Morphological and chemical variations of sweet flag (*Acorus calamus* L.) in the Czech and Finnish gene bank collection

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ABSTRACT: The aim of this study was to analyze both the morphological and chemical characteristics of *Acorus calamus* plants collected from the natural sites in the Czech Republic and to compare them to the sweet flags growing in Finland, Slovenia and Canada, which are concentrated in the Gene Bank collections in Finland. The sweet flags of Finnish, Slovenian and Czech origin were found to be phenotypically and chemically very similar. They all represent the European triploid type of *Acorus calamus* var. *calamus*. The average essential oil content was established at the value of 1.50% of dry material in Finland and 1.91% in the Czech Republic. This amount is not sufficient according to the Czech norm for *Radix calami* quality (minimal amount is 2%) but the content of carcinogen β-asarone, which was established at 9–21% in the essential oil in all the analyzed sweet flags, did not exceed the maximal recommended value 0.5% of dry drug mass.

Keywords: *Acorus calamus*; variability; essential oil; β-asarone

Sweet flag (*Acorus calamus* L., *Araceae*) is an aromatic perennial herb, indigenous to Central Asia, India and Himalaya. Its distribution in Europe was strongly affected by the Tatars during the XIIIth to XIVth century and now it is native to marshy regions in northern temperate zones. It grows wild on the edges of swamps, on the banks of rivers and ponds.

The rhizomes as well as the leaves have a pleasant, slightly sweet odour that is caused by the content of an essential oil. Dried roots have long been used in medicinal preparations as antispasmodic, in antiulcer preparations and for the flavouring of bitter liqueurs and appetizers.

Three caryotypes of *A. calamus* exist and the essential oil composition can play a significant role in their discrimination. β-asarone is the most discussed compound of the essential oil, due to its toxic and carcinogenic effect. The diploid caryotype, known also as var. *americanus* (Raf.) Wulff, ($2n$ = 24) and grown in North America and Siberia, contains only traces of β-asarone in its rhizome essential oil. The triploid caryotype (var. *calamus*, $3n$ = 36), present in Central Europe and Kashmir, contains 9–13% of β-asarone, and tetraploid var. *angustata* Engl. (or var. *angusta* Bess., $4n$ = 48) contains 70–96% of β-asarone (Röst 1979; Hanelt et al. 2001).

Acorus calamus is a common item in the international drug market and the raw material is originated from the natural populations. In a worldwide report carried out by McALPINE THORPE & WARRIER LIMITED (1996) sweet flag belongs among the endangered medicinal plants and its population in every 7th country has been reported to decrease. In Europe, the populations of *Acorus* have become rare in several countries due to its habitat loss caused by drying up of wet areas. According to Lange (1998) it is a protected plant in Bulgaria, Hungary and it is in the endangered category in Bosnia-Herzegovina and Switzerland. In Turkey, due to previous collecting, it is now rare in several natural areas. In the Ukraine

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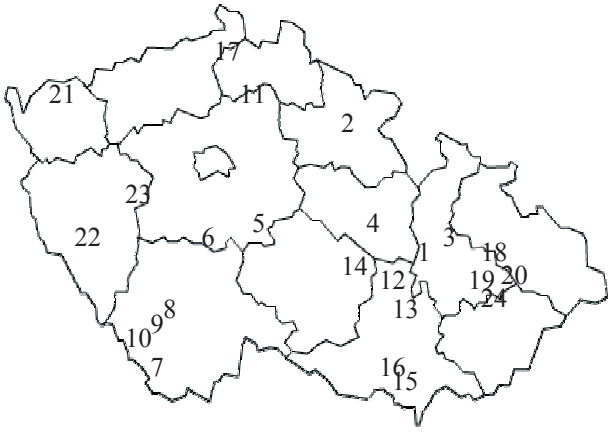

Fig. 1. Original localities of sweet flags in the Czech Republic

Fig. 2. Original localities of sweet flags in Finland

the natural populations have weakened and some protection is desirable. In Slovenia from the earlier reported 32 natural habitats 11 had disappeared until 1996 and the area of the existing populations is not larger, than 30 m^2 (Rode et al. 1996).

This catastrophic decrease of sweet flag populations calls for an immediate conservation of its genetic resources and for starting targeted sweet flag field cultivation so that the raw material demand can be satisfied. The aim of this study was to analyze both the morphological and chemical characteristics of *Acorus calamus* collected from the natural sites in the Czech Republic and to compare them to the sweet flags growing in Finland.

MATERIAL AND METHODS

The Czech Republic

The evaluation and collecting of Czech sweet flags started in 1997 (Petříková et al. 2000). All of the found natural localities were characterized (geographic coordinates, altitude, characterization of biotopes) and photodocumented; the samples of rhizomes (300–400 g) for chemical analysis, 10–15 small cuttings with growing points and also the samples of soil and water for mineral content analysis were taken. The collected cuttings were placed in Olomouc as the gene-bank *ex situ* samples which were made one for each population, and this collection consists of 26 samples collected from all over the country at present; their origin is presented in Fig. 1.

The biotopes of the natural populations were mostly banks of ponds or streams but some were found also on wet meadows or in forests. According to the elevation as well as the results of soil and water analysis the localities are very diverse (pH_{KCl} of soil ranged between 4.0–7.1, for example!) and big differences in yield and quality of essential oil should be expected.

The sweet flag cuttings were replanted into the growing containers in the Gene Bank in Olomouc, where a special basin was built to keep them in optimal wet conditions and to protect sprouting of rhizomes and mixing.

The rhizome samples were cleaned, cut and dried at laboratory conditions, and the drying ratio and essential oil content and quality were analyzed. The essential oil content was analyzed by stem distillation according to Pharmacopoeia Bohemoslovenica VI (1987) and the quality was evaluated by HP 5890 gas chromatograph with flame ionization detector and capillary column HP-INNOVAX (60 m × 0.53 mm ID × 1.0 μm film thickness). The oven temperature was programmed from 60°C (3 min) at 6°C/min up to 240°C (25 min). The identity of components was assigned by comparison of their retention time with standards.

Finland

The Finnish sweet flags were collected during 2002 in the South-West of Finland in seven natural populations (Fig. 2). The biotopes of the natural popula-

Table 1. Morphological characteristics of sweet flags from the Czech Republic

No.	Locality	Leaf length (m)		Thickness of rhizome cross section (mm)		Height of rhizome cross section (mm)	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	Horka na Moravě	1.11	0.075	19	1.840		
2	Červený Kostelec	0.99	0.142	22	2.650		
3	Ospělov	0.78	0.109	17	1.940		
4	Opatov	1.14	0.101	22	1.850		
5	Bojanov	1.12	0.180	15	4.340	14	3.540
6	Pičín	0.95	0.080	19	6.810	16	3.610
7	Křemže	0.79	0.300	17	7.550	14	5.590
8	Slavče	1.04	0.050	20	5.320	18	3.530
9	Bohouškovice	1.08	0.160	25	5.070	21	4.110
10	Stupná	1.17	0.120	15	3.460	13	2.510
11	Žehrovka-Nebákov	0.79	0.130	15	1.870	14	1.620
12	Karolín	1.47	0.130	18	5.980	18	6.370
13	Rudice	0.94	0.140	17	4.810	15	3.760
14	Lelekovice-Útěchov	1.21	0.150	21	3.590	18	2.520
15	Kostice	0.99	0.115	20	3.940	17	2.820
16	Lednice	0.98	0.170	18	6.490	15	4.530
17	Rybniště	1.17	0.146	20	1.600		
18	Visalaje-Obidova	0.62	0.110	16	2.830	13	2.070
19	Staré Hamry	0.80	0.070	16	2.700	13	2.130
20	Horní Bečva	0.74	0.110	14	4.260	12	3.030
21	Karlovy Vary	0.81	0.100	23	3.510	18	3.090
22	Kamýk	0.83	0.110	18	10.600	16	10.400
23	Mýto	1.11	0.160	20	4.070	18	3.930
24	Dolní Paseky	1.18	0.110	15	2.210	13	2.320
Mean		0.99	0.128	18.4	4.100	15.6	3.800

tions were quite similar; all of them were situated on river banks, except for the accession No. 28 at Piikkiö. It was situated on the bank of a gulf, but on a large grassy meadow.

Seven to ten of rhizomes per each population were transplanted into the field of Agrifood Research Finland, Mikkeli. The measurements of both morphological and chemical characteristics were carried out after two growing seasons, in August/September 2004. As a contrast to the Finnish sweet flags one accession originated from Canada and one from Slovenia (from the botanical garden in Zalec) were added to the evaluation.

Two plants of each locality were dug up; the rhizomes with roots were washed, weighed and dried at 40°C. The gas chromatography of the essential oils was performed with a Shimadzu capillary GC-14b DC apparatus equipped with FID detector and Supelco SE 30 quarzcolumn (30 m × 0.25 mm ID × 0.25 µm film thickness) at the CORVINUS University Budapest, Department of the Medicinal and Aromatical Plants. Oven temperature was programmed from 110°C (3 min) at 8°C/min up to 220°C (5 min). Injector temperature was 220°C and the detector temperature was 250°C. Nitrogen was used as carrier gas at a flow rate of 1 ml/min, Splitter 67:1.

GC/MA analyzes were carried out on Hewlett-Packard 5890/II GC-5971A MSD with Supelcowax 10 column, 60 m × 0.25 mm ID × 0.32 µm film thickness, also at the CORVINUS University Budapest, Department of Food Chemistry and Nutrition. Ionization energy was 70 eV. Temperature programming was from 60°C up to 240°C at 4°C/min. Component identifications were made by comparison of their mass spectra and retention indices with those of authentic compounds, and with data in the NITS and Wiley 138.L Library as described by Hethelyi et al. (2002).

Table 2. Morphological characteristics of sweet flags cultivated in Finland

No.	Locality	Leaf length (mm)		Leaf width (mm)		Thickness of rhizome cross section (mm)	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
25	Marttila	0.88	0.075	11.7	3.9	20.1	2.1
26	Aura	0.74	0.088	13.1	2.2	15.7	2.0
27	Paimio	0.81	0.105	15.5	4.1	19.9	1.3
28	Piikkiö	0.74	0.126	12.9	2.7	18.5	1.3
29	Turku I	0.83	0.161	18.4	3.9	20.3	2.4
30	Turku II	0.77	0.109	15.6	2.0	19.3	1.7
31	Pori	0.82	0.124	16.6	1.3	22.0	2.4
Mean		0.80	0.113	14.8	2.9	19.4	1.9
32	Zalec (SLO)	0.85	0.108	16.6	1.9	22.2	3.0
33	Ontario (CAN)	0.93	0.132	13.6	1.4	17.5	2.1

Statistical evaluation

One-factor analysis of variance at the significance levels $\alpha = 0.05$ and $\alpha = 0.01$ (in Excel software environment) was used for statistical evaluation of comparison of native Czech and Finnish sweet flags.

RESULTS AND DISCUSSION

Morphological characteristic

Selected results of morphological characteristic evaluation are presented in Tables 1 and 2.

Characterization of leaves: The average leaf length of all 7 accessions from Finland was 0.80 m (0.74 to 0.88 m). No remarkable differences were observed between these accessions and also the Slovenian and Canadian sweet flags had leaves with similar length (0.85 and 0.93 m, respectively). The average leaf length at Czech accessions was 1.0 m (0.62–1.47 m) and no great differences were observed between them. It is feasible that the higher growth of Czech plants is due to the warmer climate in the Czech Republic; however the differences between sweet flags cultivated in the Czech Republic and in Finland are not statistically significant in this characteristic.

The average leaf width of all accessions concentrated in Finland (Finish, Slovenian and also Canadian) was 15 mm (11.7–18.7 mm) and similarly to the leaf length, no specific differences were observed in this characteristic. This characteristic was not evaluated at Czech accessions.

No differences were observed in leaf erection in Finland, either. All leaves were erect till the end of the vegetation; the observation of Czech plants brought the same results.

Only one clear morphological difference was observed between the North American (diploid) accession and the European (triploid) accessions. The leaf colour of the North American accession was

Table 3. Original localities of sweet flags in Finland and their chemical characteristics

No.	Locality	Elevation (m)	Oil content (%)		β-asarone (%)
			$\bar{x}$	s	$\bar{x}$
25	Marttila	40	1.27	0.075	11.1
26	Aura	41	1.67	0.025	11.5
27	Paimio	20	1.15	0.050	10.0
28	Piikkiö	0	1.50	0.050	10.7
29	Turku I	0	1.30	0.050	9.0
30	Turku II	0	1.87	0.075	9.7
31	Pori	20	1.77	0.075	9.7
Mean			1.50	0.057	10.24
32	Zalec (SLO)	reintroduction	1.27	0.025	10.0
33	Ontario (CAN)	reintroduction	1.10	0.100	traces

Table 4. Original localities of Czech sweet flags and their chemical characteristics

No.	Locality	Elevation (m)	Oil content (%)		β-asarone (%)
			$\bar{x}$	s	$\bar{x}$
1	Horka na Moravě	385	1.80	0.047	18.4
2	Červený Kostelec	435	1.20	0.047	17.4
3	Ospělov	380	1.30	0.205	11.3
4	Opatov	430	1.70	0.170	21.3
5	Bojanov	425	2.40	0.100	18.2
6	Pičín	420	2.05	0.000	19.8
7	Křemže	521	2.63	0.175	18.5
8	Slavče	518	1.50	0.100	14.8
9	Bohouškovice	572	2.80	0.100	20.3
10	Stupná	528	1.90	0.100	15.2
11	Žehrovka-Nebákov	325	1.65	–	18.9
12	Karolín	462	2.45	0.375	14.1
13	Rudice	420	1.40	0.100	17.6
14	Lelekovice-Útěchov	312	1.30	0.100	12.5
15	Kostice	155	1.90	0.100	15.2
16	Lednice	173	1.80	0.000	19.7
17	Rybniště	463	1.70	0.236	17.9
18	Visalaje-Obidova	740	1.30	0.100	12.6
19	Staré Hamry	600	2.20	0.300	13.1
20	Horní Bečva	680	1.70	0.100	13.2
21	Karlovy Vary	447	2.10	0.100	18.2
22	Kamýk	472	2.92	0.080	12.8
23	Mýto	443	2.00	0.000	11.9
24	Dolní Paseky	500	2.25	0.150	13.7
Mean			1.91	0.121	16.11

lighter green compared to the sweet flags originated in Finland and Slovenia.

Characterization of rhizomes: The length of all rhizomes measured in Finland (Finish, Slovenian and Canadian) ranged between 0.18 and 0.49 m. The average thickness was 19.7 mm (15.7–22.2 mm) and the length of the hairy roots grown from the nodes ranged between 0.43–0.53 m. No remarkable differences were found in the rhizomes between the accessions; however two growing years may have been too short to spot the differences.

The length of the rhizomes picked up from original biotopes was strongly influenced by soil type and other natural conditions at the localities and therefore this characteristic was not evaluated in the Czech Republic. As the sweet flag rhizome does not have circle but elliptic shape, its thickness (a higher diameter) and height (a lower diameter) were measured separately. The average thickness of rhizome cross section was 18.4 mm (14.0–25.0 mm) and average height of rhizome cross section was measured as 15.6 mm (12.0–21.0 mm). The ratio between these two rhizome size values stays very constant (1.0–1.2:1) at all evaluated populations and no remarkable differences were found between the rhizomes. No statistically significant differences between Czech and Finnish sweet flags were found in rhizome thickness.

The range of drying ratio (from fresh to dry), which was studied in Czech sweet flag rhizomes, was surprisingly high. The weight loss of rhizomes from Visalaje (acc. No. 18) was in ratio 2.48:1, whereas the rhizomes from Karolín (acc. No. 12) lost weight in ratio 7.53:1. Nevertheless, the average rhizome drying ratio (5:1) obtained was in accordance to literature (Leifertová, Motejlek 1993).

Generative parts: In contrast to the literature data none of the Finnish accessions developed flowers in the collection. During the collecting time, flowering

Table 5. Significance of differences in essential oil content in the *Radix calami* drug

No.	Locality	1	2	3	4	5	6	7	8
33	Ontario (CAN)	0.67^{++}	0.05	0.18	0.49^{+}	1.73^{+}	0.92^{+}	2.41^{+}	0.20
32	Zalec (SLO)	0.26^{++}	0.02	0.14	0.19	1.07^{++}	0.46^{++}	1.63^{+}	0.04
31	Pori	0.02	0.29^{+}	0.45	0.11	0.42^{+}	0.09	0.80^{+}	0.11
30	Turku II	0.02	0.41^{+}	0.58	0.15	0.31	0.04	0.64	0.17
29	Turku I	0.35^{++}	0.01	0.13	0.25	1.24^{+}	0.57^{++}	1.82^{+}	0.07
28	Piikkiö	0.15^{++}	0.07	0.20	0.13	0.84^{+}	0.31^{++}	1.33^{+}	0.03
27	Paimio	0.11^{+}	0.10	0.23	0.11	0.75^{+}	0.26^{++}	1.22^{+}	0.03
26	Aura	0.04^{+}	0.24	0.33	0.09	0.55^{+}	0.14^{++}	0.97^{+}	0.05
25	Marttila	0.39^{++}	0.02	0.14	0.28	1.30^{+}	0.61^{++}	1.90^{+}	0.08
24	Dolní Paseky	0.26^{+}	1.29^{++}	1.33^{+}	0.54	0.09	0.09	0.25	0.63
23	Mýto	0.04^{+}	0.71^{++}	0.77^{+}	0.22	0.18	0.00^{++}	0.45	0.27^{+}
22	Kamýk	1.44^{++}	3.43^{++}	3.42^{++}	1.98^{++}	0.30	0.77^{++}	0.16	2.05^{++}
21	Karlovy Vary	0.11	0.93^{++}	0.98^{+}	0.33	0.13	0.02	0.36	0.40
20	Horní Bečva	0.05	0.29^{+}	0.37	0.11	0.53^{+}	0.14	0.94^{+}	0.08
19	Staré Hamry	0.21	0.45	0.53	0.27	0.69	0.30	1.10	0.24
18	Visalaje-Obidova	0.37^{++}	0.03	0.15	0.27	1.25^{+}	0.58^{+}	1.84^{+}	0.08
17	Rybniště	0.19	0.55^{+}	0.62	0.26	0.72	0.29	1.18^{+}	0.25
16	Lednice	0.01	0.39^{++}	0.47	0.11	0.38^{+}	0.06^{++}	0.74^{+}	0.11
15	Kostice	0.03	0.56^{++}	0.63	0.17	0.29	0.04	0.61	0.20
14	Lelekovice-Útěchov	0.37^{++}	0.03	0.15	0.27	1.25^{+}	0.58^{+}	1.84^{+}	0.08
13	Rudice	0.25^{+}	0.06	0.17	0.19	1.04^{+}	0.44^{+}	1.58^{+}	0.05
12	Karolín	0.98	2.51^{+}	2.53^{+}	1.40	0.34	0.58	0.34	1.50
11	Žehrovka-Nebákov	0.03	0.14^{+}	0.24	0.09	0.40	0.11^{++}	0.70	0.04
10	Stupná	0.03	0.56^{++}	0.63	0.17	0.29	0.04	0.61	0.20
9	Bohouškovice	1.15^{++}	2.97^{++}	2.97^{++}	1.65^{++}	0.20	0.58^{+}	0.11	1.73^{+}
8	Slavče	0.16^{++}	0.11	0.21	0.14	0.85^{+}	0.32^{+}	1.35^{+}	
7	Křemže	0.82^{+}	2.39^{++}	2.40^{++}	1.25^{+}	0.13	0.39		
6	Pičín	0.06^{+}	0.81^{++}	0.86^{+}	0.26	0.14			
5	Bojanov	0.41^{++}	1.66^{++}	1.69^{+}	0.75^{+}				
4	Opatov	0.14	0.38^{+}	0.45					
3	Ospělov	0.62^{+}	0.14						
2	Červený Kostelec	0.55^{++}							

[1] Horka na Moravě
$^{+}$Significant difference (at a significance level $\alpha = 0.05$)
$^{++}$Highly significant difference (at a significance level $\alpha = 0.01$)

stems were found very rarely in the natural populations as well. In the Czech Republic the flower stems with fully developed inflorescences (spadix) were routinely found at original localities but fruits (berries) were not found. It is documented that triploids are distinguished by defects in meiosis and therefore they are not able to produce functional generative cells. Therefore plants are reproduced only by rhizome segments (Dostál, Červenka 1992; Farkaš 1979).

The essential oil content and composition

According to the analytical data presented in Table 3, the average oil content of the European sweet flags (No. 25–32) in the Finnish collection was 1.50%. All the tested plants had very balanced values (1.15–1.87%) and the oil composition was also quite similar. Their main compounds were solavetivone (7.80–15.6%; $x = 11.07\%$) and β-asarone (9.00–11.50%; $x = 10.24\%$).

Table 5 to be continued

No.	Locality	9	10	11	12	13	14	15	16
33	Ontario (CAN)	2.93^{++}	0.68^{+}	0.22	2.54	0.13	0.08	0.68^{+}	0.51^{+}
32	Zalec (SLO)	2.05^{++}	0.30^{+}	0.05	0.77	0.02	0.03	0.30^{+}	0.18^{++}
31	Pori	1.08^{+}	0.05	0.02	0.96	0.17	0.26	0.05	0.01
30	Turku II	0.89^{+}	0.03	0.05	0.81	0.26	0.36^{+}	0.03	0.02
29	Turku I	2.28^{++}	0.39^{+}	0.09	1.96	0.04	0.03	0.39^{+}	0.26^{++}
28	Piikkiö	1.72^{++}	0.19	0.02	1.49	0.04	0.07	0.19	0.10^{+}
27	Paimio	1.59^{++}	0.15	0.01	1.38	0.05	0.09	0.15	0.07^{+}
26	Aura	1.29^{++}	0.07	0.00	1.03	0.10	0.16	0.07	0.02^{+}
25	Marttila	2.36^{++}	0.42^{+}	0.11	2.03	0.05	0.03	0.42^{+}	0.29^{+}
24	Dolní Paseky	0.37	0.19	0.29	0.45	0.79^{+}	0.97^{+}	0.19	0.25
23	Mýto	0.66^{+}	0.03	0.09^{++}	0.64	0.38^{+}	0.51^{+}	0.03	0.04^{++}
22	Kamýk	0.05	1.07^{+}	1.09	0.40	2.34^{++}	2.66^{++}	1.07^{+}	1.27^{++}
21	Karlovy Vary	0.53^{+}	0.08	0.16	0.55	0.53^{+}	0.68^{+}	0.08	0.11
20	Horní Bečva	1.25^{+}	0.08	0.02	1.10	0.13	0.20	0.08	0.03
19	Staré Hamry	1.41	0.24	0.18	1.26	0.29	0.36	0.24	0.19
18	Visalaje-Obidova	2.29^{++}	0.40	0.10	1.98	0.05	0.04	0.40	0.27^{+}
17	Rybniště	1.55^{+}	0.22	0.17	1.34	0.32	0.41	0.22	0.15
16	Lednice	1.02^{++}	0.03	0.02^{++}	0.91	0.18	0.27^{+}	0.03	
15	Kostice	0.85^{+}	0.04	0.06	0.78	0.29	0.40		
14	Lelekovice-Útěchov	2.29^{++}	0.40	0.10	1.98	0.05			
13	Rudice	2.00^{+}	0.29	0.06	1.72				
12	Karolín	0.34	0.78	0.88					
11	Žehrovka-Nebákov	0.90	0.06						
10	Stupná	0.85^{+}							
9	Bohouškovice								

Table 5 to be continued

No.	Locality	17	18	19	20	21	22	23	24
33	Ontario (CAN)	0.67	0.08	0.56	0.40	1.04^{+}	3.35^{++}	0.83^{+}	1.39^{+}
32	Zalec (SLO)	0.32	0.03	0.29	0.13	0.55^{+}	2.40^{++}	0.39^{++}	0.81^{+}
31	Pori	0.18	0.26	0.20	0.13	0.14	1.33^{++}	0.06	0.28
30	Turku II	0.20	0.36^{+}	0.22	0.06	0.08	1.11^{+}	0.03	0.20
29	Turku I	0.40	0.03	0.35	0.19	0.67^{+}	2.64^{++}	0.50^{++}	0.96^{+}
28	Piikkiö	0.24	0.07	0.23	0.07	0.39^{+}	2.03^{++}	0.26^{++}	0.61^{+}
27	Paimio	0.21	0.09	0.21	0.05	0.33^{+}	1.89^{++}	0.21^{+}	0.54^{+}
26	Aura	0.17	0.16	0.18	0.02	0.20	1.56^{++}	0.11^{+}	0.38
25	Marttila	0.43	0.03	0.37	0.21	0.71^{+}	2.73^{++}	0.54^{+}	1.01^{+}
24	Dolní Paseky	0.53	0.97^{+}	0.53	0.37	0.09	0.51	0.11	
23	Mýto	0.25	0.51^{+}	0.27	0.11	0.03	0.86^{++}		
22	Kamýk	1.87^{+}	2.66^{++}	1.68	1.52^{+}	0.71^{+}			
21	Karlovy Vary	0.35	0.68^{+}	0.36	0.20				
20	Horní Bečva	0.19	0.20	0.20					
19	Staré Hamry	0.35	0.36						
18	Visalaje-Obidova	0.41							

Table 5 to be continued

No.	Locality	25	26	27	28	29	30	31	32
33	Ontario (CAN)	0.06	0.35^{+}	0.23	0.19	0.07	0.63^{+}	0.49^{+}	0.10
32	Zalec (SLO)	0.02	0.09^{+}	0.04	0.02	0.01	0.26^{+}	0.17^{+}	
31	Pori	0.25^{+}	0.02	0.07	0.09	0.24^{+}	0.03		
30	Turku II	0.38^{+}	0.05	0.12	0.16	0.35+			
29	Turku I	0.02	0.15	0.07	0.05				
28	Piikkiö	0.07	0.04	0.01					
27	Paimio	0.09	0.02						
26	Aura	0.17^{+}							

The oil content of the single North American accession (No. 33) was lower – 1.10% and its composition differed from the European triploid accessions. Its highest component was solavetivone (27.2%) and it did not contain α- and β-asarones et all.

The Czech sweet flags had similar essential oil content (Table 4) and composition like the Finnish and Slovenian samples. The average essential oil content 1.91% (1.20–2.92%) is slightly higher than in Finland, however 14 from 24 analyzed samples did not exceed the norm defined for quality of sweet flag drug – *Radix calami* – which is determined as 2% of essential oil by Czech Pharmaceutical Codex (Český farmaceutický kodex 1993). It was observed that the essential oil content is higher in spring (May) than in autumn (October) and the oil content has a negative correlation with calcium content in the water and with pH (Petříková et al. 2000).

The main components of the essential oil from Czech sweet flags were β- and γ-asarones. The average content of γ-asarone was 18.65% (12.52–25.35%) and that of β-asarone was 16.11% (11.34–21.30%). The content of β-asarone in sweet flag drug did not exceed the maximal recommended value 0.5% of dry matter content (Keller, Stahl 1982).

Statistically significant differences were found between the Czech and Finnish sweet flags in the essential oil content (including the accession originated in Slovenia and Canada; Table 5) as well as in the β-asarone content in essential oil only from the triploid plants.

CONCLUSION

The sweet flags of Finnish, Slovenian and Czech origin in both collections seemed to be phenotypically and chemically very similar. They all represent the European triploid type of *Acorus calamus* var. *calamus* (Röst 1979), its average leaf length was established at 0.95 m and its average rhizome thickness at 18.5 mm.

Clear phenotypic differences were observed between the North American (diploid) accession and the European (triploid) accessions. The leaf colour of the North American accession was lighter green and there was no carcinogen β-asarone in the root essential oil, while the European accessions reach 9.0–21.3% content of β-asarone in the oil.

The evaluation of the generative parts of *Acorus calamus* in the Finnish *ex situ* collections seems to need more observation time in future. The two-year period of cultivation was not sufficient for the plant to develop generative organs. In the natural sites of Finland the flowering stems occurred unexpectedly quite rarely, whereas in the Czech Republic the flowering stems grow up without problems at original localities as well as *ex situ*.

The average essential oil content (1.50% in Finland and 1.91% in the Czech Republic) is not sufficient according to the Czech norm for *Radix calami* quality but the content of carcinogen β-asarone did not exceed the maximal recommended value 0.5% of dry drug mass.

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Morfologická a chemická variabilita českých a finských puškvorců (*Acorus calamus* L.)

ABSTRAKT: Předmětem práce je hodnocení morfologických a chemických znaků puškvorce pocházejícího z lokalit v České republice a jejich srovnání s puškvorci soustředěnými ve sbírce finské genové banky, které jsou původem z Finska, Slovinska a Kanady. Bylo zjištěno, že české, finské a slovinské puškvorce jsou si fenotypicky i chemicky navzájem velmi podobné – společně reprezentují evropský triploidní typ *Acorus calamus* var. *calamus*. Průměrný obsah silice v oddencích byl stanoven na 1,50 % suché hmoty u finských rostlin a na 1,91 % suché hmoty u českých puškvorců. Tyto hodnoty sice nesplňují požadavky na kvalitu drogy uvedené pro *Radix calami* v Českém farmaceutickém kodexu (je uveden obsah minimálně 2 %), ale obsah karcinogenního β-asaronu, který byl u všech hodnocených rostlin určen v rozmezí 9–21 % v silici, nepřevýšil maximální doporučenou hodnotu 0,5 % v suché droze.

Klíčová slova: *Acorus calamus*; variabilita; silice; β-asaron

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