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THE LIPIDS IN TISSUE CULTURES OF BRASSICA NAPUS

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Introduction

Modern biological studies have made it possible to cultivate plant tissues and organs under sterile conditions on nutritional media, in just the same manner in which microorganisms are cultivated (STREET, 1973). Thus, any plant desired for biochemical studies can now be propagated and preserved in form of its tissue culture under controlled laboratory conditions irrespective of the season and/or the weather. It is even possible to transfer a plant tissue culture, which consists of a mass of nondifferentiated cells, back into the differentiated normal plant, a phenomenon commonly known as totipotency.

Two types of tissue cultures are known, those growing on the surface of a solid agar medium, i.e., callus cultures and those growing dispersed in a liquid medium, i.e., suspension cultures.

The objectives of the present investigation were to characterize the different lipid classes of rape cultures in relation to the growth conditions, to study the fatty acid composition of the total lipids, and to test these cultures for glucosinolates which are known to occur in rapeseed.

Experimental

Tissue cultures: Mature seeds of B. napus were sterilized by soaking in 0.2 % aqueous mercuric chloride solution under low pressure for 15 minutes. The seeds were then rinsed thrice with sterile water and transferred aseptically into sterile Petri dishes for germination. After three days, the sterile seedlings were aseptically inoculated onto sterile solid RT_1-medium (MURASHIGE and SKOOG, 1962) and callus cultures were allowed to develop at 26° C in the dark for 3 weeks. The cultures were transferred onto sterile fresh medium every two to three weeks.

Suspension cultures were derived by aseptically inoculating pieces of callus culture about 0.5 g fresh weight, into 50 ml aliquots of $RT_{0.1}$-liquid medium (MURASHIGE and SKOOG, 1962) dispensed in 250 ml conical flasks, and shaking the cultures at 26° C during the whole growth period to insure adequate aeration. The suspension cultures were grown under different conditions of aeration and illumination as specified in tables 1 and 3.

Lipid extraction and analysis: The tissues were harvested by filtration and washed three times with tap water. The total lipids were extracted with propanol-chloroform (NICHOLS, 1964) and purified (FOLCH et al., 1957)

following established procedures.

The nonpolar lipids were fractionated by thin-layer chromatography on silicic acid using the solvent hexane-diethyl ether-acetic acid, 90:10:1, v/v/v (MANGOLD and MALINS, 1960); the ionic and other polar lipids were fractionated with the solvent chloroform-methanol-acetic acid, 80:25:1, v/v/v (NICHOLS, 1964). The lipid classes were detected by charring after spraying the plates with 50 % aqueous sulfuric acid, and they were identified by comparing their migration rates to those of authentic samples and by their color reactions with specific spray reagents (DITTMER and LESTER, 1964; SIAKOTOS and ROUSER, 1965). For quantitative analysis, total lipids were fractionated on layers of silicic acid, 0.5 mm in thickness, and eluted with chloroform-diethyl ether-methanol, 1:1:1, v/v/v (KATES, 1972). Aliquots were dried, and the lipids determined gravimetrically.
Aliquots of the total lipids and of individual lipid classes were subjected to methanolysis and the resulting methyl esters were purified by thin-layer chromatography on silicic acid (CHALVARDJIAN, 1964). The mixtures of methyl esters were analyzed by gas chromatography on a Hewlett-Packard instrument, model 5750 G, equipped with a flame ionization detector, using a column, 6ft x 1/8", packed with 15 % DEGS on Anakrom D, 100/120 mesh, at 176° C.

The sterols were purified by thin-layer chromatography on silicic acid using the solvent hexane-diethyl ether, 80:20, and subsequently analyzed by gas chromatography on a column 12ft x 1/8", packed with 3 % OV17 on Supelcoport, 80-100 mesh, at 265° C.

Analysis of glucosinolates: The lipid-free residues of the tissues were analyzed for glucosinolates as their aglycones (APPELQVIST and JOSEFSSON, 1967). The enzyme myrosinase required for these analyses was prepared following established procedures (SCHWIMMER, 1961).

Results and discussion

The growth-index (G.I.) values as well as the total lipid contents of suspension cultures grown for two weeks under different conditions of aeration and illumination are presented in Table 1. It is apparent that the total lipid contents are not significantly affected by the environmental conditions, but are commonly slightly higher in two weeks old cultures than in one week old cultures. The growth activity is highest during the first week of incubation and is commonly higher in aerated cultures than in nonaerated ones, but is not likely to be affected by illumination.

Analyses of the lipid classes from the different cultures revealed the abundance of sterols, steryl esters, steryl glycosides and esterified steryl glycosides in addition to highly polar unknown glycolipids which yielded sterols after acid hydrolysis (LEPAGE, 1964; GAVER and SWEELEY, 1965). Lipid classes detected in relatively low concentrations were trigly-

Table 1: Growth index values and total lipid contents of B. napus suspension cultures grown under different environmental conditions

Age of the cultures	Aerated				Non-aerated			
	in light		in dark		in light		in dark	
	GI *	Total ** Lipids (%)	GI *	Total ** Lipids (%)	GI *	Total ** Lipids (%)	GI *	Total ** Lipids (%)
1	4.45	7.0	3.69	6.9	2.28	7.1	2.21	7.0
2	1.42	8.1	1.37	7.3	1.16	7.9	1.23	8.1

* GI: Growth Index values of one week old cultures were calculated by dividing the weight of one week old tissues by the weight of inoculated samples; GI-values of two weeks old cultures were obtained by dividing the weight of two weeks old tissues by the weight of one week old tissues.

** Total lipids calculated as percentage of dry tissues' weight.

cerides, ethanol-aminephosphoglycerides, cholinephosphoglycerides, phosphatidic acids and free fatty acids. Monogalactosyldiglycerides, digalactosyldiglycerides and sulfoquinovosyldiglycerides were present in B. napus tissue cultures only in traces, if at all. These compounds are known to be abundant in green plant organs and are assumed to be involved in the process of photosynthesis (KATES, 1970). Plant tissue cultures are colorless, non-photosynthetic systems growing heterotrophically on organic media; hence the lack of galactolipids and sulfolipids in B. napus tissue cultures is not surprising. Squalene was also detected in the lipid extracts and its concentration was found to vary according to the conditions of aeration.

Gas chromatographic analyses of the total sterols in tissue cultures revealed the predominance of ß-sitosterol. In addition, small proportions of stigmasterol and campesterol, and traces of brassicasterol, if any, were present.

For the purpose of comparison, Table 2 presents the fatty acid composition of the total lipids from seeds and tissue cultures of B. napus. Compared to seeds the cultures are characterized by a much lower content of erucic acid but higher contents of lauric , myristic, palmitic and stearic acids. In general, the fatty acids of the total lipids from the cultures are more saturated than those from the seeds.

It has been reported earlier that the triglycerides extracted from tissue cultures of B. napus are characterized by a very low content of erucic acid (SHIN et al., 1971; STABA et al., 1971). The results of the present study are in accord with this finding and indicate further that the low erucic acid content is not characteristic of the triglycerides alone but of the total lipids from the cultures as a whole.

Glucosinolates could neither be detected in callus cultures nor in suspension cultures of B. napus. In this connection it is to be mentioned that small amounts of glucosinolates have been detected in suspension cultures of other Cruciferae (KIRKLAND et al., 1971).

Because of the high content of tissue cultures in sterols and other alicyclic lipids, we became interested in studying the relation between the environmental conditions prevailing during growth and the amounts of sterols and other alicyclic lipids synthesized by the cultures (RADWAN and MANGOLD, 1974). The results presented in Table 3 indicate that illumination has no considerable effect on the biosynthesis of sterols and other alicyclic lipids. On the other hand, aerated cultures are characterized by higher levels of alicyclic lipids and lower levels of squalene than non-aerated cultures. In view of the fact that the transformation of squalene into sterols in vivo requires molecular oxygen (TCHEN and BLOCH, 1956), the role of aeration in controlling the concentrations of these lipids in tissue cultures is easily understood.

It was observed that the free fatty acids as well as the fatty acids bound in

Table 2: Fatty acid composition* of the total lipids from seeds and suspension cultures of B. napus

	Chain length: Number of double bonds																
	12:0	14:0	14:1	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:1	22:0	22:1	24:0	24:1
Seeds	tr.	tr.	-	-	-	4.47	0.28	-	-	1.4	18.2	12.1	4.3	13.7	0.5	42.2	0.4	1.1
Tissue culture **	16.9	7.7	0.2	0.2	0.1	18.1	2.0	0.2	0.3	4.3	28.1	7.9	4.5	5.9	0.2	0.7	0.7	-

* Data are expressed in relative percentages

** Suspension cultures grown for two weeks aerobically in the dark

tr. = trace

\- = not detected

Table 3: Effect of illumination and aeration on the squalene and alicyclic lipids contents of B. napus suspension cultures

Age of the cultures (week)	Aerated				Non-aerated			
	in light		in dark		in light		in dark	
	Squalene	AL *	Squalene	AL *	Squalene	AL *	Squalene	AL *
1	9.8	50.6	15.6	50.0	26.0	34.0	23.4	36.2
2	4.0	53.5	10.3	61.5	26.4	25.3	25.2	27.5

Results are expressed as percentage of total lipids
* AL = Alicyclic lipids: sterols, sterylesters, sterol glycosides and esterified sterol glycosides

triglycerides, steryl esters, and esterified steryl glycosides included higher proportions of saturated very long-chain fatty acids (C_{22}-C_{26}) in the non-aerated cultures than in the aerated ones. The role of aeration in fatty acid elongation is obscure and needs further investigation.

In view of the low triglyceride content and the high level of sterols and other alicyclic lipids, tissue cultures of B. napus can not be considered suitable commercial sources of oil and can not be recommended as foodstuff.

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