EXPERIMENTAL PAPER

Setting of southern sweet-grass plantation with stem cuttings obtained by division of maternal plants

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Summary

Southern sweet-grass (*Hierochloë australis* /Schrad./ Roem. & Schult.) is a perennial, wild growing tuft grass occurring in North-East Poland, Belarus and Finland. In Poland the species is under the partial legal protection. The raw material harvested from this plant are leaves rich in coumarins, mainly in coumarin responsible for specific sweet aroma of leaves. They are used mostly for the aromatization of alcohol and tobacco products. Due to high demand for the raw material and decrease in the natural resources of the species, it is advisable to introduce the plant into cultivation. In the presented study vegetative planting stock (1-, 2-, and 4-stem cuttings) were used to set the plantation of southern sweet-grass. The influence of the planting stock type on the mass of leaves and their quality in the first and second year of plant vegetation as well as the mass of seeds from two-year-old plants were investigated. The highest number of well rooted plants was obtained from 4-stem cuttings (74.07%) and the least – from 1-stem cuttings (47.53%). Both, on one- and two-year-old plantations the plants from 4-stem cuttings were characterized by the highest mass of leaves (7.73 and 24.65 g $\times$ plant⁻¹, respectively). The plants were also characterized by the highest number of generative shoots (40.71 pcs. $\times$ plant⁻¹) and mass of seeds (4.62 g $\times$ plant⁻¹). The total contents of coumarins and phenolic acids did not depend on the type of planting stock. The contents of these compounds was higher in two-year-old plants than in one-year-old ones, whereas the content of flavonoids was higher in one-year-old plants.

Key words: *Hierochloë australis*, bison grass, cuttings, plant development, coumarins, phenolic acids, flavonoids

INTRODUCTION

Southern sweet-grass (*Hierochloë australis* /Schrad./ Roem. & Schult.) is a perennial tuft grass. This strictly European species grows at the edges of mixed and coniferous forests in East Poland, Belarus and Finland [1-4]. In Poland, the species is seriously endangered and since 1983 it is partially protected [5]. It is also included into the red list of vascular plants endangered in Poland [6].

Southern sweet-grass prefers slightly shaded sites, especially forest fellings. It begins vegetation in early spring, firstly producing generative shoots. The seeds of this plant are ready to spread in May. In the same time the leaves appear, so in the case of southern sweet-grass the generative phase (flowering and seeds setting) is followed by the vegetative growth [4, 7, 8].

The raw material collected from this plant are leaves of specific sweet aroma, used for the production of alcoholic beverages and tobacco products. The content of coumarins, with a coumarin as a dominant compound responsible for the aroma, is the main indicator of dried leaves quality [3, 4]. Despite the legal protection of this species, up to now the raw materials have been collected in high amounts only from wild growing plants [2, 3]. Due to decrease in the number of populations of this plant on natural sites and high demand for the raw material, it is advisable to introduce southern sweet-grass into cultivation. It seems that the generative propagation is the simplest method to obtain planting stock, however, in the case of southern sweet-grass the usage of vegetative plant material to establish plantation is more advisable due to unreliable germination of seeds as well as the high intraspecific variability of this species [9, 10]. Taking into consideration that only vegetative propagation allows to obtain a homogenous plant material with known morphological and chemical traits and that valuable, standardized herbal raw material may be provided only by plantations, the vegetative development, biology of flowering and seed setting of southern sweet-grass should be investigated.

The aim of undertaken study was to evaluate the usefulness of stem cuttings, obtained by simple dividing of tufts, as a plant material used to set the plantation of southern sweet-grass. The influence of cuttings type on plant development, mass and quality of leaves collected in the first and second year of plant vegetation, was investigated as well.

MATERIALS AND METHODS

Plant material

The field experiment was carried out in 2012 and 2013 at the Experimental Station of Department of Vegetable and Medicinal Plants, WULS-SGGW. In the

experiment, stem cuttings of southern sweet-grass were used. Twenty five three-year-old maternal plants (fig. 1), representing one clone, obtained in the previous works from a plant selected from among wild growing population (N 52°37.94, E 22°45.72') and described as a clone 14 [11], were dug out in Autumn, 2011 from the collection of medicinal and aromatic plants of Department of Vegetable and Medicinal Plants. The plants were divided into 1-, 2- and 4-stem cuttings (fig. 2) and planted in greenhouse in multi-cell trays filled with peat substrate of pH 6.0. Well rooted cuttings of each type were planted out in the field in May 2012. The plants were grown under the shading net, reducing the intensity of sunlight for 30%, on the river alluvial soil drained with peat of pH_(KCl) 6.4 and river sand. Nitrogen (50 kg N × ha), phosphorus (50 kg P₂O₅ × ha) and potassium fertilization (80 kg K₂O × ha) was applied (regarding the results of soil analysis in autumn) before planting. Plant density was 50 × 30 cm. The experiment was established in the randomized block design in three replications. On a single plot, 21 cuttings of one type were grown.

Developmental characteristics

In the first year of plant vegetation, the morphological observations and the harvest of leaves was performed once, in September and in the second year – twice, i.e. in mid-July (1st harvest) and at the beginning of September (2nd harvest – regrowth).

Directly before each harvest, the number of leaves per plant, their length (on 20 randomly chosen leaves per plant) and the diameter of tuft were investigated. The leaves were cut 10–15 cm above ground to protect the tillering node. Next, they were dried at 35°C. The mass of fresh and air dry leaves was determined. Air dry raw materials, separately from each plot, were subjected to chemical evaluation.

In the second year of plant vegetation, at the beginning of June, the number of generative shoots, their length and the mass of seeds (spikelets) produced by plants from each type of cuttings was investigated as well. The observations were conducted on five plants in three replications.

Determination of total contents of coumarins, flavonoids, and phenolic acids

Total content of coumarins was determined spectrophotometrically, according to Wierzchowska-Renke and Stecka [12], whereas total content of phenolic acids (expressed as caffeic acid equivalents, %) and total content of flavonoids (expressed as quercetin equivalents, %) were determined spectrophotometrically, according to Polish Pharmacopoeia VIII [13]. The presented results are mean values of three replications.

Figure 1.
Three-year-old maternal plants of southern sweet-grass (clone 14).

Figure 2.
1-, 2-, and 4-stem cuttings of southern sweet-grass

Statistical analysis

The results of field studies and chemical analysis were subjected to the one-way analysis of variance and Tukey's test at $\alpha=0.05$.

RESULTS AND DISCUSSION

Till now, southern sweet-grass has not been an object of many experimental studies. It probably results from very narrow range of the occurrence of this wild growing plant, limited only to North-Eastern Europe [1-3]. In Poland, the species is very rare, although well-known as "żubrówka" (bison grass). Dry leaves of this plant are used mainly for the production of extracts used for the aromatization of this worldwide known Polish vodka [3, 10].

Up to now, some trials on the introduction of southern sweet-grass into cultivation have been performed in Department of Biology and Pharmaceutical Biology, Medical University in Gdańsk [14, 15] and in Department of Vegetable and Medicinal Plants, WULS-SGGW in Warsaw [3, 4, 10]. The results of these experiments indicate that the content of coumarins in southern sweet-grass raw materials obtained from cultivation depend on the time of harvest, mineral fertilization, as well on drying conditions. There is a little information on the development of the plant and almost any on the reproductive abilities of the species [8, 9].

In the presented work, the trials on the vegetative propagation of southern sweet-grass as well as the investigation on the development of the plant were undertaken. 1-, 2- and 4-stem cuttings, obtained by simple division of maternal plants (clone of selected plant), were used as a planting stock to establish the plantation. The highest number of well rooted plants was obtained from 4-stem cuttings (74.07%), and the least from 1-stem cuttings (47.85%). There were no significant differences between 1- and 2-stem cuttings according to this trait (fig. 3). It was probably related to the biology of this tuft grass. In this type of grasses the tillering node, which has a meristematic functions, is situated on the stem near the ground level. The shoots and roots of the grasses develop from the node, and each newly developed shoot with a tillering node produce its own roots, generative shoots and leaves. In consequence, it produce a new plant still connected with the maternal one [7, 16]. The injury of tillering node during vegetative plant division may results in necrosis and withering of cuttings which is more probable for plants obtained from 1-stem cuttings than from 4-stem ones.

Values followed by the same letter do not differ significantly at $\alpha=0.05$.

Figure 3.

Percentage of rooted cuttings obtained from three-year-old maternal plants [%]

The type of cuttings used in the experiment influenced the plant development in first and second year of vegetation. Plants obtained from 1-stem cuttings were characterized by weaker growth parameters, i.e. lower number of leaves, smaller diameter of tuft (tab. 1, 2), although the leaves of plants from 1- and 2-stem cuttings were longer than those from 4-stem cuttings (tab. 3). These parameters influenced the weight of raw materials collected from investigated plants. The highest mass of leaves was obtained from the plants obtained from 4-stem cuttings (fig. 4, 5). In the first year of vegetation, the mass of air dry leaves varied from 7.43 (plants from 1-stem cuttings) to 16.93 g $\times$ plant⁻¹ (plants from 4-stem cuttings), and in the second year, the cumulative mass from two harvests varied from 24.65 (plants from 1-stem cuttings) to 38.87 g $\times$ plant⁻¹ (plants from 4-stem cuttings). The mass of raw materials collected from 1- and 2-stem cuttings in the second year of plant vegetation was similar. According to Węglarz et al. [10], the weight of southern sweet-grass herb depends on the intraspecific variability of the species. However, irrespectively of a very high diversity, the mass of raw material is distinctly lower in the first year of plant vegetation (from 2.5 to 28.0 g $\times$ plant⁻¹) than in the second one, with two harvests performed (from 6.0 to 68.0 g $\times$ plant⁻¹).

Table 1.

Number of leaves [pcs. $\times$ plant⁻¹]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	83.67b	105.14b	139.00a	109.27C
Two-year-old	1 st harvest	203.00c	316.33b	362.00a	293.78A
	2 nd harvest	183.33c	269.67b	353.67a	268.89AB

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$ Values in rows followed by the same letter do not differ significantly at $\alpha=0.05$

Table 2.

Diameter of tuft [cm]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	6.9a	6.9a	7.2a	7.0B
Two-year-old	1 st harvest	14.6b	15.3ab	17.4a	15.8A
	2 nd harvest	14.5b	14.7b	17.1a	15.4A

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$ Values in rows followed by the same letter do not differ significantly at $\alpha=0.05$

Table 3.

Leaf length [cm]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	28.6a	28.5a	27.9b	28.3AB
Two-year-old	1 st harvest	30.7ab	31.9a	29.6b	30.7A
	2 nd harvest	26.0b	25.6b	28.9a	26.8B

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$ Values in rows followed by the same letter do not differ significantly at $\alpha=0.05$

Values followed by the same letter do not differ significantly at $\alpha=0.05$

Figure 4.

Fresh mass of leaves [$\text{g} \times \text{plant}^{-1}$]

Values followed by the same letter do not differ significantly at $\alpha=0.05$

Figure 5.

Air dry mass of leaves [$\text{g} \times \text{plant}^{-1}$]

The raw materials collected from southern sweet-grass are rich in coumarin compounds, i.e.: coumarin, 3,4-dihydrocoumarin, bergapten [3, 4, 11]. This particular group with coumarin as a main compound, is responsible for the specific

sweet, cheery-like aroma of southern sweet-grass herb [17, 18]. The content of coumarin in the raw material depends on the origin of the herb, developmental phase of plants during harvest, plant age, fertilization and drying conditions [3, 4, 10, 14, 15]. Wierzchowska-Renke [15] reported that during three years of vegetation of southern sweet-grass in *ex situ* conditions, the highest total content of coumarin was observed in the herb harvested from two-year-old plants (1.63%). However, those data concern the herb collected during seed setting. Harvest performed in this developmental stage of wild growing plants is specially harmful. It limits the reproductive ability of populations on their natural sites. Due to high constitute of generative shoots in the total mass of raw material it is also not recommended to harvest the leaves at this particular stage from cultivations. In the study of Węglarz et al. [10], total content of coumarin compounds in the herb harvested after seed setting ranged from 0.22 to 0.60% in the first year of vegetation and from 0.30 to 1.50% in second year, depending on population and time of harvest.

In present study, total contents of coumarins, phenolic acids and flavonoids in leaves during two years of plant vegetation was evaluated. The contents of these compounds in investigated plants did not depend on the type of cutting but on plant age and the term of harvest. The highest total content of coumarin in leaves was observed in 2-year-old plants, especially in the second harvest (mean 0.90%). In the raw materials collected in the first year of vegetation the content of coumarins was three times lower (tab. 4).

Table 4.

Total content of coumarins [%]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	0.30	0.33	0.32	0.32C
	1 st harvest	0.48	0.55	0.43	0.49B
Two-year-old	2 nd harvest	0.96	0.90	0.83	0.90A

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$

Values in rows do not differ significantly at $\alpha=0.05$

The data on the content of phenolic acids and flavonoids in southern sweet-grass leaves are scarce. In the study of Podyma et al. [3], five phenolic acids, i.e.: chlorogenic, o-coumaric, ferulic, ellagic and rosmarinic and two flavonoids, i.e.: astragalin and rutin were identified in the leaves of this plant. In present study, total contents of phenolic acids and flavonoids were relatively low. Total contents of phenolic acids, similarly to coumarins, were the highest in second year of plant vegetation (second harvest) and the total content of flavonoids was the highest in leaves collected in the first year of plant vegetation (tab. 5, 6).

Table 5.

Total content of phenolic acids [%]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	0.30	0.35	0.32	0.32B
	1 st harvest	0.39	0.45	0.35	0.40B
Two-year-old	2 nd harvest	0.52	0.59	0.60	0.57A

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$ Values in rows do not differ significantly at $\alpha=0.05$

Table 6.

Total content of flavonoids [%]

Age of plants		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings	Mean
One-year-old	1 st harvest	0.41	0.37	0.38	0.39A
	1 st harvest	0.18	0.19	0.21	0.19C
Two-year-old	2 nd harvest	0.26	0.27	0.26	0.26B

Values in column (mean value) followed by the same capital letter do not differ significantly at $\alpha=0.05$ Values in rows do not differ significantly at $\alpha=0.05$

Table 7.

Developmental characteristics in the second year of plant vegetation

The investigated trait		Plants from 1-stem cuttings	Plants from 2-stem cuttings	Plants from 4-stem cuttings
Number of generative shoots [pcs. $\times$ Plant ⁻¹]		27.43b	32.86b	40.71a
Length of generative shoots [cm]		57.04a	56.57a	61.83a
Mass of seeds [g $\times$ plant ⁻¹]		2.41c	3.74b	4.62a

Values in rows followed by the same letter do not differ significantly at $\alpha=0.05$

The information on generative development of southern sweet-grass, especially on seed setting, are very rare. In this study, the influence of the type of cuttings on the generative development of plant in the second year of vegetation was investigated. Southern sweet-grass begins flowering very early, in April. At the end of May or at the beginning of June, the seeds of this plant are ripen, ready to spread. Then the species produce leaves [8, 10]. The investigated plants started flowering on April 12, the full blooming stage was observed one week later and the seeds were ripen at the beginning of May. The number of generative shoots and the mass of seeds depended strongly on the type of cuttings. The highest

number of generative shoots and the highest mass of seeds was produced by plants grown from 4-stem cuttings in comparison to plants from 1- and 2-stem cuttings (tab. 7). Due to lack of complex data on the quality of southern sweet-grass seeds (dormancy, germination, vigour), further investigation on the generative plant development, including seed setting, should be undertaken.

CONCLUSIONS

- Simple division of southern sweet-grass tufts into stem cuttings seems to be an effective method of vegetative propagation of the plant.
- The plants obtained from 4-stem cuttings, both in the first and second year of vegetation, were characterized by the highest mass of leaves (they produced more leaves and bigger tufts) than the plants from 1- and 2-stem cuttings.
- The total contents of coumarins and phenolic acids in southern sweet-grass leaves did not depend on the type of cuttings but on the plant age. The contents of these compounds were higher in 2-year-old plants in comparison to 1-year-old ones.
- The total contents of flavonoids did not depend on the type of cuttings and it was higher in one-year-old plants.
- The plants from 4-stem cuttings were characterized by the highest number of generative shoots and the highest mass of seeds per plant.

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ZAKŁADANIE PLANTACJI TURÓWKI LEŚNEJ Z SADZONEK PĘDOWYCH UZYSKANYCH PRZEZ PODZIAŁ ROŚLIN MATECZNYCH

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Streszczenie

Turówka leśna (*Hierochloë australis* /Schrad./ Roem. & Schult.) jest wieloletnią, dziko rosnącą trawą kępkową występującą na terenie północno-wschodniej Polski, na Białorusi i w Finlandii. W Polsce jest to gatunek objęty częściową ochroną prawną. Częścią użytkową u tej rośliny są liście bogate w związki kumarynowe, a przede wszystkim w kumarynę, odpowiedzialną za słodki, specyficzny zapach surowca. Liście turówki leśnej wykorzystywane są głównie do aromatyzowania alkoholi i tytoniu. Ze względu na duże zapotrzebowanie na surowiec oraz na kurczące się zasoby naturalne tej rośliny celowe jest wprowadzenie jej do uprawy. W niniejszej pracy oceniono przydatność sadzonek wegetatywnych (jedno-, dwu- i czteropędowych) do zakładania plantacji turówki leśnej. Określono wpływ tych sadzonek na przyrost masy liści i ich jakość w pierwszym i drugim roku wegetacji roślin oraz masę nasion uzyskanych z roślin dwuletnich. Najwięcej ukorzenionych roślin uzyskano z sadzonek czteropędowych (74,07%), a najmniej z sadzonek jednopędowych (47,53%). Zarówno na plantacji jedno- jak i dwuletniej rośliny uzyskane z sadzonek czteropędowych wytworzyły najwyższą masę liści (odpowiednio: 7,73 i 24,65 g $\times$ roślina⁻¹). Rośliny te charakteryzowały się także najwyższą liczbą pędów generatywnych (40,71 szt. $\times$ roślina⁻¹) i najwyższą masą nasion (4,62 g $\times$ roślina⁻¹). Ogólna zawartość kumaryn i kwasów polifenolowych nie zależała od użytego materiału sadzonkowego. Zawartość tych grup związków była wyższa u roślin dwuletnich niż jednorocznych, natomiast ogólna zawartość flawonoidów była wyższa u roślin jednorocznych.

Słowa kluczowe: *Hierochloë australis*, żubrówka, sadzonki, rozwój roślin, kumaryny, kwasy fenolowe, flawonoidy