MICROPROPAGATION OF *OENOTHERA BIENNIS* L. AND AN ASSAY OF FATTY ACIDS

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ABSTRACT

The species *Oenothera biennis* L. was micropropagated in *in vitro* cultures. After transferring to the ground plants developed correctly and generated fruits with seeds. The content of oil and γ -linolenic acid in seeds was determined.

KEY WORDS: *Oenothera*, *Onagraceae*, *in vitro* micropropagation, oil, fatty acids.

INTRODUCTION

Oenothera biennis L. – evening primrose (*Onagraceae*) is a common plant in the Polish flora. It can be found on sandy waste land, rubbles, roadsides and industrial dumps. The genus *Oenothera* L. is represented by many species and varieties. In Poland Rostański (1965 a, b; 1985) is engaged in their botanical identification.

Apart from *Oenothera biennis*, other numerous *Oenothera* L. species gain medicinal importance because of nutritious and pharmacological beneficial properties of their oil, conditioned by presence of γ -linolenic acid (Horrobin 1990; Lamer-Zarawska and Hojden 1989 b).

The great interest in evening primrose in our country is evidenced by abstracts of many papers (including clinical and pharmacological ones) presented at the Symposium in Łódź-Tuszyn, 1992. They concerned investigations on oil of *Oenothera paradoxa* Hudziok.

On the ground of many year's studies on cultivation and selection of local *Oenothera* species (Lamer-Zarawska and Hojden 1989, a; Hojden and Lamer-Zarawska 1990, Lamer-Zarawska et al. 1989, 1990), *Oenothera paradoxa* Hudziok was introduced into cultivation in Poland. This species has many valuable features: high content of oil in seeds, increased γ -linolenic acid content in oil, advantageous cultivation parameters such as one-year's vegetation, high yield, leaf fall at fruit ripening etc. Among other things oil from this species is used in Polish phytotherapy as capsules named „Oeparol” (Agropharm). The same name has a series of cosmetics (mostly creams and gels).

The aim of our studies was micropropagation of the species *O. biennis* L. in *in vitro* culture and later ground cultivation of plants to obtain seeds as medicinal material. We wanted to determine the oil content and composition of the fraction of fatty acids in these seeds. This paper starts a series of studies on micropropagation of various species and varieties of *Oenothera* L.

MATERIAL AND METHODS

In vitro cultures

The tissue culture was established from *O. biennis* L. seeds (total amount – 300) obtained in 1990 from the collection of the Department of Pharmaceutical Biology and Botany, Medical Academy in Wrocław. The preparation of seeds, their sterilisation and conditions of the cultures' growth have been described earlier (Skrzypczak et al. 1988). Seedlings (about 240) obtained from the seeds were used to start micropropagation and callus culture. Basic medium of Murashige and Skoog (MS, 1962) was used, supplemented with various regulators of plant growth and development (Table 1). About 10 explants were put on each modification of medium. Experiments were repeated usually 2-3 times. The plantlets obtained by means of micropropagation were transferred from *in vitro* conditions to pots with soil. Then in May 1991 they were planted on experimental plots of the Department of Pharmaceutical Biology and Botany, Medical Academy in Wrocław. Plants were cultivated in biennial cycle. For control and comparison, plants from seeds sowed directly to the soil were grown. In autumn 1992 seeds from both types of plants were collected.

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TABLE 1. Explants and growth regulators used in tissue culture of *Oenothera biennis* L.

Explant	Results	MS medium with growth regulators (mg dm ⁻³)					
		BAP	NAA	IAA	IBA	Kin	2,4-D
parts of seedlings without rootlets	adventitious buds	2	0.2	0.2			
		1	1				
		1		1			
		1		0.1			
		1	0.5				
		0.5		0.5			
shoots	rooted shoots				0.5		
parts of seedlings without rootlets, hypocotyls, parts of rootlets and leaves	formation of callus					1	1
						1	0.5
						0.5	0.5
		1		1			
		1		0.1			
		1					0.5
							0.5
		1					0.25
		0.2			5		

Abbreviations:

MS – Murashige and Skoog; BAP – benzylaminopurine; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; Kin – kinetin; NAA – α -naphthaleneacetic acid; 2,4-D – 2,4-dichlorophenoxyacetic acid.

Chemical analysis

Oil content and composition of fatty acids fraction were determined from 0.5 g seeds obtained from 3 specimen. Acids were changed into methyl esters (with the help of boron fluoride) and separated by means of gas chromatography (GC).

Separation parameters

GC-HP-58-90 (Hewlett-Packard), flame-ionization detector, capillary column (25 m long) covered by liquid phase film (0.2 μ m thick), column filler – Carbowax 20 μ , temperature of column – 190°C, of detector and doser – 250°C; carrying gas – helium, speed of gas flux – 25 m/sek. 0.5 μ l of 1% solution of methyl esters in n-hexane was put on column.

RESULTS AND DISCUSSION

The tissue culture was derived from whole, undamaged seeds. They were placed on MS medium without addition of growth regulators. After 10-14 days the seeds (80%) started to germinate and after 4 weeks they developed into seedlings. Parts of the seedlings with hypocotyls and without rootlets were placed on MS medium with addition of various growth regulators (Table 1). Most of the adventitious buds (4-10) were obtained on MS medium with BAP (1 mg dm⁻³) and IAA (0.1 mg dm⁻³) or with BAP (1 mg dm⁻³) and NAA (1 mg dm⁻³). Callus appeared on hypocotyls and then (after about 14 days) adventitious buds started to develop (Fig. 1). On MS medium without addition of growth regulators the buds developed into shoots after 2-4 weeks (Fig. 2). The

shoots were made to produce roots on MS medium with addition of IBA. The first roots appeared after 4-7 days. During 2-3 weeks all shoots were rooted and roots attained a length of 2-3 cm (Fig. 3). Duration of the culture, from placing the seeds on medium to transfer of the plants to the ground amounted to about 4 months (Fig. 4). By the first year of vegetation plants developed grand leaf rosettes and by the second year they bloomed and formed seeds. There were no important differences in morphology and yield between plants of *in vitro* and ground origin.

During our investigations with *in vitro* cultures we obtained callus culture of *O. biennis*. Parts of seedlings obtained *in vitro* (e.g. Fig. 5 and 6) were placed on MS medium with various growth regulators (Table 1). The best medium for callus formation turned out to be an MS one enriched with BAP (1 mg dm⁻³) and 2, 4-D (0.25 mg dm⁻³) and also one supplemented with kin (1 mg dm⁻³) and 2,4-D (0.5 mg dm⁻³). Callus tissues were pale-green, sometimes with pink tint on the surface. They grew slowly and didn't differentiate.

Table 1 shows only these variants of media which gave satisfactory results. Much more of them were used during our investigations. For example, apart from MS medium with IBA, MS medium enriched with IAA was used to root the shoots. However, the obtained roots were thick, short, stiff and broke off when planting into the soil. Sometimes rooting started on MS medium without addition of growth regulators. Plants formed few roots and didn't take root in the soil.

There were no infections in *Oenothera biennis in vitro* cultures. However, we had a lot of troubles with, sterilisation of seeds of other *Oenothera* species (unpublished data).

Plate I Micropropagation of *Oenothera biennis* L.

Fig. 1. Adventitious buds developing from hypocotyl on MS medium with BAP (1 mg dm^{-3}) and IAA (0.1 mg dm^{-3}); bar = 1 cm.

Fig. 2. Developing shoots on MS medium without growth regulators; bar = 1 cm

Fig. 3. Rooted regenerated shoots on MS medium with IBA (0.5 mg dm^{-3}); bar = 1 cm.

Fig. 4. *Oenothera biennis* L. plant from *in vitro* culture; bar = 1 cm.

Plate II Callus culture of *Oenothera biennis* L.

Fig. 5. Callus forming from segments of leaf on MS medium with 2,4-D (0. 25 mg dm⁻³) and BAP (1 mg dm⁻³); bar = 1 cm.

Fig. 6. Callus forming from segments of rootlets on MS medium with 2,4-D (0. 25 mg dm⁻³) and BAP (1 mg dm⁻³); bar = 1 cm.

TABLE 2. Content of oil and fatty acids in seeds of *Oenothera biennis* L.

Species	oil content [%]	content of fatty acids [%]				
		palmitic	stearic	oleic	linolic	γ-linolenic
<i>O. biennis</i> L. from tissue culture	24.0	6.59	1.73	10.06	71.92	8.15
<i>O. biennis</i> L. from ground cultivation	22.5	6.54	1.70	10.84	71.53	7.72

The content of oil in dry seeds was determined by the method described earlier (Lamer-Zarawska et al. 1989). Fatty acids fractions were separated by means of GC after conversion into methyl esters. The results are shown in Table 2. The content of oil and γ-linolenic acid was a little higher in seeds from plants derived from *in vitro* cultures (3 specimen – 3 plants obtained from ground cultivation after *in vitro* propagation) than in comparative material from ground cultivation.

Species *Oenothera biennis* L. can be multiplied by means of *in vitro* cultures in a relatively short period. Preliminary results of chemical assay are encouraging to later investigation, because plants obtained from *in vitro* cultures usually have lower content of secondary metabolites than ground plants.

The content of γ-linolenic acid in callus culture should be checked, too. Japanese authors (Matsumura et al. 1987; Osamu and Tadashi 1987) have found that γ-linolenic acid was biosynthesized in callus culture of *O. biennis*. The cul-

ture grew in dark on MS medium enriched with 2, 4-D and kin or NAA and kin. The content of γ-linolenic acid was 6.7% (Osamu and Tadashi 1987). Magnus et al. (1992) proved that products of alanine and lysine conjugation are active in initiation and development of callus from *Oenothera* leaves.

We found no literature reports on micropropagation of *O. biennis*. Japanese authors (Suzuki et al. 1990) were engaged in *in vitro* propagation of *Oenothera erythrosepala* Borbás by shoot apex culture on MS medium enriched with NAA and BAP in various concentrations.

During our investigations we encountered some problems which should be solved. Studies on *in vitro* cultures of genus *Oenothera* L. are continued in our Departments. The effects of temperature regimes during cultivation on the fatty acid composition of evening primrose oil are also studied according to the work of Levy (1993).

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MIKROROZMNAŻANIE GATUNKU *OENOTHERA BIENNIS* L.
I ANALIZA KWASÓW TŁUSZCZOWYCH

STRESZCZENIE

Z fragmentów siewek z hypokotylami, bez korzonków, otrzymanych *in vitro* uzyskano rośliny i tkanki kalusowe *Oenothera biennis* L. (*Onagraceae*). Stosowano pożywkę podstawową Murashige i Skooge'a wzbogacaną różnymi regulatorami wzrostu. Rośliny wysadzone do gruntu rozwijały się prawidłowo i w drugim roku wegetacji wydały nasiona, w których oznaczono zawartość oleju. Metodą chromatografii gazowej rozdzielono frakcję kwasów tłuszczowych po ich uprzednim przeprowadzeniu w estry metylowe. Zawartość oleju, a w nim kwasu γ -linolenowego, była nieco wyższa w nasionach roślin z kultur *in vitro* w porównaniu z nasionami rośliny gruntowej.

SŁOWA KLUCZOWE: *Oenothera*, *Onagraceae*, mikro rozmnażanie *in vitro*, olej, kwasy tłuszczowe.