture, callus culture, raspberry, *Rubus idaeus*, plant growth regulators

* Corresponding author – Ewa Dziadczyk, e-mail: ewa.dziadczyk@poczta.umcs .lublin.pl

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

STRESZCZENIE

Celem prezentowanych badań była optymalizacja warunków kultury *in vitro* umożliwiających uzyskanie zawiesiny komórkowej maliny (*Rubus idaeus* L.). Jako inokulum do zainicjowania kultury zawiesinowej zastosowano ustabilizowaną kulturę kalusa uzyskaną z eksplantatów liściowych na zmodyfikowanej pożywce wg Murashige i Skooga (1962). W pierwszym etapie badań testowano 5 kombinacji regulatorów wzrostu (auksyn i cytokinin) dodawanych do pożywki stymulującej powstawanie tkanki kalusowej, w celu uzyskania kultury kalusa odpowiedniej do indukcji zawiesiny komórkowej. Najlepszą tkankę kalusową (szybko mnożącą się, o luźnej strukturze) uzyskano w kombinacji uwzględniającej uzupełnienie pożywki do kultury *in vitro* auksyną IAA w stężeniu 4,0 mg l^{-1} oraz cytokininą BAP w stężeniu 1,0 mg l^{-1} . W drugim etapie badań testowano warunki kultury *in vitro* umożliwiające odpowiednią dyspersję tkanki kalusowej w płynnej pożywce, skutkującą uzyskaniem populacji pojedynczych komórek oraz małych agregatów komórkowych w hodowli. W tym celu testowano 4 warianty składu pożywki, różniące się rodzajem i stężeniem zastosowanych hormonów roślinnych należących do klasy auksyn i cytokinin. Najlepszy wynik uzyskano w płynnej pożywce uzupełnionej syntetyczną auksyną 2,4-D w stężeniu 1,0 mg l^{-1}, także pożywka zawierająca auksynę IAA w stężeniu 8,0 mg l^{-1} oraz cytokininę BAP w stężeniu 1,0 mg l^{-1} dała dobry wynik.

Słowa kluczowe: kultura zawiesiny komórkowej, kultura kalusa, *Rubus idaeus*, regulatory wzrostu roślin

Abbreviations: IAA – 3-indoleacetic acid; 2,4-D – 2,4-dichlorophenoxyacetic acid; NAA – 1- naphthalene acetic acid; BAP – 6-benzylaminopurine; GA_3 – gibberellic acid; KIN – kinetin-6-furfurylaminopurine; thidiazuron – 1-phenyl-3-(1,2,3- thidiazol-5-yl)-urea; picloram – 4-amino 3,5,6-trichloropicolinic acid; MS – Murashige and Skoog (1962) medium, RCM – callus induction medium

INTRODUCTION

The method of plant cell and tissue culture has been used for over half a century by scientists in many areas of experimental biology. It also became an important part of plant biotechnology. The commercial application of *in vitro* culture has been focused on three main fields: large-scale micropropagation of crop plants, production of plant secondary metabolites, and efficient regeneration of economically important species through somatic embryos (1, 9, 22, 24). Cell suspension culture is one of *in vitro* methods applied both in basic research and commercial plant biotechnology. As a unique model system composed of populations of single cells and small cell aggregates, it has found wide application in basic studies like biochemical, physiological, and morphological investigations of plant cell behaviour in strictly defined conditions (8). On the other hand, many protocols using plant cell culture for commercial exploitation have been developed as a suitable tool for obtaining an efficient regeneration of economically important plants by somatic embryogenesis and production of different plant derived organic compounds (2, 18, 23, 24).

Raspberry (*Rubus idaeus* L.) is a member of the genus *Rubus* and family *Rosaceae.* The genus *Rubus* is one of the most diverse plant genera. It comprises about 500 species with ploidy levels ranging from diploid to dodecaploid (10). The most economically important species in this genus are the red raspberry (*Rubus idaeus* L.) and the black raspberry (*Rubus fruticosus* L.). There are two botanical varieties of the *Rubus idaeus* L. species: the European red raspberry *R. idaeus* L. var. vulgatus Arrhen. and the native American red raspberry *R. idaeus* L. var. strigosus Michx. (19). Hundreds of commercial raspberry cultivars have been bred by hybridization between these two botanical varieties (10, 19). The red raspberry is diploid (2n=2x=14) with a very small genome (275Mbp) and therefore is a useful species for molecular and genomics investigations (10).

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

In recent years there has been an increasing demand for different berry fruits like raspberry because of its health benefits. Raspberries are a rich source of antioxidant compounds, such as anthocyanins, catechins, flavonols, flavones, and ascorbic acid, which can be essential components of diet protecting against a variety of serious human diseases (10). Raspberries are also very valuable dessert fruits and raw material for food processing industry. For these reasons, research concerning the economically important *Rubus* species has been focused among others on application of different *in vitro* culture methods for obtaining good material for breeding and food industry.

A number of protocols of *in vitro* micropropagation for some *Rubus* species have been developed (6, 7, 15, 19, 21, 25, 26). To obtain effective procedures of *in vitro* proliferation of shoots authors have tested two main factors: the type of explants and the type and concentration of plant growth regulators. Sobczykiewicz (19) applied meristem cultures from both apical and axillary buds for *Rubus idaeus* shoot propagation. Similarly, Martinussen et al. (15) used meristem cultures for micropropagation of *Rubus chamaemorus*. Another type of explants were single-node stem sections (6, 7) used for *Rubus pubescens* and *R. chamaemorus*. Similarly, Wu et al. (26) used stem segments with lateral buds and shoot tips for micropropagation of 32 different *Rubus* genotypes. Vujovic et al. (25) developed the method of *in vitro* propagation of blackberry (*Rubus fruticosus*) from leaf explants by regeneration of adventitious shoots. Some methods of obtaining *Rubus* sp. cell suspension cultures have been published (3, 5, 11, 27). Borejsza-Wysocki and Hrazdin (3) investigated rigorously key factors influencing the establishment of cell suspension cultures of *Rubus idaeus* cv. Royalty such as the type of primary explants and formulation of culture media. Cortelazzo et al. (5) used suspension culture of *Rubus fruticosus* for biochemical investigations. They evaluated peroxidase activities both in the cytoplasm of cells and in the plant cell wall and additionally in the culture medium. Similarly, Zheng et al. (27) applied *R. idaeus* cv. Royalty cell culture for basic molecular and biochemical research of three aromatic polyketide synthase genes and proteins encoded by these genes.

The aim of our investigation was to find appropriate culture conditions for induction of cell suspension culture of raspberry cv. Nawojka. In the first step, we tested 5 combinations of plant growth regulators added into modified Murashige and Skoog (1962) medium to obtain callus tissue suitable for induction of cell suspension. In the second step, we investigated chosen factors of *in vitro* culture to find the appropriate conditions for dispersing the selected callus tissue into single cells and small aggregates and promoting sustained division of cells in liquid medium.

MATERIAL AND METHODS

Plant material

Rubus idaeus cv. Nawojka plants micropropagated in *in vitro* culture were the source of primary explants used for initiation of callus and cell suspension culture. The initial microplants of cv. Nawojka were kindly donated by Dr. Jan Danek from the Fruit Experimental Station in Brzezna, Poland.

Micropropagation of raspberry shoots

In order to obtain a sufficient number of plants to establish callus cultures, we applied the method of *in vitro* clonal propagation through axillary shoot proliferation. Micropropagation was conducted on modified MS solid medium designated RM1. This medium was composed of mineral salts, vitamins, and myo-inositol according to MS and 20 g l^{-1} of sucrose as an organic source of carbon and energy. For stimulation of rapid shoot proliferation from axillary buds, we applied plant growth regulators in a combination optimized in our previous experiments (not published): cytokinin BAP and auxin IAA both at a concentration of 1 mg l^{-1} and gibberellic acid GA_3 at a concentration of 0.01 mg l^{-1}. Agar (Cat. No. 194615, MP Biomedicals, LLC) at a concentration of

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

7.8 g l^{-1} was used as a gelling agent. The pH of the medium was adjusted to the value 5.65 prior to sterilisation by autoclaving at 121^0 C and 103 kPa for 18 min. Microshoots were propagated in 450 ml glass jars containing 80 ml of RM1 medium and maintained in a growth-room at 23^0 C under a 16-h photoperiod (16 h light/8 h darkness). The culture was started from 5 microplants and subcultured at 3-week intervals onto fresh medium. After some passages, the multiplied shoots were divided into individual plantlets and transferred to the medium designated RM0. This medium contained the same components as for micropropagation but without plant hormones. The plantlets were subcultured every 3 weeks onto fresh RM0 medium to ensure vigorous growth and rooting. After 2–3 months, leaves were suitable for harvesting as primary explants to induce callus cultures.

Establishment of callus cultures

Callus cultures were initiated from leaves of raspberry (cv. Nawojka) plants grown aseptically on RM0 solid medium without plant hormones. Only young and healthy leaves were chosen for isolation of leaf explants. Leaf sections (10 mm x 5 mm) were prepared after cutting off all edges of the blade and the main vein. The explants were placed onto solid callus induction medium (RCM). This medium was composed of macro- and micro-salts, vitamins, myo-inositol, and sucrose according to MS and additionally different combinations of plant growth regulators. To find proper conditions for obtaining the callus culture suitable for induction of a cell suspension, we tested five variants of plant hormones combinations added to the culture medium: 1) 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP; 2) 0.25 mg l^{-1} 2,4-D; 3) 0.5 mg l^{-1} 2,4-D; 4) 2.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP; 5) 4.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP. Callus cultures were prepared in 80 mm Petri dishes, each containing 15 ml of the tested medium and 5 leaf explants. 30 leaf pieces were tested for each combination of plant hormones. The cultures were incubated in a growth-room at 23^0 C in the dark and subcultured to fresh medium every four weeks. After five months of the culture, half of the explants were transferred to the light, under a 16-h photoperiod.

Induction of cell suspension cultures

The established callus culture obtained on solid modified MS medium supplemented with 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP was used as an inoculum for induction of cell suspensions. All cultures were initiated by transferring 1.0 g of an undifferentiated callus tissue to 300ml Erlenmeyer flask containing 80 ml of liquid medium. To find appropriate conditions for induction of raspberry cell suspension, four combinations of plant hormones were tested: 1) 8.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP; 2) 8.0 mg l^{-1} IAA and 1.0 mg l^{-1} KIN; 3) 0.5 mg l^{-1} 2,4-D; 4) 1.0 mg l^{-1} 2,4-D. The basal medium was composed of half-strength macro-elements, unmodified micro-elements, vitamins, myo-inositol, and sucrose according to Murashige and Skoog (1962). In order to ensure adequate aeration, the cell cultures were agitated on an orbital shaker at the speed of 120 rpm. The spent medium was removed and replaced with the fresh medium every two weeks.

RESULTS AND DISCUSSION

The established callus culture consisting of loosely joined undifferentiated cells that easily dispersed during agitation in liquid medium is an excellent inoculum to induce a cell suspension culture and therefore it is the most often applied source of cells for initiation of this type of culture (8). The composition of the medium employed for initiation of callus tissue is one of the key factors that induce callus formation in *in vitro* culture. A majority of explant types require addition of plant growth regulators into culture medium to initiate callus

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

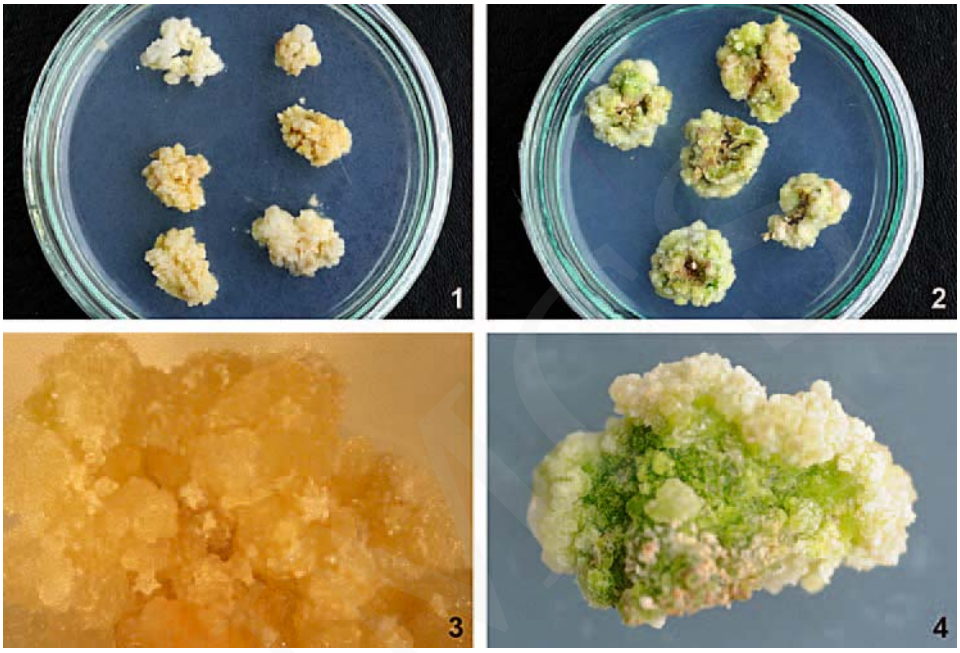

Fig. 1. Callus culture of raspberry established on the modified MS (1962) medium supplemented with 4.0 mg l-1 IAA and 1.0 mg l-1 BAP, after seven months of culture

Fig. 2. Callus culture of raspberry established on the modified MS (1962) medium supplemented with 4.0 mg l-1 NAA and 2.0 mg l-1 BAP, after seven months of culture

Fig. 3. Healthy and friable callus tissue of raspberry obtained on the best medium (supplemented with 4.0 mg l-1 IAA and 1.0 mg l-1 BAP)

Fig. 4. Healthy but hard in texture callus tissue of raspberry obtained on medium supplemented with 4.0 mg l-1 NAA and 2.0 mg l-1 BAP

formation. Auxins; IAA, 2,4-D or NAA and cytokinins; kinetin, BAP or zeatin in various combinations have most often been applied (4). In our investigation, we tested five combinations of plant growth regulators added to basal Murashige and Skoog medium in order to obtain raspberry callus suitable for induction of a cell suspension. The results obtained by us clearly showed that the induction and proliferation of raspberry callus tissue were strongly dependent on the composition of plant hormones employed in the tested media. The best result was acquired on the modified MS medium supplemented with 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP. After three weeks of incubation, the induction of callus tissue occurred on all cultured explants, first at the wounded edges. Growing callus was healthy and cream-yellow coloured. During the first passage onto the fresh medium, half of the explants were taken as whole explant pieces, whereas only growing calli were isolated and subcultured from the other explants. After separation from primary explants, the isolated calli exhibited significantly decreased growth in comparison

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

to these growing on the entire explants. After five months of culture in the dark, half of the calli were transferred to the light. The incubation in the light improved the quality and growth of callus tissue. Finally, after seven months of culture, the callus culture was well established. This culture consisted of undifferentiated loosely packed and easily fragmenting parenchyma cells. It was characterised by rapid growth of unorganized callus tissue and light (cream-yellow) coloration (Fig. 1 and Fig. 3.). Therefore, we stated that this culture was suitable for induction of a cell suspension. About 20% of the calli from this culture turned green after being transferred to the light, but this callus tissue grew slower and was hard in texture and therefore it was less competent for starting a cell culture.

The other media with a different composition of growth regulators tested by us for induction of callus cultures yielded worse results. Addition of only auxin 2,4-D at the concentration of 0.25 mg l^{-1} or 0.5 mg l^{-1} to the basal medium resulted in darkening and browning of the explants despite transferring the culture to the fresh medium. After three months of culture, the explants cultured in these two combinations died. Similarly, the culture medium supplemented with the auxin NAA and cytokinin BAP both at the concentration of 2.0 mg l^{-1} proved inappropriate for obtaining healthy callus tissue. On this medium, the induction and growth of callus was very slow. During first months of culture, the explants turned brown and finally over 90% of them died. A better result was obtained on the medium containing the higher concentration of NAA – 4.0 mg l^{-1} and 2.0 mg l^{-1} BAP. During the initial months of the culture, the growth of callus tissue was slow but it was healthy, and after being transferred to the light, it showed a visible increase in propagation. Finally, after seven months of incubation, this callus culture was well established. It consisted of healthy undifferentiated cells and was green and white coloured. However, unlike the callus culture established on the best medium supplemented with 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP, this callus was hard in texture and therefore was evaluated as inadequate for induction of a cell suspension (Fig. 2 and Fig. 4.).

Many protocols have been developed that clearly showed an importance of friable callus as a source of inoculum for obtaining a fine suspension culture (3, 12, 13, 14, 20). Borejsza-Wysocki and Hrazdin (3) obtained rapid proliferation of healthy and soft raspberry callus by application of both auxin and cytokinin. Out of the tested compositions, the best callus suitable for induction of cell culture was obtained on the medium with auxins 2,4-D (2.0 mg l^{-1}) and IBA (1.0 mg l^{-1}) and cytokinin 2iP (1.0 mg l^{-1}). Llamoca-Zarate et al. (14) gained the best soft and friable callus of *Opuntia ficus-indica* on MS medium supplemented with 0.5 mg l^{-1} 2,4-D; 0.2 mg l^{-1} kinetin, and 0.25 mg l^{-1} picloram. Out of some combinations tested, Karam and et al. (13) acquired the best result by applying auxin IAA (0.5 mg l^{-1}) together with thidiazuron (1.5 mg l^{-1}) for induction and growing of *Salvia fruticosa* callus culture. Stella and Braga (20) also verified the influence of some

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

combinations of growth regulators on callus tissue proliferation of tropical woody species (*Rudgea jasminoides*). Fast growing and friable callus was achieved in their investigation only on half-strength nitrogen MS medium supplemented with 2.0 mg l^{-1} of picloram. On the other hand, Kanwar et al. (12) obtained friable callus from *Robinia pseudoacacia* explants on solid MS medium supplemented with 0.5 mg l^{-1} 2,4-D. The cited results clearly show that the exogenous requirements of plant explants cultured for obtaining appropriate callus tissue depend primarily on the plant species, type of explant, and the composition of culture medium.

In our investigation, the friable callus grown on modified MS medium supplemented with 4.0 mg l^{-1} IAA and 1.0 mg l^{-1} BAP was proved a suitable inoculum for starting cell suspension culture in raspberry. It dispersed easily in liquid media and exhibited continued cell division; however, the degree of tissue dispersion and growth was dependent on the tested combination of growth regulators applied in the liquid medium. From among the investigated variants, the best result was obtained in modified MS medium supplemented with 1.0 mg l^{-1} 2,4-D. In this medium combination, cell propagation and growth was so high that this culture required transferring into the fresh medium every 7–10 days. After some passages, the cell culture looked homogeneous and consisted of rapidly dividing spherical cells and cell aggregates (Fig. 5 and Fig. 6). In addition, another medium formulation with the same composition as for callus establishment with only one modification that involved increment of the auxin IAA concentration to 8.0 mg l^{-1} proved good for induction of cell culture. It induced good dispersion of callus tissue and continued growth of single cell population and small cell aggregates (Fig. 7 and Fig. 8). However, this culture grew slower than the suspension in the medium supplemented with 1.0 mg l^{-1} 2,4-D. Replacement of cytokinin BAP by kinetin in another combination of plant hormones added to liquid medium gave rise to changes in the morphology of growing cells. The proliferating cells were big and elongated, and produced irregular cell aggregates (Fig. 9. and Fig. 10).

The composition of liquid medium is another essential factor that influences the initiation of cell suspension culture. Similarly to our investigation, Kanwar et al. (12) stated that liquid MS medium supplemented with auxin 2,4-D (at a concentration of 0.5 mg l^{-1}) was the best for separation of *Robinia pseudoacacia* cells from callus tissue in liquid medium. Additionally, Salman (17) obtained maximum growth of the suspension culture from friable callus of *Gypsophila paniculata* on MS medium supplemented with auxin 2,4-D (1.0 mg l^{-1}) and cytokinin BAP (0.2 mg l^{-1}). Stella and Braga (20) as well as Karam et al. (13) also applied the Murashige and Skoog basal medium for establishment of cell suspension culture, but they found 2.0 mg l^{-1} of picloram and 1.5 mg l^{-1} of thidiazuron together with 0.5 mg l^{-1} of IAA to be the best combination of plant growth regulators for induction of *Rudgea jasminoides* and *Salvia fruticosa* cell culture. In contrast to the cited results, Borejsza-Wysocki and Hrazdin (3) found

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

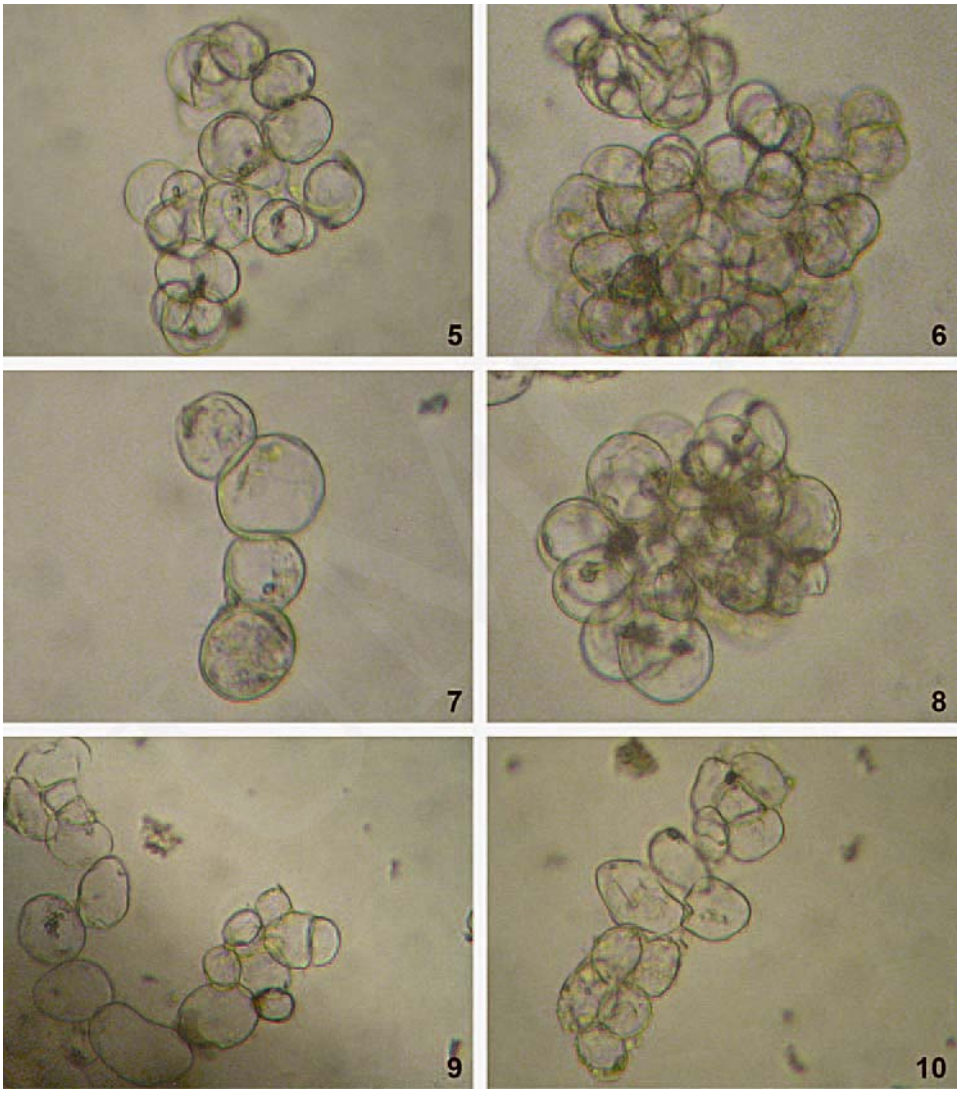

Fig. 5 and Fig. 6. Rapidly dividing spherical cells and cell aggregates of raspberry obtained in suspension culture in the presence of auxin 2.4-D (1.0 mg l-1); (x500)

Fig. 7 and Fig. 8. Isodiametric cells and cell aggregates of raspberry obtained in suspension culture in the presence of auxin IAA (8.0 mg l-1) and cytokinin BAP (1.0 mg l-1); (x750)

Fig. 9 and Fig.10. Elongate cells of raspberry obtained in suspension culture in the presence of kinetin instead of cytokinin BAP; (x500)

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

Anderson (1980) basal medium to be better than Murashige and Skoog (16) medium. The best combination of plant hormones for induction and maintenance of raspberry cell culture appeared to be the composition of two auxins 2,4-D (2.0 mg l^{-1}) and IBA (1.0 mg l^{-1}) together with cytokinin 2iP (1.0 mg l^{-1}). Presented data strongly indicate that the composition of liquid medium is one of the main factors affecting initiation and establishing of *in vitro* cell suspension culture. The cell suspension culture of raspberry cv. Nawojka obtained by us from friable callus tissue can be a suitable material for subsequent basic investigation or commercial application.

REFERENCES

1. Altman A. 2003. From plant tissue culture to biotechnology: scientific revolutions, abiotic stress tolerance, and forestry. In Vitro Cell. Dev. Biol.-Plant. 39: 75–84.
2. Anand R. P., Ganapathi A., Anbazhagan V. R., Vengadesan G., Selvaraj N. 2000. High frequency plant regeneration via somatic embryogenesis in cell suspension cultures of cowpea, *Vigna unguiculata* (L.) Walp. In Vitro Cell. Dev. Biol.-Plant. 36: 475–480.
3. Borejsza-Wysocki W., Hrazdin G. 1994. Establishment of callus and cell suspension cultures of raspberry (*Rubus idaeus* cv. Royalty). Plant Cell Tiss Organ Cult. 37: 213–216.
4. Brown J. T. 1990. The Initiation and Maintenance of Callus Cultures. In: Methods in Molecular Biology. Vol. 6, Plant Cell and Tissue Culture. J. W. Pollard, J. M. Walker (eds.). Humana Press, Clifton, New Jersey, 57–63.
5. Cortelazzo A. L., Marais M.-F., Joseleau J.-P. 1996. Changes in peroxidases in the suspension culture of *Rubus fruticosus* during growth. Plant Cell Tiss Organ Cult. 46: 27–33.
6. Debnath S. C. 2004. Clonal propagation of dwarf raspberry (*Rubus pubescens* Raf.) through *in vitro* axillary shoot proliferation. Plant Growth Regul. 43: 179–186.
7. Debnath S. C. 2007. A two-step procedure for *in vitro* multiplication of cloudberry (*Rubus chamaemorus* L.) shoots using bioreactor. Plant Cell Tiss Organ Cult. 88: 185–191.
8. Evans D. E., Coleman J. O. D., Kearns A. 2003. Plant Cell Culture. BIOS Scientific Publishers, 81–87.
9. Gamborg O. L. 2002. Plant tissue culture. Biotechnology. Milestones. In Vitro Cell. Dev. Biol.-Plant 38: 84–92.
10. Graham J., Woodhead M. 2009. Raspberries and Blackberries: The Genomics of *Rubus*. In: Genetics and Genomics of Rosaceae, Plant Genetics and Genomics: Crops and Models 6, DOI10.1007/978-0-387-77491-6 24, K. M. Folta, S. E. Gardiner (eds.), Springer Science+ Business Media, 507–524.
11. Joseleau J. P., Chambat G., Cortelazzo A. L., Faik A., Priem B., Ruel K. 1995. Oligosaccharides from xyloglucan affect the development of *Rubus fruticosus* cell suspension culture. Current Issues in Plant Molecular and Cellular Biology: 433–443.
12. Kanwar K., Kaushal B., Abrol S., Deepika R. 2008. Plant regeneration in *Robinia pseudoacacia* from cell suspension cultures. Biologia Plantarum 52 (1): 187–190.
13. Karam N. S., Jawad F. M., Arikat N. A., Shibli R. A. 2003. Growth and rosmarinic acid accumulation in callus, cell suspension, and root cultures of wild *Salvia fruticosa*. Plant Cell Tiss Organ Cult. 73: 117–121.

Pobrane z czasopisma Annales C - Biologia **http://biologia.annales.umcs.pl**
Data: 26/11/2026 10:03:15

14. Llamoca-Z´arate R. M., Studart-Guimarães C., Landsmann J., Campos F. A. P. 1999. Establishment of callus and cell suspension cultures of *Opuntia ficus-indica*. Plant Cell Tiss Organ Cult. 58: 155–157.
15. Martinussen I., Nilsen G., Svenson L., Junttila O., Rapp K. 2004. *In vitro* propagation of cloudberry (*Rubus chamaemorus*). Plant Cell Tiss Organ Cult. 78: 43–49.
16. Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.
17. Salman M. N. 2002. Establishment of callus and cell suspension cultures from *Gypsophila paniculata* leaf segments and study of the attachment of host cells by *Erwinia herbicola* pv. *Gypsophilae*. Plant Cell Tiss Organ Cult. 69: 189–196.
18. Shekhawat M. S., Shekhawat N. S. 2011. Micropropagation of *Arnebia hispidissima* (Lehm). Dc. and production of alkannin from callus and cell suspension culture. Acta Physiol. Plant. 33: 1445–1450.
19. Sobczykiewicz D. 1992. Micropropagation of Raspberry (*Rubus idaeus* L.). In: Biotechnology in Agriculture and Forestry. Vol. 18. High Tech and Micripropagation II. Y. P. S. Bajaj (ed.), Springer-Verlag Berlin Heidelberg, 339–350.
20. Stella A., Braga M. R. 2002. Callus and cell suspension cultures of *Rudgea jasminoides*, a woody Rubiaceae tropical. Plant Cell Tiss Organ Cult. 68: 271–276.
21. Tsao C. W. V., Reed B. M. 2002. Gelling agents, silver nitrate, and sequestrene iron influence adventitious shoot and callus formation from *Rubus* leaves. In Vitro Cell. Dev. Biol.- Plant. 38: 29–32.
22. Vasil I. K. 2008. A history of plant biotechnology: from the Cell Theory of Schleiden and Schwann to biotech crops. Plant Cell Rep. 27: 1423–1440.
23. Vengadesan G., Ganapathi A., Anbazhagan V.R., Anand R.P. 2002. Somatic Embryogenesis in Cell Suspension Cultures of *Acacia sinuata* (LOUR.) MERR. In Vitro Cell. Dev. Biol.-Plant 38: 52–57.
24. Verpoorte R., Contin A., Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. Phytochemistry Reviews 1: 13–25.
25. Vujovic T., Ruzic D., Cerovic R., Momirovic G. S. 2010. Adventitious regeneration in blackberry (*Rubus fruticosus* L.) and assessment of genetic stability in regenerants. Plant Growth Regul. 61: 265–275.
26. Wu J. H., Miller S. A., Hall H. K., Mooney P. A. 2009. Factors affecting the efficiency of micropropagation from lateral buds and shoot tips of *Rubus*. Plant Cell Tiss. Organ. Cult. 99: 17–25.
27. Zheng D., Schröder G., Schröder J., Hrazdina G. 2001. Molecular and biochemical characterization of three aromatic polyketide synthase genes from *Rubus idaeus*. Plant Molecular Biology 46: 1–15.