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Generative propagation and fertilisation of Stipeae species – wild grasses with ornamental potential

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Abstract: The wild grasses are of increasing interest among landscape architects. The appropriate plant selection is crucial for the subsequent survival and growth of plants in grassy gardens. The aim of the experiment was to assess the influence of seed age (1–3-year old seeds) on the germination of three *Stipeae* species: *Eriocoma occidentalis* subsp. *californica*, *Stipa pulcherrima* and *Hesperostipa curtiseta*. The seed weight and germination percentage showed a decline over a period of seed storage. There was 31–84% (depending on species) loss in germinability of 3-year old seeds in comparison to 1-year old seeds. After germination, plants were repotted and treated with Osmocote (a slow-release fertiliser, N15 + P10 + K12). The results indicated that fertilisation significantly increased the number of roots, stems, leaf length, leaf dry weight, chlorophylls and proline content. None of the tested species flowered in the year of sowing but in the next growing season. Plants fertilised in the previous year formed more and of better quality inflorescence stems.

Keywords: Poaceae; wild species; *Eriocoma*; *Hesperostipa*; *Stipa*

In recent years, landscape architects are open to unknown perennials offering interesting and close to natural alternatives to well-known and popular high-coloured plants. The tested species represent wild flora, which enriches the dry grasslands and steppe vegetation. Introducing them to the human environment, we increase biodiversity and create mini landscapes that resemble wild nature. The tribe Stipeae belongs to the subfamily Pooideae in Poaceae family and includes 527 species which distribute mostly on dry open grasslands and steppe vegetations all over the world (Jacobs et al. 2007; Peterson et al. 2019). Most Stipeae species are distinct for their characteristically clump shape formed of basal foliage from which upright inflorescence stems (enriched by long awns) arise in early to mid summer. Their lissome leaves and flowers moving with every breeze bring sound and movement to gardens and larger landscapes. *Eriocoma occidentalis* subsp. *californica* (formerly *Stipa californica*), *Stipa pulcherrima* and *Hesperostipa curtiseta* (formerly *Stipa curtiseta*) are of ornamental potential. They represent three little-known taxa within Stipeae tribe (Gould et al. 2013; Peterson et al. 2019) which propagation has not been described. Traditional vegetative propagation of grasses is more often used in horticultural practice than generative method (Kapczyńska et al. 2020) as in many cases uniform and true to type offspring can be obtain only through asexual propagation. However, it is important to start testing the reproduction of little-known taxa with natural methods, i.e. using seeds – this method is very efficient and doesn't require expensive propagation structures. Germination rate

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is usually different with the different grass species (Jiao et al. 2009) and there is no universal scheme of proceeding as it is regulated by complex interactions between endogenous factors and exogenous environmental conditions (Lamichhane et al. 2018; Zhou et al. 2020) – therefore it should be developed and tested separately for each individual genotype.

The presented results first implement the biological progress which in horticultural production can be facilitated by introducing new or less-well-known plant species to cultivation, ones that feature ornamental qualities and may have a broad range of uses. Second, expand the ornamental grasses palette for a new wave of naturalistic landscape design and to propose drought-tolerant grass species that could be adapted in the future to a wide range of urban and suburban difficult climatic conditions. Third, promote the endangered grasslands species eg. *Stipa pulcherrima* (Novák, Prach 2010) in order to preserve biodiversity in the human environment. Finally, expand knowledge about the tribe Stipeae and its species in terms of propagation, cultivation and plant morphology, flowering process and crop potential.

The objective of this study was to determine the effects of seed age on the germination process and of fertilisation on the growth and flowering of the three ornamental grasses.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse (seed germination) and on the experimental plot (fertilisation study) at the Faculty of Biotechnology and Horticulture University of Agriculture in Krakow, Poland (lat. 50.08°N, long. 19.95°E, elevation 219 m) in 2015 and 2016. The seeds of *Eriocoma occidentalis* subsp. *californica* (Merr. & Burtt Davy) Romasch., *Stipa pulcherrima* K. Koch and *Hesperostipa curtiseta* (A.S. Hitchc.) Barkworth were received from the Plant Breeding and Acclimatization Institute in Bydgoszcz (Poland). The seeds were harvested in 2012, 2013 and 2014, so at the time of the experiment, they were three, two and one year old, respectively. After harvest, the seeds were dry stored at room temperature. Before sowing, the weight (g) of 10 seeds was determined separately for each species harvested in the different years. On 9 June 2015, the seeds (4 × 30 seeds in each object) were sown singly in cell seedling trays (one cell 62 mm) filled with a peat substrate (Botanica Professional Comeco, Poland). The trays were placed in a greenhouse and keep watered. Daily average temperature inside the greenhouse, minimum and maximum temperature as well as radiation from June 2015 to July 2016 are presented in Figure 1. The appearance of the first leaf was used as the criterion for germination. The number of seeds germinated was counted and the germination percentage was determined. Seedlings emerged were systematically counted to calculate Piepper's index (W_{Piep}), which determines the average time (days number) of germination of a single seed (Pipper 1952).

$$W_{Piep} = s_1d_1+s_2d_2+...+s_nd_n/s_1+s_2+...+s_n$$

where:

s – number of germinated seeds in subsequent days of observation;

d – number of days from seeds sowing.

On 10th July 2015, to explore the effects of fertilisation on the growth and quality of *Stipeae* species, seedlings obtained from one-year-old seeds (16–18 cm high) were transplanted individually in standard round plastic pots (12 cm in diameter, 0.6 liter) filled with the peat substrate (Botanica Professional Comeco). The substrate has: N (N-NO_3) – 112 mg/L, P – 36 mg/L, K – 282 mg/L, Ca – 1 174 mg/L, Mg – 110 mg/L, Cl – 35 mg/L and the pH is 6.26. The substrate was supplied with a slow release fertiliser (Osmocote Plus with 5–6 months action 15N + 10P + 12K + 2MgO + microelements, Scotts Company), 5 g per each pot (fertilised plants – FERT). The control plants (CTRL) were planted in the substrate without fertiliser. The experiment was carried out with four replications, each treatment consisting of 20 plants (4 × 5 plants). For proper rooting, plants were cultivated in a greenhouse until 31st July 2015, then the pots with plants were moved to the open field conditions (experimental plot) and keep watered (monthly field 24 h average, lowest and highest temperature as well as radiation from August 2015 to June 2016 are presented in Figure 1). On 7th September 2015 the plants were evaluated. Data were collected including on the number of roots per plant, the average length of roots, the number of vegetative stems per plant, the average length of leaves. At the same time the leaf dry weight (DW), chlorophylls (Chl *a*, Chl *b*, Chl *a*+*b*) and proline content in leaves were estimated.

In the first growing season after sowing in 2015 none of *Stipeae* species flowered. To be able to assess the effect of fertilisation applied in 2015 on the flowering of plants in the following year in 2016,

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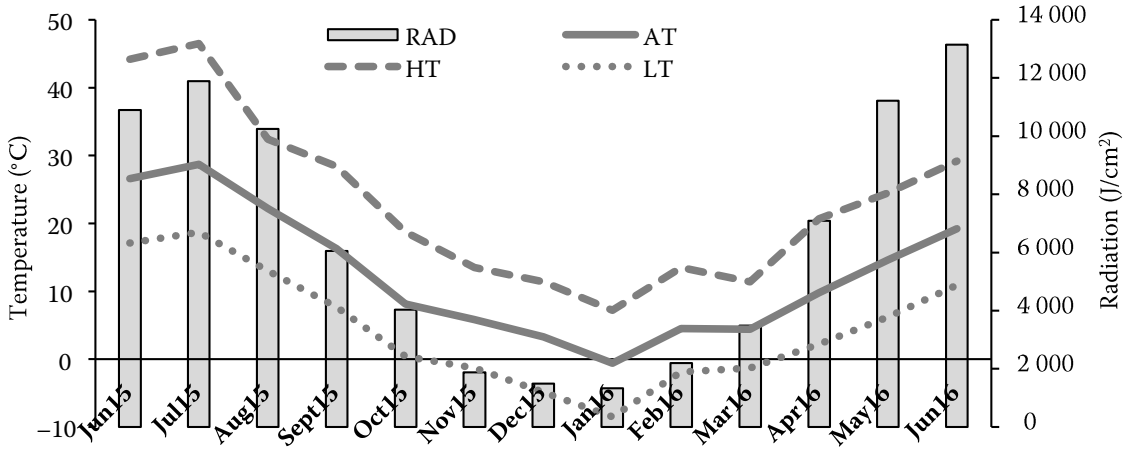

Figure 1. 24h average (A), lowest (L), highest (H) temperatures (T) and radiation (RAD) during the experiment

the pots with plants (controlled and fertilised) were buried in the ground in October 2015 to overwinter in field condition. The pots were buried to a level equal to the upper edge of the pot (to a depth of 9 cm). On 17th May 2016, the survival (%) of grasses was evaluated. Additionally, the date of the beginning of flowering (when the first inflorescence on the plant was visible), inflorescence stems per plant (no), inflorescence stem length (cm) and inflorescence length (cm) were investigated.

Determination of chlorophyll content. Chlorophylls content was determined spectrophotometrically (Spekol 1500, Analytik Jena, Germany) and calculated based on the Arnon (1949) equations. 0.25 g of leaf sample was thoroughly ground with 0.1 g of $CaCO_3$ and 10 mL of acetone in a mortar. Then the mixture was filtered through a filter paper into a volumetric flask, and the residue was washed with several portions of acetone to wash out all pigments. The obtained extract (50 mL) was diluted with distilled water so that the final concentration of acetone was 80%. Absorbance was measured against 80% acetone as blank at 645 nm and 663 nm for chlorophyll *a* and chlorophyll *b*, respectively. Three analytical replications for chlorophylls content were performed for each treatment.

Determination of leaf dry weight and proline content. Leaf dry matter (%) was determined after they were dried at 55 °C for 15 hours. Free proline in grass leaves was measured according to Bates et al. (1973). In brief, 100 mg of dry (15 h at 55 °C) leaves was extracted in 15 mL of 3% sulfosalicylic acid. 1 mL of extract was mixed with 1 mL glacial acetic acid and 1 mL of acidic ninhydrin (1.25 g ninhydrin + 30 mL glacial acetic acid + 20 mL of 2 M orthophosphoric acid) and incubated in a boiling water bath for 1 hour. After cooling, 2 ml of toluene was added and the sample was vortexed and left to phase separation. The absorbance of the toluene phase was measured at 520 nm. The proline concentration in the sample was determined using a standard curve (0–10 μg/mL) and expressed as μg/g of fresh weight. Three analytical replications for proline content were performed for each treatment.

Statistical Analysis. All data were analysed using Statistica 10.0 data analysis software system (StatSoft, Tulsa, USA). Experimental data were subjected to an analysis of variance (ANOVA), and Tukey's multiple range test was used to separate mean values at a significance level of $P \leq 0.05$. Additionally, Pearson correlation coefficient at a probability $P \leq 0.05$ was calculated to determine significant correlations among measured parameters.

RESULTS AND DISCUSSION

All seeds of the tested genotypes for three consecutive years were collected from plants growing in the same habitat but the differences in seed weight in relation to the species and seed age were noticeable (Table 1). One-year-old seeds of *S. pulcherrima* were the heaviest, while 3-year-old seeds *E. occidentalis* subsp. *californica* were 3 times lighter. Generally, seed weight decreased with seed age, which can be explained by the fact that storing seeds over a long period of time leads to a reduction in their quality, weight loss and anatomical structure changes as a result of their metabolism (Skiba et al. 2005). Seed

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Table 1. The effect of genotype and seeds age on 10 seeds weight

Genotype	Seed age (years)	Seed weight (g)
E. occidentalis	1-year-old	0.0285 ± 0.0006^{b*}
	2-year-old	0.0283 ± 0.0005^{b}
	3-year-old	0.0190 ± 0.0008^{a}
S. pulcherrima	1-year-old	0.0618 ± 0.0012^{f}
	2-year-old	0.0513 ± 0.0009^{e}
	3-year-old	0.0473 ± 0.0005^{de}
H. curtiseta	1-year-old	0.0475 ± 0.0006^{de}
	2-year-old	0.0448 ± 0.0009^{d}
	3-year-old	0.0407 ± 0.0005^{c}
Main effects**		
Genotype		< 0.0001
Seed age		< 0.0001
Genotype × Seed age		< 0.0001

*Mean values ± SD in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $P \leq 0.05$; **significant effects ($P \leq 0.05$)

longevity is an important plant criterion in the context of gene banks activities and international exchange and business (Nagel et al. 2016). Seeds of the tested species were still able to germinate three years after harvest but generally their germinability decreased (Figure 2). It was found that the germination of 1 and 2-year-old seeds of *E. occidentalis* subsp. *californica* and *H. curtiseta* were on the same level within the species and amounted 40–41.5 and 50–51.5%, respectively but their 3-year-old seeds lost viability and achieved lower than 10% germination. In the case of *S. pulcherrima* the highest germination was noticed for 1-year-old seeds (32.5%) while the germination of the older seeds was lower by nearly 10 percent. Gasque and García-Fayos (2003) also demonstrated that germination percentage of *Stipa tenacissima* was significantly affected by the duration of storage with 50% germination at harvest and less than 15% after 28 months in storage. The main factor generating the process of seed ageing is attributed to the action of reactive oxygen species (ROS) which are recognised as the main source of dysfunction of mitochondrial membranes and oxidative damage of DNA (Wojtyla et al. 2016; Kurek et al. 2019). In the present study, the highest germination rate was 51.5% only, thus prospective growers should consider the need of exposing seeds to prechill treatment as proven for *Stipa viridula* where prechilled seeds exhibited higher germinability than unchilled ones (Fulbricht et al. 1983). The seed age increased the Pipper index but significant differences were noticed only for *E. occidentalis* subsp. *californica* – average time to germination of a single 3-year-old seeds was nearly 5 days longer compared to 1 and 2-year-old seeds (Figure 3). These data agree with the results obtained by Gasque and García-Fayos (2003) and indicate that onset of germination is effected by seed age.

The results in Table 2 indicate that plants treated with the fertiliser had nearly two times more roots and three-four times more vegetative stems

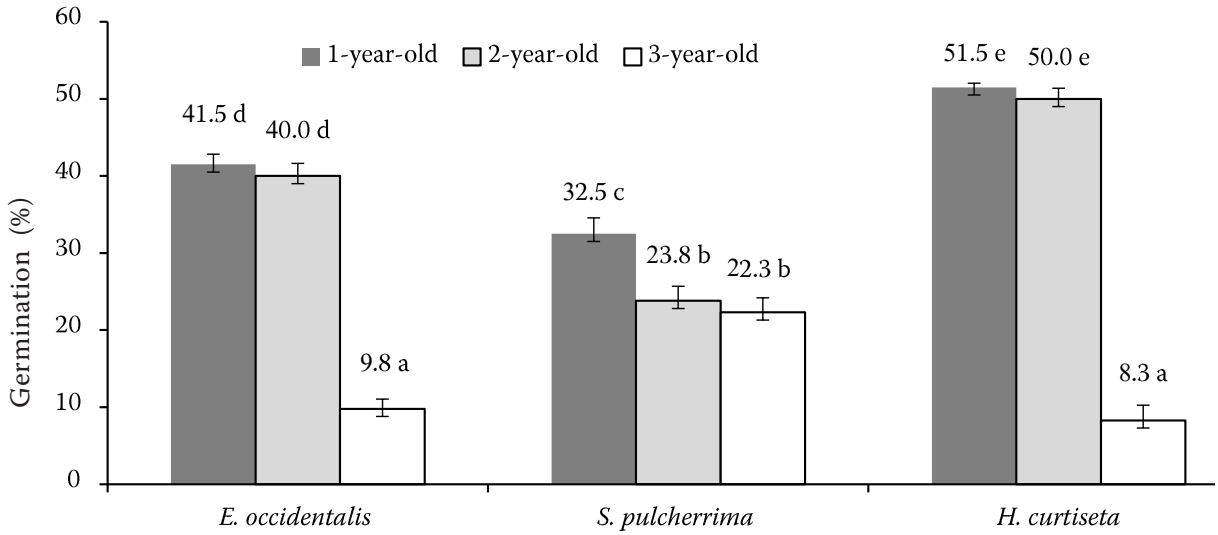

Figure 2. Effect of genotype and seed age on their germination

Mean values followed by different letter are significantly different according to Tukey's least significant difference test at $P \leq 0.05$

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Table 2. Effect of genotype and fertilisation on the growth parameters and leaf dry weight

Genotype	Treatment	Roots (No.)	Root length (cm)	Vegetative stems (No.)	Leaf length (cm)	Leaf DW (%)
E. occidentalis	CTRL	15.9 ± 0.8^{a*}	14.7 ± 0.9^{c}	6.9 ± 2.1^{a}	13.1 ± 4.4^{a}	28.3 ± 0.2^{a}
	FERT	26.3 ± 0.5^{c}	15.3 ± 0.9^{c}	21.2 ± 1.3^{c}	26.8 ± 3.8^{b}	29.4 ± 0.4^{b}
S. pulcherrima	CTRL	20.0 ± 2.1^{b}	11.7 ± 1.2^{b}	9.7 ± 3.1^{ab}	18.1 ± 2.8^{a}	28.8 ± 0.2^{ab}
	FERT	35.3 ± 2.9^{e}	12.3 ± 0.9^{b}	42.8 ± 8.7^{d}	25.3 ± 1.2^{b}	31.9 ± 0.3^{c}
H. curtiseta	CTRL	19.0 ± 1.6^{ab}	9.3 ± 0.5^{a}	4.3 ± 1.3^{a}	27.3 ± 2.4^{b}	29.5 ± 0.3^{b}
	FERT	30.3 ± 0.5^{d}	15.0 ± 0.8^{c}	17.1 ± 2.4^{bc}	49.8 ± 1.7^{c}	32.2 ± 0.2^{c}
Main effects**						
Genotype		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype × Treatment		0.0202	< 0.0001	< 0.0001	0.0002	0.0009

*Mean values ± SD in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $P \leq 0.05$; **significant effects ($P \leq 0.05$); CTRL – control plants; FERT – fertilised plants

in comparison with the control plants. It was found that leaves of fertilised plants are 7–22 cm longer than those plants cultivated in the unfertilised peat substrate. Among the tested species fertilised *H. curtiseta* had the largest number of roots (30.3) and the longest leaf (49.8 cm), while fertilised *S. pulcherrima* had the largest stem number (42.8). Roots of fertilised plants were longer than control counterpart but only in the case of *H. curtiseta* this difference was statistically significant. In all tested species, a comparison of treatments showed greater leaf dry weight in fertilised plants than in the control. All results correspond to the findings of Kapczyńska (2012) who claims that a slow release fertiliser application has a significant effect on the vegetative growth of *Stipa capillata*. Fertilisation may positively affect the photosynthetic pigments (de Souza 2016) and proline (Alaei et al. 2012; Rady 2012) content. Proline, like many others osmolytes, plays a pivotal role in stress tolerance improvement (Serraj, Sinclair 2002). Proline accumulation is species-specific and may be

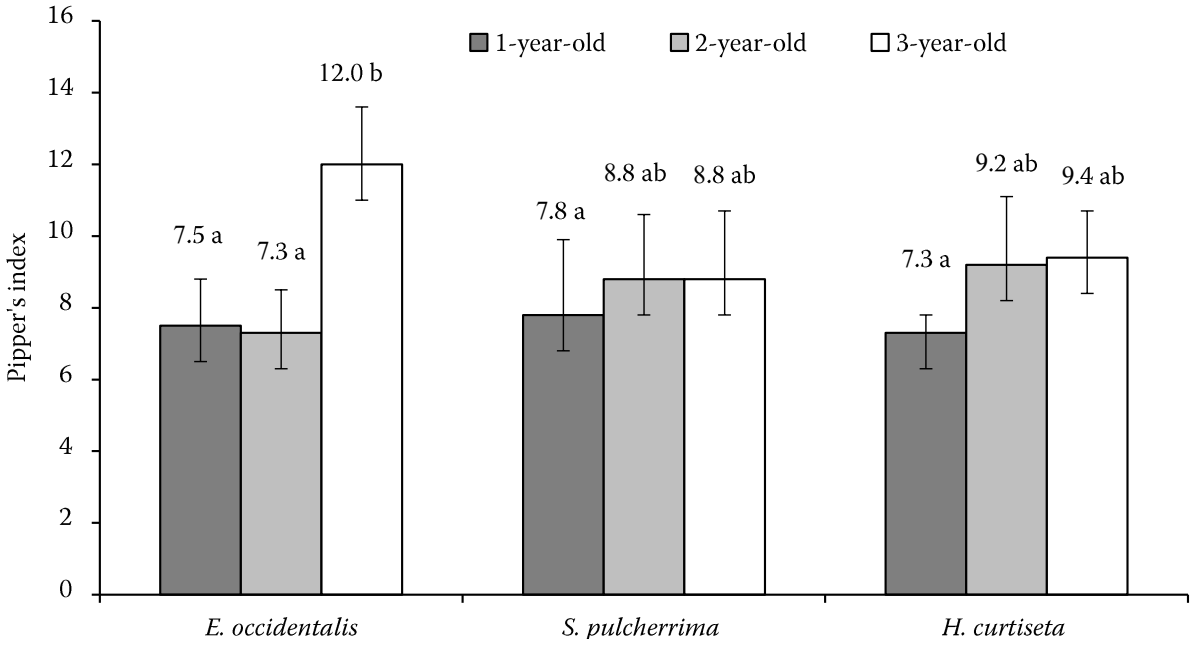

Figure 3. Effect of genotype and seed age on Pipper's index

Mean values followed by different letter(s) are significantly different according to Tukey's least significant difference test at $P \leq 0.05$

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Table 3. Effect of genotype and fertilisation on the plant pigments and proline content in leaves

Genotype	Treatment	Plant pigment content (mg/g FW)			Proline (μmol/g FW)
		Chl *a*	Chl *b*	Chl *a*+*b*	
E. occidentalis	CTRL	2.383 ± 0.006^{b*}	0.819 ± 0.035^{ab}	3.202 ± 0.036^{b}	1.088 ± 0.013^{a}
	FERT	3.119 ± 0.167^{c}	1.207 ± 0.133^{d}	4.326 ± 0.298^{c}	1.564 ± 0.171^{b}
S. pulcherrima	CTRL	2.326 ± 0.004^{b}	0.842 ± 0.029^{ab}	3.168 ± 0.032^{b}	1.449 ± 0.052^{b}
	FERT	3.133 ± 0.086^{c}	1.120 ± 0.026^{cd}	4.252 ± 0.112^{c}	3.197 ± 0.094^{c}
H. curtiseta	CTRL	1.861 ± 0.209^{a}	0.743 ± 0.034^{a}	2.604 ± 0.225^{a}	1.006 ± 0.020^{a}
	FERT	2.984 ± 0.267^{c}	0.969 ± 0.101^{bc}	3.952 ± 0.368^{c}	1.486 ± 0.053^{b}
Main effects**					
Genotype		0.0008	0.0010	0.0005	< 0.0001
Treatment		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype × Treatment		NS	NS	NS	< 0.0001

*Mean values ± SD in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $P \leq 0.05$; **significant effects ($P \leq 0.05$); NS – not significant; CTRL – control plants; FERT – fertilised plants; FW – fresh weight

higher in stress-tolerant than in stress-sensitive plants. Proline may be also accumulated under non-stressed conditions to be involved in other plant processes e.g. flowering and development (Dar et al. 2016). It was reflected in our results where fertiliser affected plant metabolism in a positive manner resulting in a higher level of proline and chrolophyll contents (Table 3) that were then positively reflected in the growth of *Stipeae* plants. Moreover, positive correlations between proline content and most other plant parameters were described (Table 4).

After the first winter the highest survival was noticed for fertilised *S. pulcherrima* and *H. curtiseta* (91.3 and 92.0%, respectively), much lower for not fertilised S. *pulcherrima* (59.3%) and the lowest for not fertilised *H. curtiseta* (41.0%) (Table 5). In the case of *E. occidentalis* subsp. *californica*, irrespective of treatment, 80% of plants resumed the growth. The winter conditions during plant rest were typical for the region (Figire 1). The average temperature in the period from November to February ranged from –0.6 °C to 5.8 °C. The lowest temperature (–8.5 °C) during wintertime was recorded in January. Referring the temperature to the grass survival it can be stated that the tested species can be successfully cultivated in the open ground conditions of temperate climate. In the first season of the experiment, none of the plants flowered. This occurrence is known in the grass world. Bartolome (1981) reported that only two-year-old plants of *Stipa pulchra* exhibit flowering ability. Similar report was for *Stipa leucotricha* (Fowler, Clay 1995). It can be concluded that tested genotypes must experience low temperatures to flower and produce seeds. In the second season, control and fertilised plants started to flower at the same time. *E. occidentalis* subsp. *californica* begin flowering in the first week of June, *S. pulcherrima* and *H. cur-*

Table 4. Correlation coefficient between measured parameters irrespective of genotype and treatment

	Chl *a*	Chl *b*	Chl *a*+*b*	Vegetative stems (No.)	Leaf length (cm)	Roots (No.)	Root length (cm)	Leaf DW (%)
Proline	0.64	0.61	0.65	0.93	NS	0.82	NS	0.62
Chl *a*		0.90	0.99	0.73	NS	0.75	0.63	0.58
Chl *b*			0.95	0.71	NS	0.67	0.52	NS
Chl *a*+*b*				0.74	NS	0.74	0.61	0.53
Vegetative stems (No.)					NS	0.86	NS	0.62
Leaf length (cm)						0.57	NS	0.58
Roots (No.)							NS	086
Root length (cm)								NS

NS – not significant; DW – dry weight

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Table 5. Effect of genotype and fertilisation on the survival ability and flowering quality

Genotype	Treatment	Survival ability (%)	Inflorescence stems per plant (No.)	Inflorescence stem length (cm)	Inflorescence length (cm)
E. occidentalis	CTRL	80.7 ± 1.2^{c*}	10.7 ± 0.6^{d}	26.0 ± 1.0^{a}	13.7 ± 0.6^{a}
	FERT	81.0 ± 1.0^{c}	19.3 ± 0.5^{e}	52.0 ± 1.0^{b}	25.3 ± 0.6^{d}
S. pulcherrima	CTRL	59.3 ± 1.2^{b}	5.3 ± 0.6^{b}	24.0 ± 1.0^{a}	15.0 ± 1.0^{a}
	FERT	91.3 ± 1.2^{d}	7.3 ± 0.6^{c}	50.7 ± 0.6^{b}	18.7 ± 0.6^{b}
H. curtiseta	CTRL	41.0 ± 1.0^{a}	3.7 ± 0.6^{a}	83.0 ± 1.0^{c}	22.0 ± 1.0^{c}
	FERT	92.0 ± 2.0^{d}	5.3 ± 0.6^{b}	112.3 ± 1.5^{d}	23.7 ± 0.6^{d}
Main effects**					
Genotype		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype × Treatment		< 0.0001	< 0.0001	0.0414	< 0.0001

*Mean values ± SD in columns followed by different letter are significantly different according to Tukey's least significant difference test at $P \leq 0.05$; **significant effects ($P \leq 0.05$); CTRL – control plants, FERT – fertilised plants

tiseta in the third week of June (data not shown). Due to the relatively early flowering time, they can be good alternatives to many popular ornamental grass species that bloom in late summer e.g. *Miscanthus* or *Pennisetum*. Moreover, they can be attractive to landscape managers because of their high resistance to drought, which results from their natural environmental habitat (dry steppe vegetation). According to Kapczyńska (2012), the flowering parameters of unfertilised ornamental grasses (*Melica* sp.) are lower in comparison with those of the fertilised plants, which is consistent with our present results (Table 5). For example, fertilised *E. occidentalis* subsp. *californica* produced nearly two times more inflorescence stems, longer by 26 cm and inflorescences longer by 11.6 cm than control plants. The research shows that fertilisation in the previous year affects the overall condition of grasses, and consequently the quality of flowering in the following year. Analysing the linear correlation between the measured morphological features in the second season of cultivation only one significant correlation was found – it was a positive correlation between inflorescence stem length and inflorescence length (data not shown).

Little information is available on the morphological and nutritional characteristics of grasses grown under nursery conditions. Thetford et al. (2011) claim that some ornamental grasses show minimal response to supplemental irrigation or fertilisation and what's more, that weak foliage accompanying with falling-over inflorescences may be observed during an excess of water and nutrients. On the contrary, Harvey et al. (2004) found that the growth and flowering of *Haconechloa macra* are enhanced by fertilisation. That is why choosing the right type and dose of fertiliser (without over or under application) is an essential element in suitable cultivation techniques at the nursery production stage and at the subsequent growth of plants in the urban landscape.

CONCLUSION

The tested species can be readily established from seeds and this can be the practical method for the largest landscapes management. The best results were obtained with the youngest material – the viability of the seeds decreased with age. The application of a slow-release fertiliser had a positive effect on the vegetative growth of all grasses investigated and did not affect the time of flowering but determined the yield and quality of inflorescences. We recommend the application of a slow-release fertiliser in the spring to improve grass quality and strength to survive in difficult urban conditions.

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