

CATHARANTHUS ROSEUS (L.) G. DON – PLANT REGENERATION AND ALKALOIDS CONTENT

MIROŚŁAWA FURMANOWA, HANNA OŁĘDZKA,
JOANNA JÓZEFOWICZ, AGNIESZKA PIETROSIUK

Department of Biology and Pharmaceutical Botany,
Medical Academy, Banacha 1, 02-097 Warsaw, Poland

(Received: May 9, 1994. Accepted: August 24, 1994)

ABSTRACT

We describe here a regeneration of plantlets of *Catharanthus roseus* (L.) G. Don from shoot tips and axillary buds. Shoot tips were excised from 7-day-old seedlings and were incubated in solid Nitsch and Nitsch (NN) medium supplemented with kinetin, benzyladenine (BA), indole-3-butyric acid (IBA) and β -indolylacetic acid (IAA) in various combinations. After two months in culture, regenerated rooted plantlets were cut and transferred to a new medium; the explants contained shoot tips or axillary buds. Four passages were done. We obtained about 200 rooting plantlets from one seedling. Then the plantlets were transferred to the soil and they grew under a foil tent. After five months of vegetation they were collected, dried and weighed. Chemical investigations of leaves of these plants were done. The vindoline and catharanthine were dominant alkaloids in the juvenile stage of plants (before blooming). Total amount of alkaloids, equal 2.95%, was gravimetrically determined in leaves of plants, after 4th passage, regenerated *in vitro* on NN medium supplemented with kinetin and IBA.

KEY WORDS: *Catharanthus roseus*, vindoline, catharanthine, vinblastine, tissue culture.

INTRODUCTION

Catharanthus roseus (L.) G. Don – periwinkle (*Apocynaceae*) is cultivated in many regions. This species contains mono-, and dimeric indole alkaloids of which vinblastine and vincristine (Fig. 1) are used as anticancer drugs for the treatment of various leukemias. Other monomeric alkaloids have hypotensive and antiarrhythmic activity. In the light of the above, periwinkle became the subject of interest of biotechnology in different areas, the most important is the production of indole alkaloids from cell cultures and micropropagation of these plants.

The differentiation and regeneration of periwinkle in tissue culture were studied in few laboratories. The first investigation was undertaken by Dhruva et al. (1977) who observed the callus initiation and differentiation into roots. Ramavat et al. (1978) described the shoot formation in *Catharanthus roseus* callus culture. The regeneration of plants from haploid and diploid callus cells of *Catharanthus roseus* was obtained by Abou-Mandour et al. (1979). For this process different combinations of kinetin and IAA were necessary.

Krueger et al. (1982) obtained leaf organ cultures from sterile seedlings of *Catharanthus roseus*. They were established from cotyledon stage seedlings on MS (Murashige and Skoog, 1962) Revised Tobacco (RT) medium with BA. One month later the resulting callus was transferred to RT medium with BA then the leaf organs were maintained on a rotary shaker. This culture shows abundant proliferation and produced vindoline and a complex variety of other alkaloids (ajmalicine, sibirin, tetrahydroalstonine and serpentine).

Leela (1985) studied the induction of pollen embryoids for rearing the haploids and isolating the lines with the increased alkaloid content.

Miura and Hirata (1986) patented the capability of producing substantial amount of indole alkaloids by an organ culture of *Catharanthus roseus*.

Endo et al. (1987) obtained root and shoot cultures from seedlings. Root cultures and cell suspension cultures, derived from the root cultures, showed similar alkaloid spectra to the roots of intact plants. The differences in alkaloid synthesis in the above mentioned cultures and in natural roots were basically quantitative rather than qualitative. In all cases no vindoline was detected. Shoot cultures produced catharanthine, ajmalicine and 3', 4'-anhydrovinblastine an immediate precursor of vinblastine. Endo et al. (1987) investigated also the effect of light on the content of alkaloids in *Catharanthus* cultures. In case of cell suspension culture, production of alkaloids, catharanthine and ajmalicine, in the dark was larger than that in the light. In contrast, when the shoot culture was transferred to the dark, the vindoline concentration decreased drastically, but the concentration of ajmalicine increased.

Hirata et al. (1987) drew attention to the formation of indole alkaloids in multiple shoot cultures of *Catharanthus roseus* which were maintained for 18 months on MS medium with BA. The reduction in the next passage of benzyloadenine increased the alkaloid content (vindoline and catharanthine), but suppressed the growth index of shoot culture. Purification and identification of alkaloids: vindoline, catharanthine and ajmalicine were done by means of thin-layer chromatography (TLC), high performance liquid chromatography

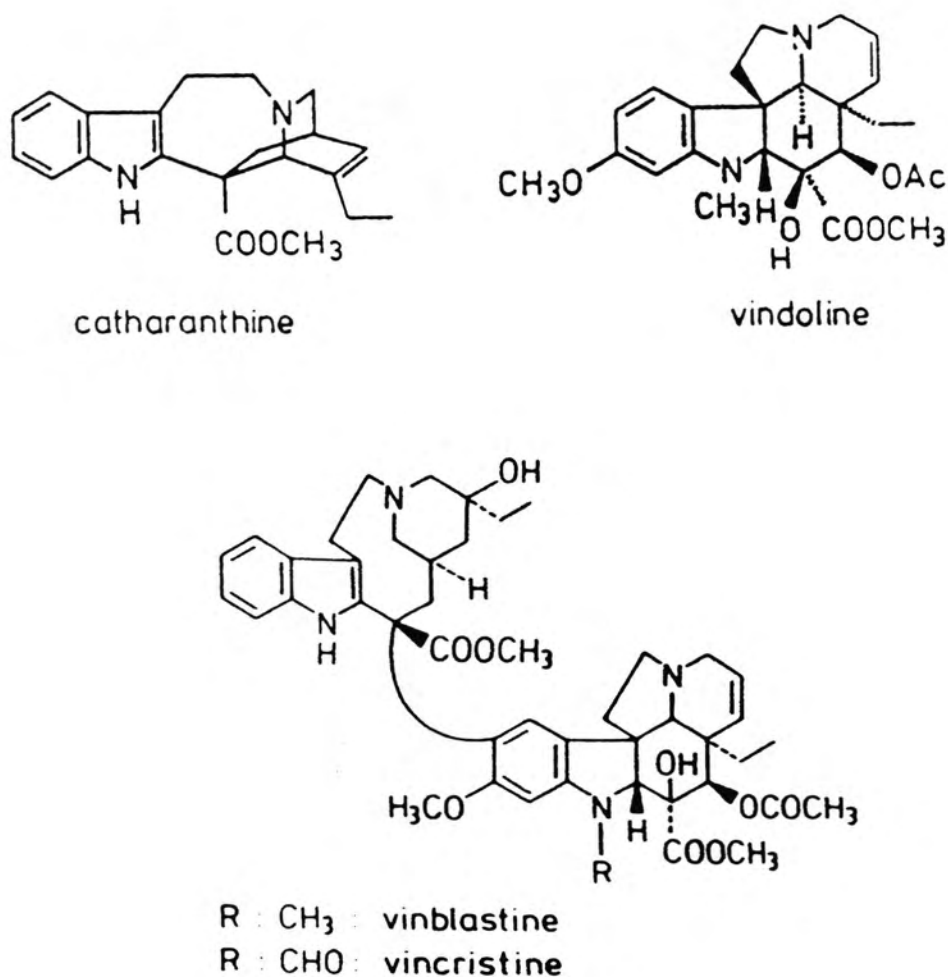


Fig. 1. Formula of vinblastine, vincristine vindoline, and catharanthine.

(HPLC) and by mass spectrometry. Simple shoots were separated from the unorganized tissue and stuck into medium with IAA. Plant regeneration was achieved by potting the rooting shoots directly in a mixture of vermiculite and sand.

Miura et al. (1988) investigated further the formation of indole alkaloids in multiple shoot cultures. The authors (l.c.) detected vinblastine in the extract of one of the multiple shoot culture lines (MSC-B-1) of *Catharanthus roseus*. The production of this alkaloid was lower than that in the parent plant. The regenerated plants obtained from the MSC-B-1 line had the same productivity for vinblastine as the parent plant.

Hirata et al. (1991, 1992) discovered that under near-ultraviolet light, $\gamma = 370$ nm, the levels of leurosine and vinblastine in multiple shoot culture of *Catharanthus roseus* increased and vindoline and catharanthine *in vivo* precursors for dimeric alkaloid synthesis decreased.

According to Bajaj et al. (1988) most useful, from the pharmaceutical point of view, is the propagation of medicinal plants from an existing meristem, since, due to this method, identical plants with desired traits may be obtained. We used the same method for micropropagation of *Catharanthus roseus*. Effects of various phytohormones on plantlets formation from shoot tips and axillary buds of *Catharanthus roseus* were

investigated by Olędzka et al. (1991). Furmanowa et al. (1991) searched bisindole alkaloids in micropropagated *in vitro* *Catharanthus roseus* plants.

Hirata et al. (1994) showed that growth and morphological differentiation, such as formation and development of shoots, were affected by phytohormones, and that these effects resulted in the changes in levels of alkaloids culture. Near ultraviolet light may specifically stimulate the synthesis of bisindole alkaloids from vindoline and catharanthine in multiple shoot cultures.

The purpose of our study was to receive a large quantity of plant material highly appreciated in medicine. These plants cannot be grown in our climate in the ground, only in greenhouses or under foil tents.

In regenerated *in vitro* plants we searched dimeric alkaloids, vincristine and vinblastine and monomeric alkaloids, vindoline and catharanthine (Fig. 1). These two monomeric alkaloids can be used for biosynthesis of dimeric alkaloids. It was demonstrated by Kutney et al. (1985) that utilizing cellfree extracts from *Catharanthus roseus* 3', 4'-anhydrovinblastine is formed in the enzymatic process via the coupling of catharanthine and vindoline. Subsequent enzymatic transformation to vinblastine, leurosine and catharine was achieved. These re-

sults stimulated us to regenerate the *Catharanthus roseus* plants and select from the ontogenic process the stage containing suitable, important alkaloids.

MATERIALS AND METHODS

Plant Regeneration

The primary culture was initiated from seedling shoot tips. Seeds of a *Catharanthus roseus* (L.) G. Don received from the Institute of Medicinal Plants in Poznań (Poland) were sterilized on the surface by immersion in 70% ethanol for 1 minute and then in 5% calcium hypochlorite for 10 minutes. They were rinsed three times with sterile water and allowed to germinate at 25°C in the dark. After 7 days of germination shoot tips of seedlings were cut and laid on 250 cm³ of Nitsch and Nitsch (NN) (1969) medium solidified with 0.7% agarose and supplemented with kinetin (0; 0.1; 0.5 and 1 mg/dm³), BA (0; 0.1; 0.5 and 1 mg/dm³), IBA (0; 0.1; 0.5 and 1 mg/dm³) and IAA (0.5 mg/dm³) in various combinations. The pH was adjusted to 5.6 before adding the agar and autoclaving at 120°C for 15 minutes. They were cultured at 25°C in the light (12 h/day, 2000 lx). Within 8 weeks well rooted plantlets containing about 7 nodes with dark green leaves arose from shoot tips and were used as mother plants

for the next step of propagation. In the second generation plantlets with four-five of nodes developed after 8 weeks from the single node explant (Fig. 2A). After the third passage the number of nodes was the same. Well rooted plantlets regenerated on the best media (NN with kin. 0.1 mg/dm³ + IBA 0.5 mg/dm³; NN with BA 0.1 mg/dm³ + IBA 0.5 mg/dm³; NN with kin. 0.5 mg/dm³ + IAA 0.5 mg/dm³) were transferred to pots with sterile mixture of soil and sand and covered with glasses. After the 2 week's period of acclimatization in non sterile conditions they were transferred to soil; 90% of plantlets survived and grew in the field under a foil tent for five months. This part of investigation was done in the Anticancer Plant Laboratory, Agriculture University, Warsaw.

Culture of plants treated with colchicine

Sterile, seven days old seedlings were treated with 0.5% colchicine water solution for 19 h. Shoot tips of seedlings were cut and laid on NN medium, supplemented with 1. kin. 0.1 mg/dm³ + IBA 0.5 mg/dm³, 2. kin. 0.5 mg/dm³ + IAA 0.5 mg/dm³. After four passages a part of the plants was dried for chemical investigation, the second part was transferred to the soil, and after five months of vegetation the plants were collected and dried for chemical investigation.



Fig. 2. In vitro culture of *Catharanthus roseus*.

A. Single node explants on the Nitsch and Nitsch medium with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³.

B. Plantlets regenerated from shoot tip on the medium NN supplemented with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³. Linear scales = 10 mm.

TABLE 1 The total contents of alkaloids in the leaves of *Catharanthus roseus* plants cultured in various conditions.

	Growth conditions	Medium	Total content of alkaloids in %	Identified alkaloids
1.	plantlets regenerated <i>in vitro</i> after 4 passage	NN + BA 0.1 + IBA 0.5	2.57	VDL, CAT
2.	plants regenerated <i>in vitro</i> , cultivated in foil tent for 5 months	NN + BA 0.1 + IBA 0.5	2.16	VDL, CAT
3.	plantlets regenerated <i>in vitro</i> after 4 passage	NN + KIN. 0.1 + IBA 0.5	2.95	VDL, CAT
4.	plants regenerated <i>in vitro</i> , cultivated in foil tent for 5 months	NN + KIN. 0.1 + IBA 0.5	1.43	VDL
5.	colchicinated plantlets regenerated <i>in vitro</i> , after 4 passage	NN + KIN. 0.1 + IBA 0.5	2.22	VDL, CAT
6.	colchicinated plants regenerated <i>in vitro</i> , cultivated in foil tent for 5 months	NN + KIN. 0.1 + IBA 0.5	1.26	VDL
7.	control plants cultivated only in the ground for 2 months		1.09	VDL, CAT
8.	control plants cultivated only in the ground for 5 months		1.86	VDL
9.	plantlets regenerated <i>in vitro</i> after 4 passage	NN + KIN. 0.5 + IAA 0.5	2.92	VDL, CAT
10.	plants regenerated <i>in vitro</i> , cultivated in foil tent for 5 months	NN + KIN. 0.5 + IAA 0.5	1.32	VDL
11.	colchicinated plants regenerated <i>in vitro</i> , cultivated in foil tent for 5 months	NN + KIN. 0.5 + IAA 0.5	0.85	VDL

Culture of control plants

The seeds were planted in the pot with soil. After two months of growth a part of plants was collected and dried for chemical investigation. The remaining part of plants was transferred to the soil under a foil tent. After two and five months of vegetation the plants were collected and dried for chemical investigation.

Chemical investigation

The leaves of *Catharanthus roseus* plants regenerated in *in vitro* culture in our department and control plants were used for chemical investigation. The plants of *Catharanthus roseus* were cultured in various conditions and on various culture media (Table 1). The thin layer chromatography (TLC) procedure and the total contents of alkaloids were indicated in the above mentioned plants material. This chemical investigation was carried out by a method described by Ruzkowska et al. (1994). The essence of this method is the extraction of dry plant material by neutral hydroxyl solvent, purification alkaloid fraction two times with application of salt out of water phase on a certain stage of extraction and then the alkaloid

determination by gravimetric or colorimetric analysis. Alkaloids were identified using TLC and color reaction with ceric ammonium sulfate (CAS) (Farnsworth et al. 1964). The solution of citrates of periwinkle alkaloids (pH 2) was extracted by ethylene dichloride. The water phase was adjusted to pH 6 with 10% NH₄OH and then was extracted by benzene for obtaining dimeric alkaloids and a part of catharanthine. The above mentioned extracts were evaporated to dryness and the dry residue was dissolved in an appropriate volume of ethylene dichloride and benzene for TLC accordingly. This alkaloid extract was fractionated on a precoated silica gel plate (Kieselgel 60, 0,2 mm, Merck). The following TLC solvent systems were used: (1) C₆H₆ – EtOH – EtOAc – NH₄OH (5:23:100: 2,3), (2) C₆H₁₂ – EtOH (1:1), (3) C₆H₁₄ – C₃H₆O (3:1), (4) CHCl₄ – MeOH (10:1). The products were identified by comparing with standards: vindoline, catharanthine, vinblastine and vincristine.

RESULTS AND DISCUSSION

The best plant regeneration was obtained on NN medium with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³ used earlier by

Olszowska and Furmanowa (1987) for *Thymus vulgaris* micropropagation. On this medium an average of 200 plantlets were obtained from one shoot tip of seedling after six months. The leaves were large and dark green (Fig. 2B). When BA was used instead kinetin, a multiplication of shoots was observed. In our observation the highest branchings of shoots were obtained when BA 0.1 mg/dm³ with IBA 0.5 mg/dm³ was used. The leaves were smaller and yellow-green.

The presence of the indole alkaloids in the investigated extracts was attested with color reaction with CAS, and then by comparison of colour of spots with the standard samples and measurement of R_f values. Monomeric alkaloids, such as vindoline and catharanthine were main alkaloids in leaves of the 11 tested groups of plants, but vindoline was always dominant (Table 1). Catharanthine was not found in five groups: (6) colchicinated plantlets regenerated *in vitro* on NN medium with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³, and after 4 passages transferred to foil tent for five months; (8) control plants cultivated only in the ground (foil tent) from May until September (5 months); (4) plants regenerated *in vitro* on NN medium with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³ then cultivated in foil tent for five months; (5) plants regenerated *in vitro* on NN medium with kinetin 0.5 mg/dm³ and IAA 0.5 mg/dm³ and (11) colchicinated plants regenerated *in vitro* on the medium mentioned in (5) point, cultivated in foil tent for five months. In these five groups the contents of alkaloids were smaller (0.85% to 1.86%). These five groups of plant were blooming during the collection.

Our observations show that catharanthine, monomeric alkaloid is typical for the juvenile (before blooming) stage of plants. The young plantlets regenerated *in vitro* contain catharanthine and vindoline independently on a medium used. After five months of cultivation in foil tent all plants had well developed flowers. At this stage of the ontogenic process catharanthine was not detected. Only in one instance, blooming plants cultivated in the foil tent, previously micropropagated on NN medium with BA, contained catharanthine. It could be caused by the growth regulator used in juvenile stage or season or soil conditions. This problem needs to be solved in course of further investigations. The determination of the growth stage in which the catharanthine production is the highest, is very important as this alkaloid, using the biotechnological method, can be coupled with vindoline to the 3', 4'-anhydrovinblastine – precursor of vinblastine (Kutney et al. 1985).

The total contents of indole alkaloids was higher in plantlets regenerated *in vitro* (2.22 to 2.95%) than in plants regenerated *in vitro* which were in the next step cultivated in foil tents or in control plants cultivated only in the ground (0.85 to 2.16%). The plantlets regenerated on the NN medium with kinetin 0.1 mg/dm³ and IBA 0.5 mg/dm³ or with kinetin 0.5 mg/dm³ and IAA 0.5 mg/dm³ contained a higher total content of alkaloids, equaling 2.95%, and along with other nonidentified alkaloids, both vindoline and catharanthine were present in these plantlets. In no one of the tested groups of plants, the anticancerous alkaloids vincristine (VCR) and vinblastine (VBL), were produced. Despite the fact that in our investigations the R_f value of one of the spots approximated a R_f value of vinblastine sample, they differed one from another with colour.

The results of chemical investigation are summarized in Table 1.

Our achievement, compared to the studies by Hirata et al. (1987) and Miura et al (1988), is the fact that we obtained rooted plants *in vitro*, which can be transferred to the ground

or under a foil tent and developed throughout their whole vegetative process. In those plants we found vindoline (VDL) and catharanthine (CAT); we did not discover any dimeric alkaloids, which were present in the unrooted shoots cultivated by Miura et al. (1988). This can be explained by the different medium composition and culture condition used in our studies. Our results indicated that plant tissue culture can be used for *Catharanthus roseus* plant improvement and selection of the growth stage with optimal composition of secondary products. The fast method of micropropagation of *Catharanthus roseus* plants elaborated by us can be applied by breeders.

ACKNOWLEDGEMENTS

This study was supported by the Committee of Scientific Research in Warsaw, grant no.: 4 0324 91 01.

The authors wish to thank: Prof. Dr hab. J. Kozłowski, Institute of Medicinal Plants, Poznań, for kindly supplying *Catharanthus roseus* seeds, Prof. Dr hab. A. Sadowska, Agriculture University, Warsaw, for her help in cultivating plants under foil tent, Dr J. Ruzkowska, Warsaw University, for her participation in alkaloids determination. We are also grateful Dr. W. Gustowski, Medical Academy, Warsaw, for the vindoline and catharanthine standard substances. We thank Mrs A. Purzycka and Mrs A. Łukasiewicz for technical assistance, and Mr I. Rudnicki for the photographs.

LITERATURE CITED

- ABOU-MANDOUR A.A., FISCHER S., CZYGAN F.C., 1979. Regeneration of intact plants from haploid and diploid callus cells of *Catharanthus roseus*. *Z. Pflanzenphysiol. Bot.* 91: 83-88.
- BAJAJ Y.P.S., FURMANOWA M., OLSZOWSKA O., 1988. Biotechnology of the micropropagation of medicinal and aromatic plants. In: Bajaj Y.P.S. (ed) *Biotechnology in Agriculture and Forestry*, Vol. 4: Medicinal and Aromatic Plants I. Springer-Verlag, Berlin Heidelberg, New York, Tokyo pp. 60-103.
- DHRUVA B., RAMAKRISHNAN T., VAIDYANTHAN C.S., 1977. Studies in *Catharanthus roseus* callus cultures, callus initiation and differentiation. *Current Science* 46 (11): 364-365.
- ENDO T., GOODBODY A., MISAWA M., 1987. Alkaloid production in root and shoot cultures of *Catharanthus roseus*. *Planta Medica* 53 (5): 479-482.
- FARNSWORTH M. R., BLOMSTER R. M., DAMRATOSKI D., MEER A.W., CAMMARATO L.Y., 1964. VI. Evaluation by means of thinlayer chromatography and ceric ammonium sulfate spray reagent. *Lloydia* 24 (7-8): 302-314.
- FURMANOWA M., JÓZEFOWICZ J., OŁĘDZKA H., PIETROSIUK A., 1991. Poszukiwanie alkaloidów bisindolowych w roślinach *Catharanthus roseus* (L.) G. Don mnożonych *in vitro*. VI Konferencja naukowa: Roślinne Kultury Tkankowe w Polsce 1991. Radzików, 32. (in Polish)
- HIRATA K., YAMANAKA A., KURANO N., MIYAMOTO K., MIURA Y., 1987. Production of indole alkaloids in multiple shoot culture of *Catharanthus roseus* (L.) G. Don. *Agric. Biol. Chem.* 51 (5): 1311-1317.
- HIRATA K., HORIUCHI M., ANDO T., ASADA M., MIYAMOTO K., MIURA Y., 1991. Effect of near-ultraviolet light on alkaloid production in multiple shoot cultures of *Catharanthus roseus*. *Planta Medica* 57: 499-500.
- HIRATA K., HORIUCHI M., ANDO T., ASADA M., MIYAMOTO K., MIURA Y., 1992. Stimulation of dimeric alkaloid production by near-ultraviolet light on alkaloid production in multiple shoot cultures of *Catharanthus roseus*. *J. Ferment. Bioeng.* 74: 222-225.

- HIRATA K., MIYAMOTO K., MIURA Y., 1994. III *Catharanthus roseus* L. (Periwinkle): Production of Vindoline and Catharanthine in Multiple Shoot Cultures. In: Bajaj Y.P.S. (ed) Biotechnology in Agriculture and Forestry, Vol. 4: Medicinal and Aromatic Plants I. Springer-Verlag, Berlin Heidelberg, pp. 46-55.
- KRUGER R.I., CAREW D.P., LUI I.H.C., STABA E.J., 1982. Initiation and maintenance and alkaloid content of *Catharanthus roseus* leaf organ cultures. *Planta Medica* 45 (1): 56-57.
- KUTNEY J.P., 1985. Studies in plant tissue culture – potentially important sources of clinically important anticancer agents. III International Conference on Chemistry and Biotechnology and Biology Active Natural Products, September 16-21, 1985 Sofia, Bulgaria, (1): 168-179.
- LEELA G., 1985. Anther culture of *Catharanthus roseus* L. – development of pollen embryoids. *Current Science* 54 (13): 641-642.
- MIURA Y., HIRATA K., 1986. An organ culture of *Catharanthus roseus* capable of producing substantial amount of indole alkaloids. European Patent 0 200 225 A2.
- MIURA Y., HIRATA K., KURANO N., MIYAMOTO K., UCHIDA K., 1988. Formation of vinblastine in multiple shoot culture of *Catharanthus roseus*. *Planta Medica* 54 (1): 18-20.
- MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473.
- NITSCH J.P., NITSCH C., 1969. Haploid plants from pollen grains. *Science* 163: 85-87.
- OLEŹDZKA H., JÓZEFOWICZ J., FURMANOWA M., 1991. Działanie różnych fitogormonów na obrazowanie prądków i wierzchołków pobiegów z pazusznymi pączkami u *Catharanthus roseus*. *Biologia kulturowych kłitek i biotechnologia roślin. Akademia Nauk SSSR. Moskwa. 201-206.* (in Russian).
- OLSZOWSKA O., FURMANOWA M., 1987. Mikrorozmnażanie tymianku pospolitego (*Thymus vulgaris* L.) z fragmentów pędów z węzłami. *Herba Polonica* 33 (2): 137-144. (in Polish).
- RAMAVAT K.G., RAJ BHANSLI R., ARYA H.C., 1978. Shoot formation in *Catharanthus roseus* (L.) G. Don callus cultures. *Current Science* 77 (3): 93-94.
- RUSZKOWSKA J., MACIEREWICZ B., MATUSZEWSKA M., BĄBEL K., WRÓBEL J.T., 1994. Phytochemical screening of *Catharanthus roseus* (L.) G. Don cultivated in Poland. Part I. *Polish Journal of Applied Chemistry* (in press).

CATHARANTHUS ROSEUS (L.) G. DON – REGENERACJA ROŚLIN I ZAWARTOŚĆ W NICH ALKALOIDÓW

STRESZCZENIE

W pracy opisano regenerację roślinek *Catharanthus roseus* (L.) G. Don z pączków szczytowych i bocznych. Z siedmiodniowych siewek odcinano pączki szczytowe i inkubowano na stałej pożywce Nitsch i Nitsch (NN) z dodatkiem kinetyny, benzyloadeniny i kwasu β -indoliloctowego w różnych kombinacjach. Po dwóch miesiącach hodowli, zregenerowane, ukorzenione roślinki cięto i przenoszono na świeżą pożywkę; eksplantaty zawierały pączki szczytowe i boczne. Przeprowadzono cztery pasaże. Otrzymano 200 ukorzenionych roślinek z jednej siewki. Następnie roślinki przenoszono do ziemi, w której rosły pod namiotem foliowym. Po pięciu miesiącach wegetacji zebrano je, wysuszone i zważono. Badania chemiczne liści przeprowadzono metodą grawimetryczną i chromatografią cienkowarstwową. Windolina i katarantyna okazały się dominującymi alkaloidami w roślinach będących w stadium juwenilnym (przed kwitnieniem). Całkowitą zawartość alkaloidów, równą 2,95%, oznaczono grawimetrycznie w liściach roślin, po czwartym pasażu, zregenerowanych *in vitro* na pożywce NN z dodatkiem kinetyny i IBA.

SŁOWA KLUCZOWE: *Catharanthus roseus*, windolina, katarantyna, winblastyna, hodowla tkankowa.