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FATTY ACIDS AND TRIACYLGLYCEROLS IN SEEDS OF PINACEAE SPECIES

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Abstract—The fatty acid and fully hydrogenated triacylglycerol composition in the seeds of *Pinus massoniana*, *P. merkusii*, *P. caribea*, *P. kesyia*, *P. dalatensis*, *P. krempfii*, *P. koraiensis* and *P. siberya* were determined. The non-methylene-interrupted polyenoic fatty acids amounted to 12.0–26.4% of total acids. The distribution of triacylglycerols of the seeds according to their molecular weights was similar to that of common seed oil. The possible chemosystematic application of the fatty acid composition of seeds of Pinaceae is discussed.

INTRODUCTION

A distinctive feature of Pinaceae plants (Gymnospermae) is the presence of a significant amount of non-methylene-interrupted polyenoic (NMIP) fatty acids (FAs) in lipids obtained from the leaves (needles) and seeds [1–6]. The most interesting NMIP acid found in the seeds of Pinaceae is pinolenic acid (18:3 Δ5,9,12) [7, 8]. It takes part in eicosanoid biosynthesis, influences blood pressure [9] and is used in a dietary nutrition [10]. The presence of uncommon NMIP acids in the total lipids of Pinaceae allows data on FA composition to be used for the chemometric comparison of a number of plant species by multivariate statistical analysis [11]. Therefore, the step-by-step study of FAs in Pinaceae is important not only for plant biochemistry, but also for plant chemosystematization.

It is known that six species of the genus *Pinus* occur in Vietnam, and two of them (*P. dalatensis* and *P. krempfii*) are endemic. A comparative study of FA composition of the seeds of these Vietnamese species has not been performed, and data on triacylglycerol composition not published. We investigated the FA and triacylglycerol composition of the seed oils of six *Pinus* species from Vietnam and compared it to that of two *Pinus* species from the far eastern region of Russia.

RESULTS AND DISCUSSION

The total lipid contents of the seeds with testa are shown in Table 1. The amount of total lipids ranged from 10.3% (*P. merkusii*) to values exceeding 21% (*P. massoniana* and *P. caribea*). The FA composition of total seed lipids of eight *Pinus* species was similar in spite of the geographical differences (Table 1). The main FAs with straight chains were 18:2 ($n-6$) and 18:1 ($n-9$). Saturated FAs accounted for less than 10% of the total FA. The seed lipids of the investigated species were rich in NMIP FAs. The main NMIP FAs were 18:3 (5,9,12), 20:3(5,11,14) and 18:2 (5,9) with a smaller amount of 20:2 (5,9). The greatest contents of 18:2(5,9) and 18:3(5,9,12) were detected in *P. massoniana* (4.5 and 18.4%, respectively); *P. kesyia* was rich in 20:3 (5,11,14) (5.2%). Two species from the far eastern region (*P. koraiensis* and *P. siberya*) were characterized by a low content of 20:3 (5,11,14), as compared to Vietnamese species, but, in general, the total profile of seed FAs of all investigated species was highly similar.

The distribution of triacylglycerol (TG) molecular species according to their molecular weights, after full catalytic hydrogenation, is given in Table 2. Six molecular species of TG (from C-48 to C-58) were identified. The main TG was trioctadecylglycerol (C-54, up to 77.2%). We did not find C-60 TG, i.e. C-20 acyl residues were incorporated into the TG molecule only in combination with shorter-chain FAs, but not separately. There was no significant difference between the total TG profiles of all studied species and that of common seed oils, which have a similar ratio of C-18 and C-20 fatty acids [12]. Hence, the presence of NMIP acids in total FAs hardly influences the distribution of TG according to molecular weights.

The use of lipid composition of needles for comparison among Pinaceae species have been reported [11, 13]. It is possible that the FA composition of the seed lipids, in contrast to leaf lipids, may contain some

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Table 1. Fatty acid composition of seeds of Pinaceae (% of total FA content) and content of total lipids (%) in wet seeds

	Species number							
Fatty acid	1	2	3	4	5	6	7	8
14:0	0.1	0.1	0.1	0.1	0.1	0.1	tr.	tr.
16:0*	4.1	5.3	5.4	5.5	7.1	6.1	4.7	4.4
16:1 ($n-9$)	0.2	0.1	0.1	0.1	0.1	0.1	tr	tr
16:1 ($n-7$)*	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.1
18:0*	1.8	4.6	2.0	1.4	1.8	2.2	2.0	2.6
18:1 ($n-9$)*	17.0	16.7	19.3	18.7	20.7	24.3	27.0	25.1
18:1 ($n-7$)*	0.8	0.5	0.7	0.7	1.4	0.4	0.3	0.5
18:2 NMI*,†	4.5	1.7	2.5	2.7	2.8	3.3	2.0	1.7
18:2 ($n-6$)*	47.4	54.9	46.0	43.6	47.4	51.1	45.3	43.2
18:3 NMI*	18.4	10.3	18.3	18.2	12.2	7.3	14.9	18.1
18:3 ($n-3$)*	0.4	1.3	0.3	0.3	0.4	0.4	0.2	0.2
20:0*	0.2	0.4	0.4	0.3	0.4	0.4	0.3	0.3
20:1 (n − 9)*	0.3	0.4	0.7	0.8	0.8	0.6	1.1	1.3
20:2 NMI*	0.4	0.3	0.3	0.5	0.4	0.1	0.1	0.1
20:2 ($n-6$)*	0.6	0.8	0.6	1.0	0.7	0.7	0.5	0.6
20:3 NMI*	3.1	2.6	2.5	5.2	1.9	1.3	0.8	0.9
20:3 ($n-6$)	0.2	—	0.3	0.5	—	0.2	—	0.1
Other	0.5	—	0.5	0.3	1.4	1.3	0.7	0.8
Total lipids	21.8	10.3	21.8	17.5	14.2	15.6	20.9	18.1

1: *Pinus massoniana*; 2: *P merkusii*; 3: *P. caribea*; 4: *P. kesyia*; 5: *P. dalatensis*; 6: *P. krempfii*; 7; *P. koraiensis*; 8: *P. siberya*. Species 1–6 were collected in Vietnam, and species 7 and 8 were collected in Russia (far eastern region).

tr = traces (<0.05%).

*Use for principal component analysis.

†Non-methylene-interrupted fatty acids: 18:2 Δ5,9; 18:3 Δ5,9,12; 20:2 Δ5,11; 20:3 Δ5,11,14.

specific FAs, which are often correlated to plant family [14]. To establish a chemotaxonomical significance of the seed FAs for a determination of the relationship between Pinaceae species and other families of Gymnospermae, we processed our data (Table 1) and the data on the FA composition of Gymnospermae seeds published earlier [2], using principal component analysis. Fourteen components used for multivariate data treatment are indicated in Table 1. Figure 1 is a plot of the plant species against the first two principal component axes. Of the total variation in the data, 64.6% is accounted for by these first two principal components (PC); 51.3% of the total variation in the data is along the first PC axis. The differences in the content of 18:3 NMI and 18:3 ($n = 3$) are the main cause of this variation; smaller contribution is made by 16:1 ($n-7$), 18:2 NMI and 20:3 NMI; 13.3% of the variation is along the second PC axis. This variation is caused by differences in the content of 16:1 ($n-7$), 18:3 ($n-3$) and 20:3 NMI, and to a lesser degree, 20:2 ($n-6$).

Figure 1 shows a distinct group of species that consists of eight investigated species of the genus *Pinus* from Vietnam (1–6) and far eastern region (7, 8), and four *Pinus* species from Japan described by Takagi and Itabashi [2]. *Pinus jezoensis* (15), *Larix leptolepis* (16) and *Cedrus deodara* (17), included into this group (Fig. 1), also belong to the family Pinaceae. Other species of Gymnospermae are located on the plot relatively far from the above group of Pinaceae. When principal

Table 2. Triacylglycerol composition (%) of the seeds

	Triacylglycerols					
Species	C-48	C-50	C-52	C-54	C-56	C-58
P. massoniana	tr	4.3	21.7	64.7	8.8	0.5
P. caribea	tr	0.3	15.0	71.5	12.1	1.1
P. merkusii	0.2	1.1	15.3	73.5	9.0	tr
P. kesyia	tr	1.0	15.0	65.8	17.3	1.0
P. dalatensis	tr	2.6	15.4	71.8	9.7	0.5
P. krempfii	tr	1.3	16.2	75.6	7.0	tr
P. koraiensis	tr	0.4	16.6	76.1	6.6	0.3
P. siberya	tr	2.0	13.5	77.2	7.3	tr

tr = traces (<0.05%).

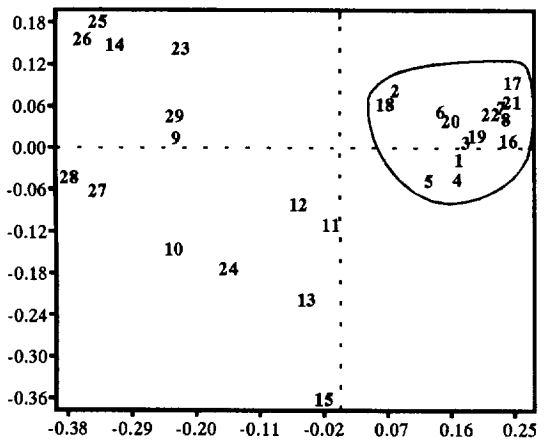

Fig. 1. Plot of chemometric data from the seeds of 29 species of Gymnospermae against the first two axes in principal components analysis. 1, *Pinus massoniana*; 2, *P. merkusii*; 3, *P. caribea*; 4, *P. kesyia*; 5, *P. dalatensis*; 6, *P. krempfii*; 7, *P. koraiensis*; 8, *P. siberya*; 9, *Cycas revoluta*; 10, *Ginkgo biloba*; 11, *Taxus cuspidata*; 12, *Taxus canadensis*; 13, *Torreya nucifera*; 14, *Podocarpus macrophylla*; 15, *Podocarpus nagi*; 16, *Picea jezoensis*; 17, *Larix leptolepis*; 18, *Cedrus deodara*; 19, *P. densiflora*; 20, *P. thunbergii*; 21, *P. koraiensis*; 22, *P. pentaphylla*; 23, *Taxodium districhum*; 24, *Sciadopitys verticillata*; 25, *Cryptomeria japonica*; 26, *Chamaecyparis pisifera*; 27, *Juniperus rigida*; 28, *J. chinensis*; 29, *Ephedra sinica*.

component analysis was performed separately for Pinaceae species (plot not shown), there was a good correlation between two samples of *P. koraiensis* from Japan and far eastern region, but the species did not group according to sampling areas.

Thus, the FA composition of seeds may be used as a chemotaxonomic characteristic of a plant species of Gymnospermae. The data on seed FA composition clearly reflect a close botanical relationship in the family Pinaceae. The differences among Pinaceae species hardly depend on climatic and geographic factors, but are species-specific.

EXPERIMENTAL

Plant seeds were received from the Forest Science Institute of Vietnam and from the Research Department of the Botanical Garden, Vladivostok (Russia) in May 1992. Total lipids were extracted with $CHCl_3$–MeOH (1:2) using the method of ref. [15]. Total lipid contents of seeds were determined gravimetrically. TG frs of total lipids were obtained by prep. TLC with hexane–Et_2O–HOAc (8:2:0.1). Hydrogenation of the TG was carried out in MeOH over PtO_2 for 2 hr.

FA methyl esters (FAME) were prepd by consecutive treatment of total lipids with 1% NaOMe in MeOH and 5% HCl in MeOH according to ref. [16] and purified by TLC in C_6H_6. Analysis of FAME was carried out by GC with FID, a fused silica capillary column (30 m × 0.25 mm i.d.) with Supelcowax 10M, split ratio 1:30 and carrier gas He (1 ml min^{-1}). The column and detector temp. was 210°; the injector temp. was 240°. Identification of FAME was confirmed by chromatographic comparison with authentic standards and calculation of ECL [17]. GC of hydrogenated TG was performed on a fused silica capillary column (1.2 m × 0.32 mm i.d.) with OV-1 (bonded) and He as carrier gas. The injector temp. was 370°. The column and detector temp. was programmed from 290 to 330° at 2° min^{-1} and maintained at 330° for 10 min. The TG was identified by use of authentic standards.

Data for FA peak areas used for multivariate statistical treatment were scaled by taking the logarithm of numbers. Principal components analysis of normalized peaks was performed using the program A Multivariate Statistics Package Plus, ver. 21k.

REFERENCES

1. Diamantoglou, S. and Kull, U. (1981) *Z. Pflanzenphysiol.* **103**, 157.
2. Takagi, T. and Itabashi, Y. (1982) *Lipids* **17**, 716.
3. Vickery, J. R., Whitfield, F. B., Ford, G. L. and Kennett B. H. (1984) *J. Am. Oil Chem. Soc.* **61**, 573.
4. Andrikopoulos, N. K., Demopoulos, C. A., Siafakakapadai, A. and Kapoulas, V. M. (1985) *Phytochemistry* **24**, 2953.
5. Janick, J. and Whipkey, A. (1988) *Hortscience* **23**, 734.
6. Sagreronieves, L. (1992) *J. Sci. Food Agric.* **59**, 413.
7. Hase, A., Alapeijari, M., Kaltia, S. and Matikainen, J. (1992) *J. Am. Oil Chem. Soc.* **69**, 832.
8. Yoon, T. H., Im, K. J., Koh, E. T. and Ju, J. S. (1989) *Nutrit. Res.* **9**, 357.
9. Sugano, M., Ikeda, I., Wakamatsu, K. and Oka, T. (1994) *Br. J. Nutrit.* **72**, 775.
10. Holman, R. T. (1981) *Chem. Ind.* **20**, 704.
11. Grahl-Nielsen, O., Mjaavatten, O. and Ovstedal, D. O. (1991) *Nord. J. Botany* **11**, 613.
12. Padley, F. B., Grunstone, F. D. and Harwood, J. L. (1986) in *The Lipid Handbook* (Padley, F. B., Gunstone, F. D. and Harwood, J. L., eds), pp. 98–103. Chapman and Hall, London.
13. Chalchat, J. R., Garry, R. P. and Gorunpvic, M. S. (1994) *Pharmazie* **49**, 852.
14. Aitzetmuller, K. (1993) *J. High Resolut. Chromatogr.* **16**, 488.
15. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
16. Carreau, J. P. and Dubacq, J. P. (1978) *J. Chromatogr.* **151**, 384.
17. Christie, W. W. (1988) *J. Chromatogr.* **447**, 305.