

## LARGER SEED MASS PREDICTS HIGHER GERMINATION AND EMERGENCE RATES IN SANDY GRASSLAND SPECIES WITH NON-DORMANT SEEDS

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The lack of knowledge on the traits related to the germination and establishment of native plant species represents obstacles to restoration. Seed mass, germination and emergence variability from two characteristic grasses (*Festuca vaginata* and *Stipa borysthénica*), and two dicots (*Centaurea arenaria* and *Dianthus serotinus*) of Hungarian sandy grasslands from altogether 34 localities were tested. Our results showed that seed mass had a significant positive effect on germinability of the dicots and on seedling emergence of all species. The laboratory germination capacity of *S. borysthénica* was low due to dormancy. We found a significant variability among seed traits and emergence between localities in all the species except for the germination of *S. borysthénica*. This significant variation among populations might be explained by local adaptation or maternal effects. We conclude that germination under laboratory-regulated conditions is a good predictor of seedling emergence for restoration projects, but limited to species with non-dormant seeds.

Key words: emergence, germination, grasslands, local adaptation, Pannonian region, relationship of traits, thousand seed weight

### INTRODUCTION

Grasslands cover an estimated 52.5 million km<sup>2</sup> or 40.5% of the Earth's land surface, making them one of the world's largest ecosystems (Suttie *et al.* 2005, Dengler *et al.* 2014, Török and Dengler 2018). Grasslands provide a wide range of ecosystem services, such as carbon sequestration (34% of the global carbon stock in terrestrial ecosystems; EC 2008), fodder supply, erosion control, soil fertility preservation (Lavorel *et al.* 2017), and foster recreation activities such as hunting, bird watching and hiking (White *et al.* 2000). The area of grasslands is shrinking and their biodiversity is decreasing globally and also in the EU, mainly due to land-use conversion (Maes *et al.* 2021).

The implementation of ecological restoration projects is necessary to compensate grassland loss and fragmentation. Many conservation practition-

ers advocate for passive restoration as they consider that spontaneous succession is sufficient to improve the natural condition of grasslands (Prach and del Moral 2015). However, it is not enough in many regions of Europe where the active introduction of species is necessary by sowing seeds or transferring plant material from target species or communities to the restoration site to accelerate the process and avoid invasion by non-native species (Török *et al.* 2011). Kövendi-Jakó and coworkers (2019) found a tenfold acceleration of succession due to seeding.

To cope with the loss of grasslands, large-scale restoration efforts are planned globally (UN Decade on Ecosystem Restoration) and in the EU Biodiversity Strategy 2030 (EU 2020) that require big quantities of seeds, as the most common restoration method is direct seeding (Kiehl *et al.* 2010). There is a seed shortage at the moment that must be solved (Merritt and Dixon 2011, McDonald *et al.* 2016) by developing native seed markets with ecologically based certification programs (Ladouceur *et al.* 2017). Fortunately, few countries, like Austria and Germany (De Vitis *et al.* 2017) have already developed a native seed market that can serve as a model, however, still with a multitude of problems (Mainz and Wieden 2019). The lack of knowledge on native species characteristics and behaviour provides further obstacles to restoration (Rinella *et al.* 2016); e.g. to estimate seeding quantities (Kövendi-Jakó *et al.* 2017, Pedrini and Dixon 2020), or to identify provenance guidance for seed transfer (Cevallos *et al.* 2020).

When plant material is sown or transplanted to a new site, it is essential to know if this material will fit under the new conditions. The use of seeds from local origin is usually recommended to avoid maladaptation in restoration (Broadhurst *et al.* 2008, Breed *et al.* 2018). According to a meta-analysis, in nearly two thirds of studies, local plants performed better than plants from distant regions (Leimu and Fischer 2008). Populations evolve fitness traits that provide advantage in the local habitat irrespective of other habitats, the resulting pattern and the process is called local adaptation (Kawecki and Ebert 2004). Evidence of intraspecific variation among populations, as a proof of local adaptation, has been demonstrated in several studies, and can be measured by functional traits of selected grassland species (Moreira *et al.* 2012, Wellstein *et al.* 2013, Siefert *et al.* 2015, Helsen *et al.* 2017). Intraspecific functional trait variation was found to be so large that it makes the use of mean values from databases originating from a few populations challenging (Albert *et al.* 2010).

Seed mass is an essential fitness-related trait (Violle *et al.* 2009), easily measurable and variable within plant species (Helsen *et al.* 2017). Hendrix and Sun (1989) found that intraspecific variation in seed mass can range from three to 16-fold. Intraspecific seed mass variability might exceed that of species within a genus (Ellison 2001). The variability between seeds from different provenance can be a sign of local adaptation to the environment (Moles *et*

al. 2005). Seed mass variation can also be explained by several biotic factors, including maternal genetic influence, flowering time, seed location within a plant and fruit (Obeso 2012).

The germination rate provides critical information on the early life stage of the introduced species (Gallagher and Wagenius 2016) and this knowledge is essential for estimating seeding densities for restoration (Kövendi-Jakó *et al.* 2017). The regulations on native seed transfer also require the knowledge on germinability of seed lots (Mainz and Wieden 2019). Germination is the first stage-transition that can shape later life-stage traits, thus especially important for adaptation (Donohue *et al.* 2010). Germination is generally studied in laboratory or under greenhouse conditions (Kövendi-Jakó *et al.* 2017), therefore it might be a weak predictor for field establishment (Carrington 2014). Gallagher and Wagenius (2016) found 3–12 times higher germination in the chamber than in the field for three grass species. Similarly lower emergence was detected in the field than in a germination experiment for seven loess species out of 15 seeded species, and four did not emerge at all (Valkó *et al.* 2018). Emergence variability can be measured in common garden settings when all provenances are grown under similar conditions (Kawecki and Ebert 2004, Bell and Galloway, 2008). Common garden experiment is also used to test trait variations among provenances for a particular restoration purpose (Bernik *et al.* 2021). For Pannonian sandy grassland species, germination and emergence rate were positively connected (Kövendi-Jakó *et al.* 2017). If different traits respond similarly to an environmental gradient, a strong intraspecific relationship between traits can be detected (Laughlin *et al.* 2017). Intraspecific trait variance should be included in studies when the response of species to environmental changes and thus consequences for survival are to be estimated (Violle *et al.* 2012), like during the introduction of species to a site in restoration interventions. Frequently measured plant functional traits are seed size and germination, seed production and leaf area (Baskin and Baskin 1998, Qaderi and Cavers 2002, Vergeer and Kunin 2013, Helsen *et al.* 2017). Studies should sample high number of populations to reveal trait variability (Albert *et al.* 2010).

We tested trait variability by comparing and linking seed traits (seed mass, germination) and seedling emergence from altogether 34 different locations within Hungary. In our study, four representative species of the Pannonian sand grasslands were studied. Two species of grass (*Festuca vaginata* Waldst. et Kit. ex Willd. and *Stipa borysthena* Klokov ex Prokudin), which are dominant species within the calcareous sandy areas in the Pannonian region and two forbs (*Centaurea arenaria* M. Bieb. ex Willd. and *Dianthus serotinus* Waldst. et Kit.), as accompanying species. We hypothesised 1) that larger seed mass predicts higher germinability and establishment for all studied species; 2) that laboratory germination rate is a good predictor of emergence in the common garden, and 3) intraspecific variation is significantly different among localities.

## MATERIALS AND METHODS

*Study region* – This study was conducted in the Pannonian biogeographic region, Hungary (Fig. 1). Within the Carpathian Basin, the Pannonic sand steppes are endemic, and they are classified as priority habitats by the EU (HD code 6260). The larger parts of sand steppes lie in the Mid-Danube-Tisza and Nyírség regions. Smaller patches are located in other areas within the Great Hungarian Plain, Transdanubia and the foothills of the North Hungarian Mountains (Fig. 1, Bölöni *et al.* 2011). The yearly mean temperature ranges between 10.5 and 11 °C, with yearly precipitation ranging between 500 and 600 mm (Bihari *et al.* 2018). The type of soil from the most extensive sandy regions, e.g. Mid-Danube-Tisza region and Nyírség, is identified by blown sand skeletal soil (arenosol) including low humus content (below 1%). In the Transdanubian region and the northern foothills, the sandy soils are formed by sandy loam with up to 2% organic matter content (Pásztor *et al.* 2018). Open and closed sandy grasslands characterise the Pannonic sandy steppes, commonly composing a mosaic. We collected samples in open sandy grasslands that potentially occur on dune tops, on loose sand with low humus content (Bölöni *et al.* 2011).

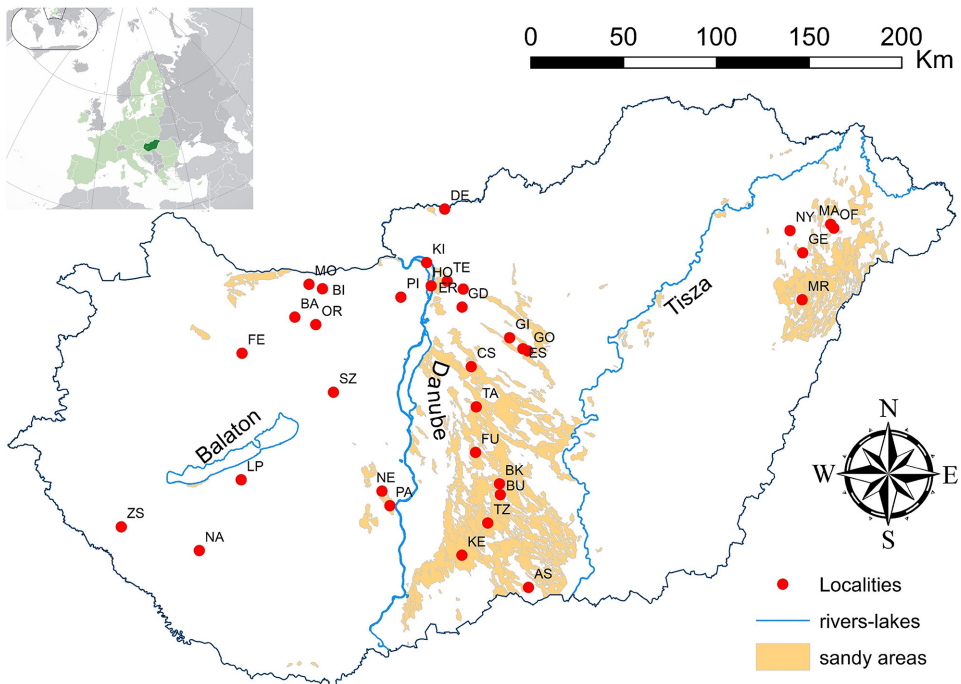


Fig. 1. Map of locations of seed collection of the four species and main sandy regions in Hungary (Pásztor *et al.* 2018). Names of localities are displayed in Table 1

*Field sampling* – We selected the two dominant grasses (*Festuca vaginata* and *Stipa borysthénica*) and accompanying forbs (*Centaurea arenaria* and *Dianthus serotinus*) that are widely distributed in open sandy grasslands throughout Hungary. Seeds were collected from altogether 34 different locations (Fig. 1; Table 1) during the 2019 growing season according to the ripening: *S. borysthénica* in May–July; *F. vaginata* in June–July; *C. arenaria* in July; *D. serotinus* in August–September. Ripening period was estimated based on Török *et al.* (2013) collection dates and checked by visits to the field. The 34 sampled locations can be found in the most representative sandy grassland patches within Hungary. Fifty seeds were collected by hand from 15 randomly selected specimens, resulting in 15 seed samples (each containing 50 seeds) at each locality. *S. borysthénica* displayed a lower average seed yield per individual, therefore, the seeds collected from neighbouring individuals were considered as one sample to achieve 50 seeds. The seeds were stored in a dry environment (temperature:  $25\pm 3$  °C, humidity:  $38\pm 4\%$ ) until they were ready to be processed.

*Laboratory processing* – An analytical balance (0.0001 g precision) was used to measure the dry weight of 50 or 25 seeds (for *S. borysthénica*) from each of the 15 individuals from each location. Due to the large seeds of the latter species, there was no need to use a high number of seeds to reach a measurable quantity. The seed mass was expressed as grams per 1,000 seeds.

A germination experiment was carried out under regulated conditions in a plant growth chamber from October 2019 to March 2020 based on Peti *et al.* (2017) and RBGK (2020). The germination experiment was carried out during three weeks for *D. serotinus*, *F. vaginata* and *C. arenaria*, and six weeks for *S. borysthénica*. The extended incubation time for the latter species was due to retarded germination. Seeds of six individuals were selected randomly per location for each species. Thirty-five seeds (*C. arenaria*, *D. serotinus*, and *F. vaginata*) and ten seeds (*S. borysthénica*) were placed on wet filter paper in sterile Petri dishes. Seed coats were not sterilised. Mechanical scarification (rubbing the seeds with sandpaper) was used to break the dormancy of *S. borysthénica*. The Petri dishes were placed inside an Aralab plant-growth climatic chamber (FITOCLIMA D1200 PHL) with regulated temperature (20 °C), light (8 h light and 16 h dark) and humidity (50%). The same conditions were used for all the species. Seeds were watered regularly, three times per week. Germination was counted once a week. A seed was considered to be germinated when the radicle reached 2 mm.

*Common garden experiment* – A cumulative seedling emergence experiment was conducted in a roof garden under outdoor conditions at the National Botanical Garden in Vácrátót (47.7106° N, 19.2305° E). Fifteen seeds were sown (5 rows  $\times$  3 columns) per sample in a pot (13  $\times$  13  $\times$  13 cm) filled with washed river sand. Fifteen pots were sown per location and species, with a total of 285 pots for *C. arenaria*, 240 pots for *D. serotinus*, 435 pots for *F. vaginata*, and 210 pots for *S. borysthénica*. For all species, sowing was carried out in

Table 1

Seed traits and emergence for each species organised by locality. Average ( $\bar{x}$ , bold), maximum (I), minimum values (l) and sample size (S, italics). At the end of the table the total average per trait per species ( $\bar{x}$ )  $\pm$  standard error is displayed. These values are compared to two references: values in Török *et al.* 2013 and value in Peti *et al.* 2017. Seed mass is expressed in grams per thousand seeds, germination and emergence in ratio. The sample size is in italics and it is represented for the seed mass by the number of measurements per location, and for the germination and emergence by the total number of seeds used in the measurements. Abbreviation: S = symbol, GE = germination, SM = seed mass, EM = emergence

Locality	S	<i>Centauria arenaria</i>			<i>Dianthus serotinus</i>			<i>Festuca vaginata</i>			<i>Stipa borjysthenica</i>		
		SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM
Ásóthalom (AS)	$\bar{x}$	<b>1.792</b>	<b>0.181</b>	<b>0.262</b>	<b>0.498</b>	<b>0.781</b>	<b>0.573</b>	<b>0.395</b>	<b>0.286</b>	<b>0.404</b>	<b>12.443</b>	<b>0.167</b>	<b>0.848</b>
	I	2.479	0.457	0.600	0.679	1.000	0.867	0.543	0.800	0.867	14.736	0.500	1.000
	l	1.400	0.029	0.067	0.246	0.400	0.267	0.180	0.000	0.000	8.988	0.000	0.667
	S	<i>15</i>	<i>210</i>	<i>225</i>	<i>15</i>	<i>210</i>	<i>225</i>	<i>15</i>	<i>210</i>	<i>225</i>	<i>14</i>	<i>60</i>	<i>210</i>
Bársonyos (BA)	$\bar{x}$	-	-	-	<b>0.635</b>	<b>0.971</b>	<b>0.609</b>	<b>0.490</b>	<b>0.533</b>	<b>0.591</b>	-	-	-
	I	-	-	-	0.807	1.000	1.000	0.626	0.853	0.933	-	-	-
	l	-	-	-	0.494	0.857	0.200	0.380	0.343	0.000	-	-	-
	S	-	-	-	<i>15</i>	<i>210</i>	<i>225</i>	<i>15</i>	<i>210</i>	<i>225</i>	-	-	-
Billepuzsza (BI)	$\bar{x}$	-	-	-	<b>0.482</b>	<b>0.719</b>	<b>0.742</b>	-	-	-	-	-	-
	I	-	-	-	0.669	1.000	0.933	-	-	-	-	-	-
	l	-	-	-	0.256	0.353	0.267	-	-	-	-	-	-
	S	-	-	-	<i>15</i>	<i>210</i>	<i>225</i>	-	-	-	-	-	-
Bugac (BU)	$\bar{x}$	<b>2.239</b>	<b>0.531</b>	<b>0.387</b>	-	-	-	<b>0.490</b>	<b>0.759</b>	<b>0.578</b>	<b>13.528</b>	<b>0.117</b>	<b>0.909</b>
	I	2.947	0.800	0.800	-	-	-	0.615	0.886	0.933	15.472	0.200	1.000
	l	1.293	0.400	0.000	-	-	-	0.306	0.000	0.067	11.876	0.000	0.733
	S	<i>15</i>	<i>210</i>	<i>225</i>	-	-	-	<i>15</i>	<i>210</i>	<i>225</i>	<i>14</i>	<i>60</i>	<i>210</i>
Bugac külső (BK)	$\bar{x}$	-	-	-	-	-	-	<b>0.434</b>	<b>0.494</b>	<b>0.587</b>	<b>13.133</b>	<b>0.033</b>	<b>0.924</b>
	I	-	-	-	-	-	-	0.520	0.829	0.867	16.500	0.100	1.000
	l	-	-	-	-	-	-	0.288	0.235	0.200	11.916	0.000	0.800
	S	-	-	-	-	-	-	<i>15</i>	<i>210</i>	<i>225</i>	<i>15</i>	<i>60</i>	<i>225</i>

Table 1 (continued)

Locality	S	<i>Centaurea arenaria</i>				<i>Dianthus serotinus</i>				<i>Festuca vaginata</i>				<i>Stipa borjssythenica</i>			
		SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM	
Csevharaszt (CS)	$\bar{x}$	<b>2.113</b>	<b>0.233</b>	<b>0.244</b>	<b>0.269</b>	<b>0.500</b>	<b>0.383</b>	<b>0.391</b>	<b>0.057</b>	<b>0.129</b>	<b>14.443</b>	<b>0.120</b>	<b>0.906</b>				
	I	3.053	0.543	0.933	0.593	0.857	0.800	0.527	0.171	0.600	16.280	0.400	1.000				
	I	1.334	0.000	0.000	0.076	0.000	0.000	0.247	0.000	0.000	11.944	0.000	0.733				
	S	15	210	225	15	210	225	15	210	225	15	60	225				
Dejtár (DE)	$\bar{x}$	-	-	-	-	-	-	-	-	-	<b>12.407</b>	<b>0.017</b>	<b>0.893</b>				
	I	-	-	-	-	-	-	-	-	-	13.684	0.100	1.000				
	I	-	-	-	-	-	-	-	-	-	11.428	0.000	0.733				
	S	-	-	-	-	-	-	-	-	-	15	60	225				
Erdőkertes (ER)	$\bar{x}$	<b>2.221</b>	<b>0.553</b>	<b>0.276</b>	<b>0.503</b>	<b>0.857</b>	<b>0.760</b>	<b>0.534</b>	<b>0.564</b>	<b>0.716</b>	<b>15.462</b>	<b>0.100</b>	<b>0.937</b>				
	I	2.895	0.800	0.533	0.581	1.000	0.933	0.745	0.941	1.000	16.920	0.300	1.000				
	I	1.710	0.257	0.000	0.410	0.486	0.467	0.321	0.257	0.467	13.580	0.000	0.800				
	S	15	210	225	15	210	225	15	210	225	15	60	225				
Erdőszőlő (ES)	$\bar{x}$	-	-	-	-	-	-	-	-	-	<b>10.919</b>	<b>0.017</b>	<b>0.764</b>				
	I	-	-	-	-	-	-	-	-	-	13.004	0.100	1.000				
	I	-	-	-	-	-	-	-	-	-	7.708	0.000	0.467				
	S	-	-	-	-	-	-	-	-	-	14	60	210				
Fenyőfő (FE)	$\bar{x}$	-	-	-	-	-	-	<b>0.494</b>	<b>0.777</b>	<b>0.676</b>	-	-	-				
	I	-	-	-	-	-	-	0.619	0.914	1.000	-	-	-				
	I	-	-	-	-	-	-	0.348	0.457	0.267	-	-	-				
	S	-	-	-	-	-	-	15	210	225	-	-	-				
Fülpáza (FU)	$\bar{x}$	<b>1.667</b>	<b>0.039</b>	<b>0.013</b>	<b>0.576</b>	<b>0.908</b>	<b>0.680</b>	<b>0.481</b>	<b>0.658</b>	<b>0.644</b>	<b>15.113</b>	<b>0.117</b>	<b>0.933</b>				
	I	2.287	0.176	0.067	0.806	1.000	0.933	0.653	0.941	1.000	16.392	0.600	1.000				
	I	1.280	0.000	0.000	0.138	0.735	0.400	0.369	0.000	0.400	12.072	0.000	0.800				
	S	15	210	225	15	210	225	15	210	225	15	60	225				

Table 1 (continued)

Locality	S	<i>Centaurea arenaria</i>				<i>Dianthus serotinus</i>				<i>Festuca vaginata</i>				<i>Stipa borysghenica</i>			
		SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM	
Gesztérd (GE)	$\bar{x}$	<b>1.904</b>	<b>0.398</b>	<b>0.373</b>	-	-	-	-	-	-	-	-	-	-	-	-	
	I	2.580	0.857	0.600	-	-	-	-	-	-	-	-	-	-	-	-	
	I	1.443	0.086	0.067	-	-	-	-	-	-	-	-	-	-	-	-	
	S	15	35	225	-	-	-	-	-	-	-	-	-	-	-	-	
Gicei-hegy (GI)	$\bar{x}$	<b>2.183</b>	<b>0.419</b>	<b>0.156</b>	<b>0.565</b>	<b>0.952</b>	<b>0.760</b>	<b>0.514</b>	<b>0.649</b>	<b>0.600</b>	-	-	-	-	-		
	I	2.775	0.829	0.400	0.678	1.000	0.933	0.631	0.857	0.867	-	-	-	-	-		
	I	1.679	0.029	0.000	0.411	0.886	0.600	0.352	0.200	0.067	-	-	-	-	-		
	S	15	210	225	15	210	225	15	210	225	-	-	-	-	-		
Göböljárás (GO)	$\bar{x}$	<b>2.374</b>	<b>0.629</b>	<b>0.342</b>	<b>0.480</b>	<b>0.900</b>	<b>0.764</b>	<b>0.479</b>	<b>0.814</b>	<b>0.707</b>	-	-	-	-	-		
	I	3.346	0.829	0.733	0.625	1.000	1.000	0.639	0.914	0.933	-	-	-	-	-		
	I	1.794	0.429	0.133	0.124	0.514	0.467	0.325	0.429	0.400	-	-	-	-	-		
	S	15	210	225	15	210	225	15	210	225	-	-	-	-	-		
Gödöllő (GD)	$\bar{x}$	<b>1.608</b>	<b>0.265</b>	<b>0.265</b>	-	-	-	<b>0.286</b>	<b>0.029</b>	<b>0.049</b>	-	-	-	-	-		
	I	2.097	0.543	0.543	-	-	-	0.436	0.057	0.267	-	-	-	-	-		
	I	1.184	0.029	0.029	-	-	-	0.225	0.000	0.000	-	-	-	-	-		
	S	15	210	225	-	-	-	15	210	225	-	-	-	-	-		
Horány (HO)	$\bar{x}$	<b>1.561</b>	<b>0.274</b>	<b>0.191</b>	<b>0.644</b>	<b>0.766</b>	<b>0.627</b>	<b>0.456</b>	<b>0.483</b>	<b>0.529</b>	<b>8.450</b>	<b>0.067</b>	<b>0.400</b>	-	-		
	I	1.997	0.471	0.467	0.772	0.941	0.867	0.594	0.714	0.867	15.520	0.400	1.000	-	-		
	I	1.168	0.171	0.000	0.532	0.514	0.400	0.283	0.343	0.067	4.124	0.000	0.000	-	-		
	S	15	210	225	15	210	225	15	210	225	14	60	210	-	-		
Kéteshalom (KE)	$\bar{x}$	<b>1.949</b>	<b>0.521</b>	<b>0.427</b>	<b>0.452</b>	<b>0.825</b>	<b>0.518</b>	<b>0.539</b>	<b>0.748</b>	<b>0.751</b>	<b>10.759</b>	<b>0.017</b>	<b>0.640</b>	-	-		
	I	2.664	0.914	0.933	0.637	0.971	0.867	0.681	0.857	1.000	15.321	0.100	1.000	-	-		
	I	1.113	0.200	0.067	0.200	0.543	0.133	0.379	0.514	0.467	7.564	0.000	0.200	-	-		
	S	15	210	225	15	210	225	15	210	225	14	60	210	-	-		



Table 1 (continued)

Locality	S	<i>Centaurea arenaria</i>				<i>Dianthus serotinus</i>				<i>Festuca vaginata</i>				<i>Stipa borjshenica</i>			
		SM	GE	EM		SM	GE	EM		SM	GE	EM		SM	GE	EM	
Kisoroszi (KI)	$\bar{x}$	1.766	0.584	0.551	-	-	-	-	0.472	0.433	0.462	0.462	9.480	0.167	0.516		
	I	2.353	0.657	0.867	-	-	-	-	0.900	0.829	0.867	0.867	15.784	0.600	1.000		
	I	0.966	0.486	0.267	-	-	-	-	0.322	0.000	0.000	0.000	4.216	0.000	0.000		
	S	15	210	225	-	-	-	-	15	210	225	225	15	60	225		
Látrány-puszta (LP)	$\bar{x}$	-	-	-	-	-	-	-	0.356	-	0.120	0.120	-	-	-		
	I	-	-	-	-	-	-	-	0.513	-	0.467	0.467	-	-	-		
	I	-	-	-	-	-	-	-	0.146	-	0.000	0.000	-	-	-		
	S	-	-	-	-	-	-	-	15	-	225	225	-	-	-		
Magy (MA)	$\bar{x}$	-	-	-	-	-	-	-	0.519	0.795	0.724	0.724	-	-	-		
	I	-	-	-	-	-	-	-	0.703	0.971	1.000	1.000	-	-	-		
	I	-	-	-	-	-	-	-	0.308	0.343	0.267	0.267	-	-	-		
	S	-	-	-	-	-	-	-	15	210	225	225	-	-	-		
Mocsa (MO)	$\bar{x}$	-	-	-	-	-	-	-	0.489	0.781	0.764	0.764	12.589	0.033	0.831		
	I	-	-	-	-	-	-	-	0.615	0.971	0.933	0.933	14.080	0.100	0.933		
	I	-	-	-	-	-	-	-	0.411	0.516	0.467	0.467	11.192	0.000	0.600		
	S	-	-	-	-	-	-	-	15	210	225	225	15	60	225		
Martinka (MR)	$\bar{x}$	2.219	0.624	0.560	-	-	-	-	0.484	0.838	0.693	0.693	-	-	-		
	I	2.860	0.743	0.867	-	-	-	-	0.619	0.914	1.000	1.000	-	-	-		
	I	1.718	0.457	0.133	-	-	-	-	0.390	0.057	0.467	0.467	-	-	-		
	S	15	210	225	-	-	-	-	15	210	225	225	-	-	-		
Nagybajom (NA)	$\bar{x}$	-	-	-	-	-	-	-	0.342	0.495	0.347	0.347	0.382	0.514	0.404		
	I	-	-	-	-	-	-	-	0.518	0.943	0.600	0.600	0.490	0.914	0.800		
	I	-	-	-	-	-	-	-	0.116	0.229	0.067	0.067	0.309	0.057	0.000		
	S	-	-	-	-	-	-	-	10	210	150	150	15	210	225		

Table 1 (continued)

Locality	S	<i>Centaurea arenaria</i>			<i>Dianthus serotinus</i>			<i>Festuca vaginata</i>			<i>Stipa borjysthenica</i>		
		SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM
Németkér (NE)	$\bar{x}$	1.439	0.059	0.120	0.458	0.809	0.716	0.445	0.771	0.684	-	-	-
	I	1.896	0.125	0.467	0.688	1.000	0.867	0.608	1.000	1.000	-	-	-
	I	0.767	0.000	0.000	0.138	0.429	0.467	0.319	0.000	0.333	-	-	-
	S	15	210	225	15	210	225	15	210	225	-	-	-
Nyíregyházi lőtér (NY)	$\bar{x}$	-	-	-	-	-	-	0.445	0.238	0.244	-	-	-
	I	-	-	-	-	-	-	0.657	0.657	0.733	-	-	-
	I	-	-	-	-	-	-	0.316	0.000	0.000	-	-	-
	S	-	-	-	-	-	-	15	210	225	-	-	-
Ófehértó (OF)	$\bar{x}$	1.839	0.405	0.489	-	-	-	-	-	-	-	-	-
	I	2.267	0.686	0.800	-	-	-	-	-	-	-	-	-
	I	0.964	0.171	0.000	-	-	-	-	-	-	-	-	-
	S	15	210	225	-	-	-	-	-	-	-	-	-
Oroszlány (OR)	$\bar{x}$	-	-	-	0.471	0.898	0.764	0.455	0.703	0.711	-	-	-
	I	-	-	-	0.592	1.000	0.933	0.727	1.000	1.000	-	-	-
	I	-	-	-	0.372	0.529	0.533	0.352	0.086	0.000	-	-	-
	S	-	-	-	15	210	225	15	210	225	-	-	-
Paks (PA)	$\bar{x}$	1.610	0.110	0.156	0.604	0.867	0.707	0.359	0.210	0.244	-	-	-
	I	2.374	0.457	0.800	0.984	1.000	1.000	0.476	0.686	0.733	-	-	-
	I	1.092	0.000	0.000	0.194	0.676	0.267	0.267	0.000	0.000	-	-	-
	S	15	210	225	15	210	225	15	210	225	-	-	-
Pilisvörös-vár (PI)	$\bar{x}$	-	-	-	0.440	0.680	0.431	0.459	0.702	0.356	14.642	0.000	0.636
	I	-	-	-	0.884	1.000	0.867	0.662	0.857	0.867	20.044	0.000	0.867
	I	-	-	-	0.076	0.367	0.000	0.313	0.029	0.000	9.096	0.000	0.267
	S	-	-	-	15	210	225	15	210	225	14	60	210

Table 1 (continued)

Locality	S	Centauria arenaria			Dianthus serotinus			Festuca ovaginata			Stipa borjshenica		
		SM	GE	EM	SM	GE	EM	SM	GE	EM	SM	GE	EM
Székesfehérvár (SZ)	$\bar{x}$	-	-	-	-	-	-	<b>0.491</b>	<b>0.268</b>	<b>0.204</b>	-	-	-
	I	-	-	-	-	-	-	0.760	0.429	0.667	-	-	-
	I	-	-	-	-	-	-	0.247	0.029	0.000	-	-	-
	S	-	-	-	-	-	-	15	210	225	-	-	-
Táborfalva (TA)	$\bar{x}$	<b>1.719</b>	<b>0.211</b>	<b>0.124</b>	<b>0.585</b>	<b>0.914</b>	<b>0.813</b>	<b>0.442</b>	<b>0.500</b>	<b>0.307</b>	-	-	-
	I	2.057	0.486	0.467	0.733	1.000	1.000	0.559	0.829	0.733	-	-	-
	I	1.410	0.029	0.000	0.484	0.829	0.400	0.304	0.229	0.067	-	-	-
	S	15	210	225	15	210	225	15	210	225	-	-	-
Tázlár (TZ)	$\bar{x}$	<b>2.077</b>	<b>0.562</b>	<b>0.471</b>	-	-	-	<b>0.483</b>	<b>0.600</b>	<b>0.573</b>	-	-	-
	I	2.743	0.800	0.733	-	-	-	0.571	0.971	0.733	-	-	-
	I	1.230	0.371	0.267	-	-	-	0.337	0.086	0.067	-	-	-
	S	15	210	225	-	-	-	15	210	225	-	-	-
Tece (TE)	$\bar{x}$	<b>1.642</b>	<b>0.123</b>	<b>0.169</b>	-	-	-	<b>0.503</b>	<b>0.685</b>	<b>0.649</b>	<b>14.655</b>	<b>0.183</b>	<b>0.986</b>
	I	2.150	0.194	0.333	-	-	-	0.612	0.914	0.933	16.996	0.400	1.000
	I	1.212	0.057	0.000	-	-	-	0.375	0.314	0.333	12.756	0.000	0.933
	S	15	210	225	-	-	-	15	210	225	15	60	225
Zsigárd-major (ZS)	$\bar{x}$	-	-	-	-	-	-	<b>0.427</b>	<b>0.731</b>	<b>0.489</b>	-	-	-
	I	-	-	-	-	-	-	0.651	0.914	0.667	-	-	-
	I	-	-	-	-	-	-	0.288	0.412	0.333	-	-	-
	S	-	-	-	-	-	-	15	210	225	-	-	-
$\bar{x} \pm SE$	<b>1.891</b> ±0.224	<b>0.354</b> 0.053	<b>0.293</b> 0.043	<b>0.500</b> 0.066	<b>0.803</b> 0.105	<b>0.637</b> ±0.084	<b>0.455</b> ±0.032	<b>0.558</b> ±0.057	<b>0.503</b> ±0.050	<b>12.716</b> ±1.736	<b>0.083</b> ±0.015	<b>0.795</b> ±0.110	
Török	1.318		0.449		0.647			13.108					
Peti	1.406	1	0.610*	0.64	0.335	0.08	12.249**	0.070***					

\* avg. of 2 locations; \*\* avg. of 3 locations, other data from one location; \*\*\*two seed lots, others one seed lot tested for germinability

September 2019. The seedling emergence was tracked in October, November, and April 2020 after the winter period. The maximum value was used in the analysis as cumulative seedling emergence rate, so mortality during the winter was not considered (only a few cases detected).

*Data analysis* – We used linear regression to examine the effect of seed mass on germination and emergence for each species separately, applying linear mixed models (LME) by using the nlme package's linear mixed models (LME; Pinheiro *et al.* 2017). Germination and emergence were used as response variables, seed mass was the explanatory variable, and sampling location was used as a random factor in the models. The variability of traits was analysed by using ANOVA to determine significant results. Squaring transformations of the response variables were used to approximate assumptions of normality and homoscedasticity when it was necessary. Pearson correlation was used to evaluate the relationship between germination and emergence for each species separately. All statistical analyses were performed using the R version 3.6.2 (R Core Team 2019).

## RESULTS

### *Effect of seed mass on germination and seedling emergence*

The overall values of seed mass, germination and emergence, as well as the standard error and data from other references (Török *et al.* 2013, Peti *et al.* 2017) are displayed in Table 1. The mean seed mass of *S. borysthénica* was much higher than that of the other studied species. Average seed mass values derived from this study are in between the values from the two references for *D. serotinus*, *F. vaginata* and *S. borysthénica*, as for *C. arenaria* thousand seed weight was greater in this study, not only on average, but considering all sites separately.

Seed mass had a significant positive effect on germination for *C. arenaria* ( $\chi^2 = 13.439$ ,  $df = 1$ ,  $p = 0.0002$ ) and *D. serotinus* ( $\chi^2 = 6.239$ ,  $df = 1$ ,  $p = 0.0125$ ), while for the two grass species we did not find such effect (Fig. 2A and B). The

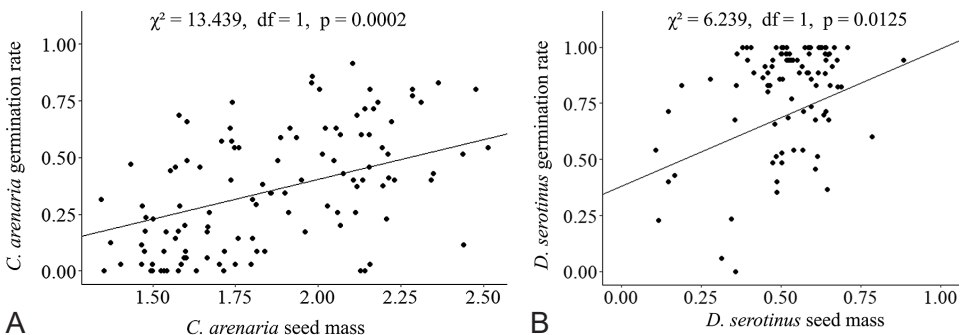


Fig. 2. The effect of seed mass on the germination; A) *C. arenaria*, B) *D. serotinus*

seed mass had a significant positive effect on seedling emergence for all species based on the results of LME (*C. arenaria*:  $\chi^2 = 65.806$ ,  $df = 1$ ,  $p = 4.976e-16$ , *D. serotinus*:  $\chi^2 = 64.232$ ,  $df = 1$ ,  $p = 6.078e-11$ , *F. vaginata*:  $\chi^2 = 37.289$ ,  $df = 1$ ,  $p = 1.018e-09$ , *S. borysthena*:  $\chi^2 = 148.41$ ,  $df = 1$ ,  $p = 2.2e-16$ ; Fig. 3A, B, C and D).

### Relationship between germination and seedling emergence

*Dianthus serotinus* had the highest germination in the growth chamber (80.25%), while in the outdoor setting, the cumulative seedling emergence of *S. borysthena* was the highest (79.62%). Higher mean germination percentages were found in the plant growth chamber for all species when compared to outdoor emergence, except for *S. borysthena*, which germinated at a much lower rate in the chamber. A significant positive correlation between germination and emergence for all species was detected (*C. arenaria*:  $R = 0.551$ ,  $p = 2.102e-10$ , *D. serotinus*:  $R = 0.316$ ,  $p = 0.0016$ , *F. vaginata*:  $R = 0.538$ ,  $p = 5.37e-14$ ), except in the case of *S. borysthena* ( $R = 0.159$ ,  $p = 0.149$ ) due to the low germination rate in the chamber (Fig. 4).

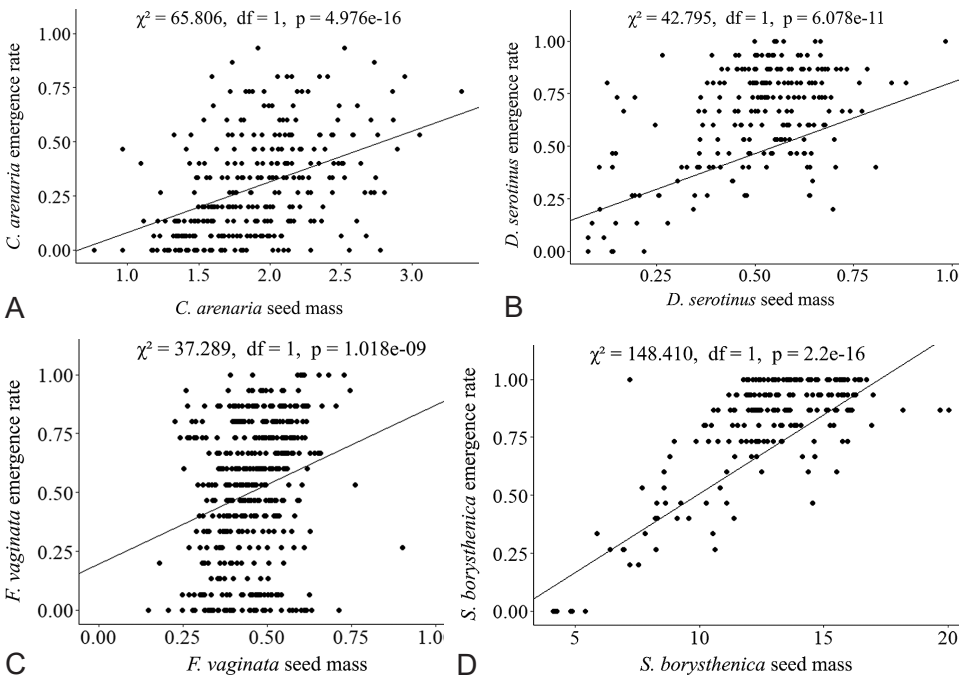


Fig. 3. The effect of seed mass on the emergence; A) *C. arenaria*, B) *D. serotinus*, C) *F. vaginata*, and D) *S. borysthena*

### Variability of seed traits and emergence between localities

The seed traits (seed mass, germination rate) and seedling emergence had a significant variability between locations (Table 2) except for *S. borysthena* germination results in the plant-growth chamber as an effect of the failure in dormancy breaking.

## DISCUSSION

Our study provides new data on the intraspecific variability of seed mass, germination capacity and seedling emergence of four species of the Pannonian biogeographic region over a large sampling area of about 40,000 km<sup>2</sup>. Our results supplement previous data collections on seed traits and emergence from single or few locations (Csontos *et al.* 2003, 2007, Török *et al.* 2013, 2016; Kövendi-Jakó *et al.* 2017, Peti *et al.* 2017, Kiss *et al.* 2018) with multisite measurements.

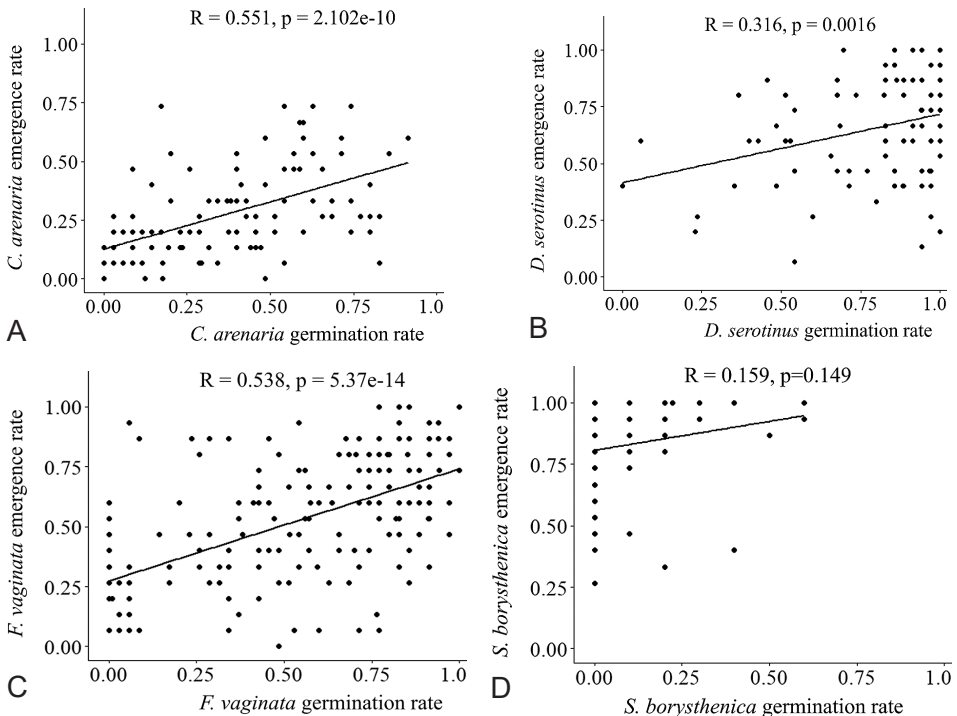


Fig. 4. Pearson correlation between germination in plant-growth chamber and emergence in common garden in all studied species a) *C. arenaria*, b) *D. serotinus*, c) *F. vaginata* and d) *S. borysthena*

Table 2  
 Variability within seed traits and emergence between localities in the studied species. Values in bold are significant. Traits: seed mass (M), seed germination (G), seedling emergence (E).

Species	<i>C. arenaria</i>			<i>D. serotinus</i>			<i>F. vaginata</i>			<i>S. borysthénica</i>		
	M	G	E	M	G	E	M	G	E	M	G	E
Traits & emergence	9.999	35.366	12.819	7.175	2.985	5.707	6.793	7.088	15.375	15.373	1.379	15.069
F-value	18	18	18	15	15	15	28	27	28	13	13	13
df												

Concerning seed mass data in the literature, only Peti *et al.* (2017) and Török *et al.* (2013) include data on the studied species, based on one measurement from one to three locations. For three species, the values of seed mass are between those of the above references, but for *C. arenaria* we measured higher thousand seed weight values in this study by 34-42%. This deviation is important and points to intraspecific variability that may originate also from year effect (Mazer *et al.* 2020).

The knowledge on the germinability of seed batches is essential for estimating seeding densities for restoration (Kövendi-Jakó *et al.* 2017, Pedrini *et al.* 2020). Seed provenance had an impact on germinability for all four studied species, as it was found for other species by Gallagher and Wagenius (2016). At this level of knowledge, the reason for site differences might be either local adaptation or differences in maternal environment (Galloway 2005, Gallagher and Wagenius 2016). Further genetic studies could shed light on the presence of local adaptation that is necessary to help decide on the regulation of seed transfer (Durka *et al.* 2017), and to help create seed zones (Cevallos *et al.* 2020). The seed dormancy of *S. borysthénica* was expected based on literature (Gasque and García-Fayos 2003), so mechanical scarification was applied. Despite this pretreatment germination was very low, similar to the rate achieved by gibberellin A3 treatment (Peti *et al.* 2017). In the lack of results from other experiments, we suggest to exempt this species from the obligation of providing germinability levels in seed batches for practice (Pedrini and Dixon 2020), as the emergence rate in the field was found very high, so low germinability under laboratory conditions is no impediment for restoration use.

Our study is filling a knowledge gap regarding emergence data in common garden for a large number of provenances. Only standardised conditions are relevant to study intraspecific variation as under field conditions competition with other species limits seedling establishment in different ways (Carrington 2014), and different populations might cope differently with competition. The detected variability in the emergence of the

seed lots from different sites detected for the four species suggests that some populations with low emergence are less suitable to be introduced to new sites in restoration trials, and in such cases a mixed approach of local and non-local seed use, called admixture provenancing is suggested (Bucharova *et al.* 2019).

The hypothesis that larger seed mass predicts higher germinability and establishment was supported by the results, except for the germination of *S. borysthenica* due to its seed dormancy. Seed mass proved to be an important fitness trait as it had a significant positive effect on the fraction of germinated seeds in the case of the two dicots and on the seedling emergence of all the species, in accordance with other studies (Greipsson and Davy 1995, Larios *et al.* 2014, Kövendi-Jakó *et al.* 2017). Higher resource availability of maternal plants induce the production of larger seeds that correlates with higher germinability and emergence (Galloway 2005, Gallagher and Wagenius 2016). However, it is not clear to what extent this advantage remains during field emergence and development and in later stages.

The second hypothesis that laboratory germination rate and emergence in the common garden are positively correlated was also partly proved, similarly to other studies (Clarke and Davidson 2004, Kövendi-Jakó *et al.* 2017). We found this interdependence for three species (*C. arenaria*, *D. serotinus*, and *F. vaginata*), but not for *S. borysthenica*. The non-significant correlation for *S. borysthenica* can be explained by the failure in its breaking seed dormancy. A positive correlation between laboratory germination and second year field establishment was found for twelve dry grassland species in a study (Kövendi-Jakó *et al.* 2017). Our results point to the importance of seed dormancy regarding the predictability of field performance based on laboratory germination, like for *S. borysthenica*. If breaking dormancy fails, germination remains low and no predictions can be made on field emergence. Further specific studies are required to test best methods for dormancy breaking as very little is known on native species behaviour (Valkó *et al.* 2018).

The third hypothesis was also confirmed, as a significant variability between locations was observed in the studied traits and emergence for all the studied species. Other studies found similar results comparing the intraspecific variability within plant traits among different localities (Mitchell and Bakker 2014, Helsen *et al.* 2017). This result might reflect local adaptation, but maternal effects can also contribute to the differences among populations, mainly in seed mass (Bischoff *et al.* 2006, Bischoff and Müller-Schärer 2010). To identify the origin of the differences, genetical analyses are required in further studies.

## IMPLICATIONS FOR PRACTICE

Based on our results, seed mass and germination in the laboratory are good predictors for cumulative seedling emergence in species showing no



dormancy. As we found sufficient emergence for *S. borysthena*, seed dormancy should not be a barrier for its use in restoration projects. The seed mass of collected seed lots can be used as a proxy for germination potential and help calculate the necessary seeding rates for restoration, also found in the study of Kövendi-Jakó and colleagues (2017). However, the international standards for native seeds in ecological restoration recommend using viability and germinability tests as well for samples of seed batches to achieve good quality propagule sources available on the seed market (Pedrini and Dixon 2020). To support this direction, further experiments are needed, among others, on the genetic variability to disentangle local adaptation versus maternal effects to guide provenancing; on finding better approaches to break seed dormancy of native species, and on native species behaviour in later life stages to improve restoration success.

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