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Variability of southern sweet-grass in respect of developmental traits and coumarin compounds accumulation

INTRODUCTION

Southern sweet-grass (*Hierochloë australis* (Schrad.) Roem. et Schult.) is a tuft grass growing wild in coniferous and mixed forest in Europe, mostly in Finland, Belarus and Poland (Ćwikliński and Głowacki, 1990; Gawłowska et al., 1989). In Poland leaves of this plant are collected exclusively from natural sites as a coumarin raw material used for extracts production, utilized in food industry for aromatization of alcoholic beverages (Przybył et al., 2011; Podyma et al. 2010; Węglarz et al., 2004; Kohlmünzer, 2000). The species, rapidly disappearing from natural sites, is seriously endangered, mostly because of excessive and uncontrolled harvesting from this sites (Polakowski, 1995). Taking into the account constantly growing demand for the raw material, as well as poor recovery of the plant populations in the natural habitat, it seems that the most effective way to protect southern sweet-grass is to introduce it into cultivation. In natural sites is seems to be relatively resistant to diseases and pests as well as to drought, so such introduction especially to organic cultivation should not be recommended.

The aim of this work was to compare 12 populations of southern sweet-grass, in the first year of plant cultivation in organic farm system, concerning their morphological and chemical traits.

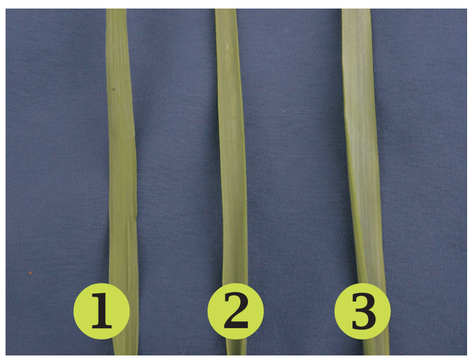
MATERIAL AND METHODS

The seeds of 12 southern sweet-grass populations used to establish the field experiment were collected from natural sites from Podlasie district, in eastern Poland. The seedlings were produced in greenhouse and planted out at the experimental, organic field of Department of Vegetable and Medicinal Plants WULS-SGGW at the beginning of September (the experiments were carried out in 2010 and 2011). The observation of morphological traits and harvesting of raw materials were done two times during vegetation period, first time at the beginning of July and the second one after two month, at the beginning of September (regrowth - second cut).

The following morphological traits were observed:
- number of leaves per plant,
- length of leaves,
- width of leaves,
- intensity of white coumarin coating on leaves.

In case of the last above mention trait (the intensity of coumarin coating on leaves) three step scale was prepared, where: 1. – green leaves without white coating; 2. – leaves with thin, white coumarin coating and 3. – leaves with clearly visible, white coating, easy to remove when touching (reference photography). The observation were done on 20 randomly chosen plants from each population.

Southern sweet-grass in cultivation



Intensity of coumarin coating on the leaves (reference photography)

The harvested leaves were analyzed on the total content of coumarin compounds (Wierzchowska-Renke, 1972) and phenolic acids (Polish Pharmacopoeia VIII, 2008). For the determination of coumarin, which is the most important quality discriminant of this raw material (Sulma and Wierzchowska-Renke, 1974), HPLC and TLC methods were applied as well.

RESULTS

The investigated populations differed significantly in the morphological traits i.e. in the number of leaves per plant (from 14.3 to 65.8 in the first time of cutting and from 40.0 to 161.8 in the second one), their length (from 23.39 to 34.86 cm in the first time of cutting and from 21.27 to 35.20 cm in the second one), width (from 0.71 to 1.19 cm in the first time of cutting and from 0.61 to 0.92 cm in the second one) and air dry mass per plant (from 0.75 to 3.48 g in the first time of cutting and from 3.67 to 17.89 g in the second one) (tab. 1,2). Most investigated population were characterised by high percentage of plants with the presence of white coumarin coating on the leaves, except for population no 5, where 60% of plants had green leaves without any coating (tab. 3).

The populations differed also distinctly in the total content of coumarin compounds in the leaves. The content of these compounds in investigated populations in first cut ranged from 0.19 to 1.62% and in second cut - from 0.37 to 3.35%. The total content of phenolic acids in the raw materials of investigated populations in the first and in the second cut was less diversified than the content of coumarins and ranged from 0.27 to 0.73 and from 0.30 to 0.74%, respectively (tab. 4,5). HPLC and TLC analysis confirmed both - high differences among the populations concerning coumarin content as well as distinct differences in the content of this compound in the leaves collected in the first harvest time, in June (from 23.03 to 51.36 mg $100g^{-1}$ d.m.) and in the second cut (regrowth), in September (from 42.73 to 164.69 mg $100g^{-1}$ d.m.) (fig. 1-3).

Tab. 1. Characteristics of vegetative organs (first time of cut)

Population No.	Fresh mass of leaves (g)	Air dry mass of leaves (g)	No. of leaves per plant	Length of leaves (cm)	Width of leaves (cm)
1.	2.00	0.97	30.40	23.39	0.84
2.	2.76	1.22	36.60	25.66	0.95
3.	2.52	0.96	44.60	24.77	0.90
4.	2.04	0.75	14.27	23.43	0.71
5.	1.70	0.76	13.00	24.86	0.74
6.	2.00	0.88	19.60	24.03	0.82
7.	2.05	0.85	33.40	27.11	0.82
8.	3.35	1.38	36.00	26.38	1.16
9.	8.70	3.48	65.80	34.86	1.19
10.	6.18	2.48	62.00	34.68	0.85
11.	2.18	0.90	22.80	29.35	0.91
12.	3.09	1.44	38.00	26.68	0.94
mean	**3.21**	**1.34**	**34.71**	**27.10**	**0.90**
CV	**2.10**	**0.83**	**16.80**	**3.96**	**0.15**

Tab. 2. Characteristics of vegetative organs (second time of cut)

Population No.	Fresh mass of leaves (g)	Air dry mass of leaves (g)	No. of leaves per plant	Length of leaves (cm)	Width of leaves (cm)
1.	5.97	2.76	66.40	25.23	0.84
2.	8.47	2.75	40.00	27.17	0.89
3.	9.13	3.73	114.60	24.07	0.94
4.	3.67	1.69	42.40	26.97	0.86
5.	6.53	2.63	35.60	24.80	0.85
6.	8.29	3.36	67.40	26.33	0.92
7.	6.32	2.48	59.80	27.90	0.86
8.	8.61	3.73	62.20	21.27	0.78
9.	17.89	7.67	139.40	30.10	0.81
10.	19.30	8.26	161.80	35.20	0.61
11.	8.94	4.29	85.40	29.53	0.66
12.	15.67	6.67	126.60	30.30	0.78
mean	**9.90**	**4.17**	**83.47**	**27.41**	**0.82**
CV	**4.97**	**2.17**	**42.13**	**3.60**	**0.10**

Tab. 3. Percentage of plants with different intensity of white coumarin coating on leaves per population (first time of cut).

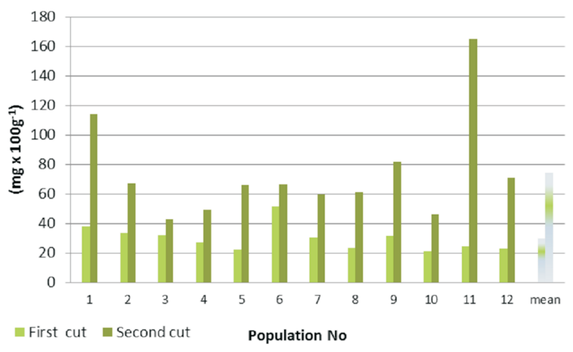
Population No.	Green leaves	Leaves with light, whitish coating	Leaves with strong white coating
1.	16.7	41.7	41.6
2.	11.1	55.6	33.3
3.	20.7	48.3	31.0
4.	27.2	36.4	36.4
5.	60.0	20.0	20.0
6.	14.3	28.6	57.1
7.	11.1	55.6	33.3
8.	0.0	47.1	52.9
9.	38.5	30.8	30.7
10.	0.0	35.3	64.7
11.	9.1	45.5	45.4
12.	8.1	33.6	58.3
mean	**18.07**	**39.88**	**42.06**

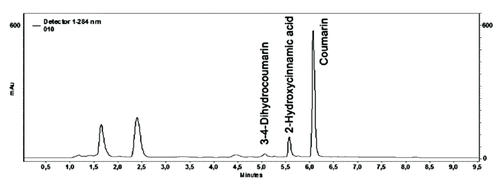
Tab. 4. Total content of biologically active compounds in southern sweet-grass leaves in the first time of cut (%)

Biologically active compounds	Population No.												mean	CV
	1	2	3	4	5	6	7	8	9	10	11	12		
coumarin compounds	0.28	0.31	0.19	1.01	1.00	1.40	1.39	1.04	1.62	0.99	1.21	0.61	**0.92**	**0.47**
phenolic acids	0.68	0.73	0.56	0.56	0.54	0.61	0.37	0.27	0.54	0.41	0.45	0.49	**0.52**	**0.13**

Tab. 5. Total content of biologically active compounds in southern sweet-grass leaves in the second time of cut (%)

Biologically active compounds	Population No.												mean	CV
	1	2	3	4	5	6	7	8	9	10	11	12		
coumarin compounds	1.76	0.69	0.37	0.60	0.83	1.28	1.42	1.17	3.35	2.66	1.92	1.23	**1.44**	**0.87**
phenolic acids	0.56	0.73	0.74	0.54	0.48	0.30	0.35	0.48	0.47	0.40	0.30	0.30	**0.47**	**0.15**





Sample HPLC chromatogram

Fig. 1. The content of coumarin in southern sweet-grass leaves (mg $100g^{-1}$)

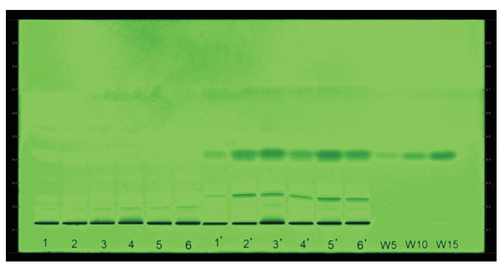


Fig 2. 1-6 extracts from southern sweet-grass leaves from population 1-6 (first time of cut)
1'-6' extracts from southern sweet-grass leaves from population 1-6 (second time of cut)
W5, W10, W15 – reference solution of coumarin in rising concentration

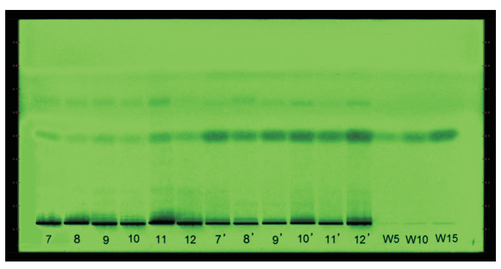


Fig 3. 7-12 extracts from southern sweet-grass leaves from population 7-12 (first time of cut)
7'-12' extracts from southern sweet-grass leaves from population 7-12 (second time of cut)
W5, W10, W15 – reference solution of coumarin in rising concentration

CONCLUSIONS

1. **The results indicate high intraspecific variability of southern sweet-grass.**
2. **There is a possibility to get relatively high yield of good quality leaves of southern sweet-grass from selected populations introduced in to cultivation.**

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