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RESEARCH PAPER

Fatty acid composition of gametophytes of *Matteuccia struthiopteris* (L.) Tod. (Onocleaceae, Polypodiophyta)

Eduard V. Nekrasov[1]*, Lyudmila A. Shelikhan[1] & Vasily I. Svetashev[2]

Eduard V. Nekrasov[1]*
e-mail: ed_nekrasov@mail.ru

Lyudmila A. Shelikhan[1]
e-mail: solecito91@mail.ru

Vasily I. Svetashev[2]
e-mail: vsvetashev@mail.ru

[1] Amur Branch of the Botanical Garden-Institute FEB RAS, Blagoveshchensk, Russia

[2] National Scientific Center of Marine Biology FEB RAS, Vladivostok, Russia

* corresponding author

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ABSTRACT

Fern gametophytes are considered as a convenient model for biotechnological studies. Ferns produce arachidonic (20:4n-6, ARA) and eicosapentaenoic (20:5n-3, EPA) fatty acids, which have biological effects in the human body. The aim of this study was comparison of fatty acid composition of gametophytes cultivated *in vitro* and young fronds of sporophytes grown outdoors of the fern *Matteuccia struthiopteris* (L.) Tod. The fatty acid composition was not different in gametophytes and sporophytes, but their content varied. Gametophytes contained more ARA (9.5 % of total fatty acids) and less EPA (0.6 %) as compared with sporophytes (5.4 % and 3.3 %, respectively). Both gametophytes and sporophytes contained potential metabolic precursors of ARA and EPA. With exception of some minor components, distribution of fatty acids in lipid fractions (non-polar, glyco- and phospholipids) was similar for sporophytes and gametophytes. Thus, gametophytes of *M. struthiopteris* can be a potential model for studies of ARA and EPA biosynthesis in ferns.

Keywords: *Matteuccia struthiopteris*, gametophyte, sporophyte, fatty acids, arachidonic acid, eicosapentaenoic acid

РЕЗЮМЕ

Некрасов Э.В., Шелихан Л.А., Светашев В.И. Состав жирных кислот гаметофитов *Matteuccia struthiopteris* (L.) Tod. (Onocleaceae, Polypodiophyta). Гаметофиты папоротников рассматриваются как удобные модели для биотехнологических исследований. Папоротники способны синтезировать арахидоновую (20:4n-6, АК) и эйкозапентаеновую (20:5n-3, ЭПК) жирные кислоты, которые играют важные функции в организме человека. Целью этого исследования был сравнительный анализ состава и содержания жирных кислот в гаметофитах, выращенных i*n vitro*, и молодых вайях спорофитов, растущих в открытом грунте, папоротника *Matteuccia struthiopteris* (L.) Tod. Состав жирных кислот не различался между гаметофитами и спорофитами, но их содержание варьировало. Гаметофиты содержали больше АК (9,5% от суммы жирных кислот) и меньше ЭПК (0,6%) по сравнению со спорофитами (5,4% и 3,3%, соответственно). Как в гаметофитах, так и спорофитах присутствовали потенциальные метаболические предшественники АК и ЭПК. За исключением некоторых минорных компонентов, распределение жирных кислот по фракциям липидов (неполярных, глико- и фосфолипидов) было сходным для спорофитов и гаметофитов. Таким образом, гаметофиты *M. struthiopteris* могут служить потенциальной моделью для изучения биосинтеза АК и ЭПК в папоротниках.

Ключевые слова: *Matteuccia struthiopteris*, гаметофит, спорофит, жирные кислоты, арахидоновая кислота, эйкозапентаеновая кислота

Fatty acid composition of ferns is characterized by presence of fatty acids which are uncommon or absent in gymnosperms and angiosperms. In contrast to the seed plants, fern green tissue contains long-chain polyunsaturated fatty acids with 20 carbon atoms and 4 (20:4n-6, arachidonic acid) or 5 (20:5n-3, eicosapentaenoic acid) double bonds. These fatty acids are widely distributed in the mosses and lower plants (algae) and play different important roles in human physiology (Zarate et al. 2017, Christie 2018). Due to the presence of the omega-3 (eicosapentaenoic acid) and omega-6 (arachidonic, γ-linoleic, dihomo-γ-linolenic) fatty acids, immature fronds of the edible fern *Matteuccia struthiopteris* (L.) Tod. were recommended as a healthful vegetable in the human diet (DeLong et al. 2011). Biosynthesis of ARA and EPA in ferns remains mostly unresolved with only one study published on the subject almost fifty years ago (High et al. 1969).

Fern gametophytes are independently living, autotrophic, haploid organisms which give rise to sporophytes. In addition, they can proliferate in the vegetative mode to produce gametophyte clones. These attributes make fern gametophytes as an attractive model for biotechnological studies and genetic manipulations (Johari & Singh 2018). Apparently, gametophytes can be used as a model for study of the long-chain polyunsaturated fatty acids biosynthesis in ferns. So far, fatty acid composition of fern gametophytes

has been determined only for one species (Sato & Furuya 1984). In that study, fatty acids were analyzed in lipid classes of gametophytes in early stages of their development – up to 15 days of germination only.

The aim of this study was comparison of fatty acid composition of gametophytes and sporophyte young fronds of the edible fern *M. struthiopteris* with special attention paid to the long-chain polyunsaturated fatty acids.

MATERIAL AND METHODS

Plant material

Spores of *M. struthiopteris* were collected by I.A. Kreshchenok (Amur Branch of Botanical Garden-Institute of FEB RAS, Blagoveshchensk) in natural populations (The Bureya River valley, Amur Province, Russia) in August 2015. For gametophyte development *in vitro*, the spores were sterilized in 70 % ethanol for 3 min, followed by the commercial bleach "Belizna" diluted with water (1:2, v/v) for 10 min. The spores were rinsed with sterile distilled water 4 times and sown in a Petri dish containing half-strength Murashige and Skoog (1/2MS) medium (Murashige & Skoog 1962) supplemented with MS vitamins, 2 % sucrose, and 0.8 % agar, at pH 5.7. For lipid extraction, fragments of the obtained gametophyte thalli were cultivated in full-strength MS medium supplemented with MS vitamins, 2 % sucrose, 0.8 % agar, containing no plant growth regulators, at a 16 h photoperiod (cool-white florescent light) and room temperature for 75 days. Two batches of gametophytes grown in different vessels were used for lipid extractions (3.38 and 1.54 g, wet weight). Their portions (2.68 and 1.35 g, respectively) were taken for determination of dry weight.

Fronds of the fern sporophytes were collected from plants growing outdoors (The collection of genetic resources of plants of Amur Branch of Botanical Garden-Institute of FEB RAS) in May 2017. The fronds were young, almost unfolded, with apices still coiled. A half of each frond (pinnae of one side of a frond) was taken for lipid extraction (5.26 g, wet weight) and another half was used for determination of dry weight (5.09 g, wet weight). Central ribs of the fronds were discarded. Dry weights (dry wt.) of the fronds and gametophytes were determined by drying the material at 110°C.

Lipid extraction and fatty acid analysis

The plant material was boiled in water for 3 min before lipid extraction. Total lipids were extracted by a modified method of Bligh & Dyer (1959) as described earlier (Kotel'nikova et al. 2004). Lipids (6.8–11.8 mg) were fractionated by solid-phase extraction (SPE) on silica gel (Supelclean LC-Si, SPE tubes 6 mL, 0.5 g, Supelco, USA) by subsequent elution with chloroform (30 ml), acetone (15 ml), methanol (7.5 ml), and chloroform – methanol – water (3:5:2, v/v/v, 7.5 ml). The methanol and chloroform – methanol – water fractions were combined. Fatty acids were analyzed as methyl esters (FAME) by gas-liquid chromatography. For the preparation of FAME, 2–4 mg of total lipids or a fraction obtained by SPE and corresponding to 2–4 mg of initial total lipids, were dissolved in 0.1 ml of toluene, then 1 ml of 2 % H_2SO_4 (wt./wt.) in methanol was added, the vial was flushed with N_2 and incubated at 50°C overnight (Svetashev & Imbs 2014). After addition of water (0.125 ml), FAME were extracted with hexane (0.5 ml and, repeatedly, 0.2 ml) and purified by thin-layer chromatography on silica gel plates in benzene. FAME were analyzed on a Shimadzu GC 2010 gas chromatograph equipped with a Supelcowax 10 capillary column (30 m x 0.25 mm, 0.25 μm film thickness, Supelco). Analysis conditions were as follows: carrier gas (He) was 30 cm/sec; split ratio was 1:20; injector and flame ionization detector temperatures were set at 250°C; the temperature program was 190°C for 47.5 min, then 5°C/min up to 220°C and held for 15 min. Fatty acids were identified by the use of standards and calculation of equivalent chain-lengths (ECL) (Stransky et al. 1997).

RESULTS AND DISCUSSION

We found that gametophytes of *M. struthiopteris* contain less total lipids than the young fronds of its sporophytes (5 vs 11.7 % wt./dry wt.). The fatty acid composition of the fern gametophytes and young fronds are shown in Table 1. Twenty nine fatty acids were identified by GLC with content ≥0.1% of total fatty acids. In gametophytes, the main fatty acids were palmitic (16:0) and α-linolenic (18:3n-3) with content of 26.1 % and 24.3 % of total fatty acids, respectively, followed by linoleic (18:2n-6), 18.5 %, arachidonic (ARA), 9.5 %, and oleic (18:1n-9), 8.1 % acids. All other fatty acids were minor and did not exceed 2 %. The content of eicosapentaenoic acid (EPA) was low (0.6 %) in gametophytes.

The fatty acid composition of sporophytes was similar to that of gametophytes. The major fatty acids of the fronds were also α-linolenic (27.2 % of total), palmitic (22.4 %), linoleic (15.3 %), and oleic (8.5 %), but the content of ARA was lower (5.4 %). There was significantly higher content of EPA (3.3 %) and chloroplast-associated fatty acids, 16:3n-3 and *t*-16:1n-13 (4.8 and 1.8 %, respectively, vs 1.0 and 0.6 % in the gametophytes).

Distribution of fatty acids in fractionations obtained by SPE was similar for the sporophytes and gametophytes (Table 1). The fractions enriched in phospholipids (elution with methanol and the mixture of chloroform – methanol – water) contained the major portions of 16:0, 18:2n-6, ARA, EPA, *t*-16:1n-13, and also the potential metabolic precursors of ARA (18:3n-6, 20:3n-6) and EPA (20:4n-3). The fractions enriched in glycolipids (elution with acetone) were high in 18:3n-3, 18:1n-9, 16:3n-3, and also included significant portions of 16:0, 18:2n-6, EPA, and ARA. Non-polar lipids (elution with chloroform) contained all the major fatty acids in percentages very close for gametophytes and sporophytes: 16:0 (9.2 and 10.8 % of total recovered in all three fractions for gametophytes and sporophytes, respectively), 18:3n-3 (5.5 and 2.7 %), 18:2n-6 (8.1 and 11.6 %), 18:1n-9 (7.6 and 10.3 %), and ARA (10.2 and 9.0 %). EPA was found in this fraction only in the fronds of sporophytes. Conspicuous differences between gametophytes and sporophytes in fatty acid distribution in the fractions were observed only for some minor components: 16:2n-6, 18:0, 18:4n-3, 20:0, 22:0, and 24:0 (Table 1). The fatty acid 18:4n-3 is a potential metabolic precursor of EPA. It was found to have different distribution

Table. Fatty acid composition of gametophytes and sporophytes (young fronds) of *Matteuccia struthiopteris* and their distribution in fractions after solid phase extraction (SPE) on silica

Fatty acid	Gametophytes				Sporophytes			
	Fatty acid content, % of total*	Distribution of fatty acids in fractions after SPE, % of all fractions			Fatty acid content, % of total	Distribution of fatty acids in fractions after SPE, % of all fractions		
		NL	GL	PL		NL	GL	PL
14:0	0.2	49.0	30.5	20.5	0.3	47.3	26.6	26.2
14:1n-7	0.2	80.1	14.8	5.1	0.3	94.9	5.1	–
14:1n-5	1.0	63.9	28.1	8.0	0.4	86.6	9.7	3.7
15:0	0.2	30.8	13.5	55.7	0.1	25.0	18.4	56.7
16:0	26.1	9.2	30.6	60.1	22.4	10.8	39.6	49.6
16:1n-9	0.3	18.5	48.0	33.5	0.7	9.7	49.5	40.9
16:1n-7	0.3	14.2	47.0	38.8	0.4	14.7	52.8	32.6
16:1n-5	0.4	8.8	40.4	50.8	0.1	11.0	44.6	44.4
t-16:1n-13	0.6	–	12.4	87.6	1.8	0.6	11.7	87.6
16:2n-6	0.3	19.7	80.3	–	0.5	–	94.7	5.3
17:0	0.3	31.6	20.2	48.2	0.1	35.6	17.2	47.1
16:3n-3	1.0	–	100.0	–	4.8	–	99.1	0.9
18:0	1.8	22.4	23.5	54.2	1.9	57.5	9.9	32.6
18:1n-9	8.1	7.6	51.1	41.3	8.5	10.3	48.9	40.7
18:1n-7	0.8	7.3	32.5	60.2	0.3	9.9	46.9	43.2
18:1n-5	0.6	8.6	44.0	47.4	0.1	–	38.2	61.8
18:2n-6	18.5	8.1	38.9	53.0	15.3	11.6	22.6	65.8
18:3n-6	1.0	15.5	32.5	52.0	2.0	13.1	16.1	70.8
18:3n-3	24.3	5.5	77.2	17.3	27.2	2.7	90.1	7.2
18:4n-3	0.1	–	–	100.0	0.1	–	52.4	47.6
20:0	0.5	33.5	13.8	52.7	0.7	87.4	2.8	9.8
20:2n-6	0.1	49.8	–	50.2	0.1	53.6	–	46.4
20:3n-6	1.8	7.7	11.3	81.1	1.3	10.7	20.5	68.8
ARA	9.5	10.2	17.9	71.9	5.4	9.0	17.5	73.5
20:4n-3	0.1	–	–	100.0	0.1	–	–	100.0
EPA	0.6	–	44.2	55.8	3.3	9.7	28.8	61.5
22:0	0.7	26.2	20.9	52.9	0.6	28.1	39.0	33.0
23:0	0.1	–	–	–	0.1	–	–	–
24:0	0.7	9.2	29.2	61.6	1.1	32.6	33.8	33.6

Notes: Abbreviations: ARA – arachidonic acid; EPA – eicosapentaenoic acid; NL, GL, and PL – fractions eluted with chloroform, acetone, and methanol, respectively, and enriched in non-polar lipids (NL), glycolipids (GL), and phospholipids (PL); '–' – not detected or less than 0.1 % of total fatty acids. *Means of two replicates are shown.

in gametophytes and sporophytes: while it was exclusively associated with the phospholipid fraction of gametophytes, it was almost equally distributed between the phospholipid and glycolipid fractions of the fern sporophytes.

In contrast to our results, 10-day-old gametophytes of *Adiantum capillus-veneris* L. were found to contain less ARA in different lipid classes than the fern sporophytes (Sato & Furuya 1984). It may be related to the age of gametophytes since we used gametophytes with developed thalli cultivated *in vitro* for a long period of time. Similar to our results, more 16:3, *t*-16:1n-13, and EPA were found in pinnae of the *A. capillus-veneris* sporophytes than in the gametophytes. Also, there was no EPA found in triacylglycerols, a major lipid class of non-polar lipids, of the *A. capillus-veneris* gametophytes (Sato & Furuya 1984).

Biosynthesis of ARA in ferns was suggested as follows: 18:2n-6 → 18:3n-6 → 20:3n-6 → 20:4n-6 (High et al. 1969), which is common for many eukaryotes (Lee et al. 2016). While biosynthesis of EPA in fern has not been investigated yet, a common pathway for eukaryotes is well known: 18:3n-3 → 18:4n-3 → 20:4n-3 → 20:5n-3 (Lee et al. 2016). Since 18:3n-6, 20:3n-6, 18:4n-3 and 20:4n-3 are all present in gametophytes and sporophytes of *M. struthiopteris*, the pathways for biosynthesis of ARA and EPA seems to be common for sporophytes and gametophytes of the fern.

CONCLUSIONS

Gametophytes of *M. struthiopteris* grown *in vitro* contain all the fatty acids found in the fern sporophytes grown outdoors. Presence of ARA, EPA, and their potential precursors in gametophytes, similar distribution of the long-chain polyunsaturated fatty acids in the fractions of polar lipids (glycolipids and phospholipids) in gametophytes and sporophytes make gametophytes of *M. struthiopteris* a potential model for studies of ARA and EPA biosynthesis in ferns.

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