

## PRESENTATION OF THE HUSEED<sup>WILD</sup> – A SEED WEIGHT AND GERMINATION DATABASE OF THE PANNONIAN FLORA – THROUGH ANALYSING LIFE FORMS AND SOCIAL BEHAVIOUR TYPES

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(Received 17<sup>th</sup> Aug 2016; accepted 25<sup>th</sup> Oct 2016)

**Abstract.** In this paper, we present the HUSEED<sup>wild</sup>, which is an online seed weight and germination database relying on our investigations concerning the regional seed collection (Pannon Seed Bank) of the Pannonian flora (available at: <http://huseed.nodik.hu:8243/en/wild>). Our data of 806 taxa (*sensu* species and subspecies) represent nearly 40% of the Pannonian flora. With our database, we contribute to former Hungarian databases with seed weight data of 50 taxa, and data of 20 taxa are first to be published in Europe. Our database is the first national germination database, and it contributes to European databases with germination data of 228 taxa. Additionally, we exemplify the ecological applicability of the dataset through analysing two plant strategies: (i) life forms and (ii) social behaviour types. Correlations between seed traits and plant strategies are investigated. Our findings are the following: (i) woody plants have significantly higher seed weight and significantly lower germination capacity than herbs (incl. forbs and graminoids); (ii) within herbs, perennials have significantly lower germination capacity than annuals and within forbs, they have significantly higher seed weight as well; (iii) herbaceous ruderals have significantly lower seed weight and significantly higher germination capacity than herbaceous stress tolerants. Our online database is being extended continuously, and its standardised data make it suitable for integration into larger European online databases.

**Keywords:** *seed mass, seed dormancy, plant growth forms, ruderals, Pannon Seed Bank*

**Abbreviations:** GP – germination percentage; SBT – social behaviour type; TSW – thousand-seed weight

**Nomenclature:** scientific plant names and authors follow the nomenclature of Flora Europaea (RBGE, 1998)

## Introduction

By definition, “plant traits” are morphological, anatomical, biochemical, physiological and phenological characteristics, which are measurable at individual level (Violle et al., 2007). Easy-to-measure “soft traits” are suitable for describing difficult-to-measure “hard traits” that correlate with them (Weiher et al., 1999). Seed weight is one of the most used soft traits (e.g. Csecserits et al., 2009; Török et al., 2013), while germination, due to dormancy, is a trait that is more difficult-to-measure and harder to interpret. However, both attract special interest because as “seed traits”, they are essential plant traits that primarily affect the early phases of a plant’s life cycle and thus the success of reproduction.

The application of plant trait databases (e.g. Csecserits et al., 2009; Kattge et al., 2011), including seed trait datasets (e.g. Csontos, 1998; Csontos et al., 2013; Török et al., 2013) is widespread in plant ecological researches. They are particularly effective in the case of analyses in community, comparative, evolutionary, conservation or invasion ecology for instance (Kattge et al., 2011). Thus, several complex European online plant trait databases including seed weight [e.g. Ecoflora (Fitter and Peat, 1994), BiolFlor (Klotz et al., 2002), LEDA (Kleyer et al., 2008), TRY (Kattge et al., 2011), D<sup>3</sup> (Hintze et al., 2013), SID (RBGK, 2016)] and germination [e.g. Ecoflora (Fitter and Peat, 1994), SID (RBGK, 2016)] datasets have been established recently. These databases contain widely accessible records of thousands of indigenous and non-indigenous species of Europe.

However, characteristics of species may differ depending on their geographical distribution (e.g. Csecserits et al., 2009; Valkó et al., 2009), thus regional databases possess great value. As a consequence, attribute databases in Hungary related to vascular plant species of the Pannonian Biogeographical Region (EEA, 2016) have been established. Such seed trait databases in Hungary are the SEED (including slenderness, flatness and weight records of seeds often based on literature data) by Csontos (1998), soil seed bank type database by Csontos (2001), and seed dispersal database by Csontos et al. (2002). Seed weight data of the Pannonian flora based on direct, original measurements are in the database of Török, P. et al. (2013, 2016). With former relevant data collections (Schermann, 1967; Csontos et al., 2003, 2007), it publishes records of approximately 70% of the Pannonian flora (Török, P. et al., 2016). On the other hand, an extensive germination database has not been established in Hungary so far.

In consequence of the above, we aimed at establishing an extendable, online database, which serves the extension of the seed weight dataset and provides basis for developing the germination dataset of the Pannonian flora. In this paper, we present the HUSEED<sup>wild</sup> database relying on our direct, original measurements concerning the regional (Pannonian Biogeographical Region) propagule collection established for *ex situ* conservation within the frame of the Pannon Seed Bank project (LIFE08 NAT/H/000288). In addition, we exemplify the ecological applicability of our seed weight and germination datasets through analysing two selected plant strategies: (i) life forms (Soó, 1964-1980) and (ii) social behaviour types (SBTs; Borhidi, 1995). Scientifically proven and presumed correlations between seed traits and plant strategies are explored as well.

## Materials and Methods

The HUSEED<sup>wild</sup> is based on our investigations concerning the wild plant seed collection maintained by the Center for Plant Diversity genebank. We established this collection within the frame of the Pannon Seed Bank project (LIFE08 NAT/H/000288) for *ex situ* conservation of wild native vascular plants of the Pannonian Biogeographical Region (see Peti et al., 2015; Török, K. et al., 2016).

### *Seed collection program*

The collection of wild plant seeds on the territory of the Pannonian Biogeographical Region was started within the frame of the project in 2011, and with the contribution of the Center for Plant Diversity, it is continuous even after finishing the project in 2014. Here we refer to the activities and outputs of the period between 2011 and 2014.

The list of target species of the collections encompassed plant groups recommended for conservation by ENSCONET (2009a), for instance protected and strictly protected species, endemisms, character species, indicator species and species threatened by climate change. Additionally, several ruderal and some invasive species were included. The latter may be of importance in the case of seed biological and ecological investigations.

During seed collections, ENSCONET (2009a) specifications for sampling, qualities and quantities were followed. Seeds were harvested from spontaneous populations in ripening phase. Our goal was to get samples which represent the population's genetic diversity, thus we gathered pooled seed samples from several mother plants of the population (further on, we refer to seed samples that represent individual populations as "accessions"). We aimed at sampling as many populations in the region as possible, thus providing the most complete data of the regional genetic material of each taxon typical in the flora. Metadata of the collections [e.g. location, date, sampling method, habitat type according to Bölöni et al. (2011), etc.] were thoroughly documented. For further details of seed collecting methodology see our former publications by Peti et al. (2015) and by Török, K. et al. (2016).

### *Seed weight measurement protocol*

Seed weight measurements were carried out within three months after collecting. Until measurement, accessions were stored in paper bags, in a dry place using silica gel.

Before seed weight measurements, the accessions were cleared of non-propagule fractions. In the case of fleshy fruits, cleaning occurred by washing, while dry fruits were cleaned by hand sorting, by sieves of various wire mesh sizes and by seed blower according to recommendations of methodological literature (Smith et al., 2003; Rao et al., 2006; ENSCONET, 2009b). For the investigations propagules which seemed visibly healthy (i.e. presumably not unfertile) based on identification guides of Bojňanský and Fargašová (2007) and Cappers et al. (2006) were used. Corresponding with standards of international databases (Kleyer et al., 2008; Hintze et al., 2013), air-dry weights of propagules (germinules or dispersules, including appendages) were measured with 0.0001 g accuracy using analytical balance.

Four replicates of samples with 100 propagules in each (400 propagules in total) per accession were counted to measure seed weight, and the results were averaged for each (i) accession and (ii) taxon, *sensu* species and subspecies (for further analyses, we used means (ii) as raw data). Seed weight was expressed as thousand-seed weight (TSW) [g].

### ***Germination protocol***

Germination testing was carried out under laboratory circumstances, using freshly-matured visibly healthy seeds, within three months after collecting. In the case of taxa where germination tests were not recommended, tetrazolium test was used (results of these tests are not shown here).

Germination tests were carried out either on the Jacobsen table with 20-30 °C operating temperature, or in Petri-dishes in germination cabinets with 15-25 °C operating temperature depending on the species. Germination substrate was wetted filter paper, and the tests took 30 days (considering the clear discharge in germination dynamics). Species-specific germination methods – proper combination of pretreatment, temperature setting and light/dark setting – were applied, which allowed the investigation of “organic dormancy” [after Nikolaeva (1977), further on, we refer to it as “dormancy”] instead of “enforced dormancy” [after Baskin and Baskin (2004)] caused by inadequate environmental factors. We used an online database of RBG Kew, named SID (RBGK, 2016) and in some cases of crop-wild relatives, standards of ISTA (2013) as a basis for selecting the species-specific germination methods. We tested two to five recommended germination methods per species, and adjusted them, if needed, to the specifics of the local flora. Further on, the most effective method was applied. Depending on the species, scarification, soaking or warm and/or cold stratification were used as a pretreatment to break non-deep dormancy. When germination of a taxon was unsuccessful in spite of several attempts, tetrazolium test was applied.

Since, we focused on *ex situ* conservation purposes during determining the proper sample size and the repeat count, we took the ENSCONET (2009b) germination recommendations for wild plant species into account. Based on this, two replicates of samples with 50 seeds in each (100 seeds in total) per accession were germinated, and the results were averaged for each (i) accession and (ii) taxon, *sensu* species and subspecies (for further analyses, we used the means (ii) as raw data). Germination capacity was expressed as germination percentage (GP) [%].

After seed weight measurements and germination tests, moisture content of the seeds were reduced to 3-7%, and were banked in cold rooms of the Center for Plant Diversity and associated institutions, namely Aggtelek National Park and Institute of Ecology and Botany of the MTA Centre for Ecological Research. The cold rooms operate on 0 °C (active store) and on -20 °C (base store) according to the FAO (2013) international genebank standard based on the FAO/IPGRI (1994) standard, which have been successfully applied by Center for Plant Diversity for decades (see Peti et al., 2015).

### ***Data processing***

We used the dataset of HUSEED<sup>wild</sup> accessed on 1 June 2015, which includes records for 1257 accessions collected between 2011 and 2014. All further reported quantifications are based on this access.

The taxon set of our database was compared on presence-absence level to taxon sets of national paper format databases of Török, P. et al. (2013, 2016), Csontos et al. (2003, 2007) and Schermann (1967), and to taxon sets of international online databases, namely LEDA (Kleyer et al., 2008) and SID (RBGK, 2016).

The raw dataset of our further analyses consisted of the single TSW means [g] and GP means [%] of all taxa available in HUSEED<sup>wild</sup> (the mean calculated for the taxon

from accessions belonging to it). We took taxa with germination result of 0% into consideration, since the lack of germination can be a species-specific strategy.

We analysed (i) life forms and (ii) SBTs in terms of seed weight and germination capacity, respectively. For gathering the Raunkier's life form (Soó, 1964-1980) and Borhidi's SBT (Borhidi, 1995) categories of species concerning the Pannonian flora, we used the FLORA database (Horváth et al., 1995). Borhidi's SBT classification is the adapted version of Grime's CSR plant functional type system (Grime, 1979) to the Pannonian flora, by supplementing the "competitor", "stress tolerant" and "ruderal" original categories with several sub-categories.

We set up different (i) life form and (ii) SBT groups by merging certain categories of the original life forms and SBTs. We compared these groups with each other based on the TSW means and GP means of taxa belonging to the given groups, respectively. In the case of life forms, the following simplified morphological life form groups were compared to each other: "woody plants", "forbs" and "graminoids". Trees and shrubs were classified as woody plants, herbaceous dicots and non-graminoid herbaceous monocots were classified as forbs, and herbaceous members of the *Cyperaceae*, *Juncaceae* and *Poaceae* families were classified as graminoids (further on, "herbs" means forbs and graminoids together). In addition, we treated "perennial", "biennial" and "annual" groups separately. In the case of SBTs, "competitor", "stress tolerant" and "ruderal" groups were compared to each other, and these groups were analysed within groups specified by life forms as well. The analyses of SBT groups within woody plants was omitted due to small number of samples.

The above mentioned groups were compared using Kruskal-Wallis test (with Tukey-Kramer significant difference criterion), and differences between the two means of two groups were tested by two-sample t-test (Zar, 2010). The rejection of the null hypothesis meant the differences in the expected values of the groups. Transformed data were used because the distribution of the original data violated the two-sample t-test's assumption of normality. In the case of TSW, the logarithms of the data showed normal distribution. In the case of GP, we normalised the data by using Box-Cox transformation (Sakia, 1992). Normality of data in each case was tested by Lillefors test (Conover, 1999). Confidence interval concerning mean of each group were determined by Bootstrap method due to the non-normal distribution of data (Devore and Berk, 2012). For statistical analyses, Statistics and Machine Learning Toolbox of Matlab R2015a software (MathWorks, 2015) was used.

## Results

### *The HUSEED<sup>wild</sup>*

We established the online HUSEED<sup>wild</sup>, which is available on the <http://huseed.nodik.hu:8243/en/wild> website. The database includes the following information: TSW mean with standard error calculated for taxon [g], TSW range (minimum–maximum) determined for taxon [g], the weighed morphological unit, GP mean with standard error calculated for taxon [%], GP range (minimum–maximum) determined for taxon [%], the applied germination method, all the above on accession-level, collection metadata (location, date) of accession. The content of the database can be searched by submitting terms (family, genus, species, subspecies, author) in the search field.

*Electronic Appendix 1* shows the extract of the online HUSEED<sup>wild</sup> concerning the single TSW data, GP data and the applied germination methods of all taxa available in it (without detailing the data of accessions). As a result of our seed weight measurements, we got TSW data of 806 taxa (*sensu* species and subspecies) from 83 different families including 32 taxa which are strictly protected and 158 taxa which are protected by Hungarian law (Környezetvédelmi Minisztérium, 2001). As a result of the germination tests, we got GP data and germination methods applicable to the local flora of 744 taxa (*sensu* species and subspecies) from 73 families including 25 taxa which are strictly protected and 143 taxa which are protected by Hungarian law. In the case of the remaining 62 taxa where germination methods were not applicable tetrazolium test was applied. Out of the 744 taxa, 613 taxa from 63 families showed GP different from zero, while 131 taxa from 10 families did not germinate at all. However, tetrazolium tests proved these latter taxa were viable as well (members of *Orobanchaceae* were not investigated since their seed sizes were not suitable for tetrazolium test). Thus, failure of their germination was interpreted as germination strategy and their data were not excluded from the analyses.

Out of the 806 taxa where seed weights were measured, 199 taxa were not listed in LEDA (Kleyer et al., 2008), 101 taxa were not listed in SID (RBGK, 2016), and 50 taxa were not listed in national databases of Török, P. et al. (2013, 2016), Csontos et al. (2003, 2007) and Schermann (1967). TSW data of 20 taxa are novel in Europe (*Electronic Appendix 1*). Out of the 20 taxa, nine taxa are protected and four taxa are strictly protected by Hungarian law. The strictly protected *\*Serratula lycopifolia* (Vill.) A. Kern. is also listed in Habitats Directive Annex II. (European Commission, 1992).

Out of the 613 taxa which had GP different from zero, GP data and germination method recommendation of 170 taxa were not listed in SID (RBGK, 2016) which is the most complete European database containing germination records we know of (*Electronic Appendix 1*). Out of these, 61 taxa are protected and 10 taxa are strictly protected by Hungarian law. Five taxa of them, the protected *Cirsium brachycephalum* Jur. and *Iris humilis* Georgi, and the strictly protected *\*Onosma tornensis* Jáv., *\*Serratula lycopifolia* (Vill.) A. Kern. and *Seseli leucospermum* Waldst. et Kit. are listed in Habitats Directive Annex II.

The 131 taxa where GP was zero came from the *Aceraceae*, *Apocynaceae*, *Balsaminaceae*, *Celastraceae*, *Cornaceae*, *Ephedraceae*, *Fagaceae*, *Orobanchaceae*, *Oxalidaceae* and *Violaceae* families. Out of these, GP data of 58 taxa were not listed in SID (*Electronic Appendix 1*). Out of the 58 taxa, 19 are protected and five are strictly protected by Hungarian law. The protected *Echium russicum* J.F. Gmel., the strictly protected *Colchicum arenarium* Waldst. et Kit., *Iris aphylla* L. and *\*Pulsatilla pratensis* (L.) Mill. subsp. *hungarica* Soó are also listed in Habitats Directive Annex II.

The most TSW and GP data in the HUSEED<sup>wild</sup> are reported for the *Compositae* (TSW: 101 taxa, GP: 96 taxa), *Poaceae* (TSW: 88 taxa, GP: 80 taxa), *Leguminosae* (TSW: 60 taxa, GP: 58 taxa), *Cruciferae* (TSW: 46 taxa, GP: 40 taxa), *Charyophyllaceae* (TSW: 45 taxa, GP: 38 taxa), *Umbelliferae* (TSW: 43 taxa, GP: 28 taxa) and *Labiatae* (TSW: 40 taxa, GP: 29 taxa) families. Additionally, the HUSEED<sup>wild</sup> includes TSW and GP data of taxa where seed collecting is difficult due to either narrow distribution area [e.g. *Angelica palustris* (Besser) Hoffm., *Onosma tornensis* Jáv. and *Teucrium sorodonia* L.], or methodological problems (e.g. *Balsaminaceae*, *Geraniaceae* and *Oxalidaceae*) including ephemeral species [e.g. *Corydalis* and *Helleborus* (for latter, only TSW data is available)].

## Application of TSW and GP data in analysing plant strategies

### Analysis of life forms

We analysed the different life form groups in terms of seed weight and germination capacity, respectively. Based on the Kruskal-Wallis test, the woody plant group differed significantly from other groups regarding both seed weight ( $P < 0.001$ , where  $P$  is the significance level) and germination ( $P < 0.001$ ), while significant differences between the forb and graminoid groups were not detectable (*Table 1*).

The groups showed the following decreasing order of magnitude regarding their TSW means: woody plants (56.9 g), forbs (3.5 g) and graminoids (2.6 g) (*Table 1*). The two-sample t-test confirmed that the TSW mean of the woody plant group was significantly higher than those of the forb ( $P < 0.001$ ) and graminoid groups ( $P < 0.001$ ). Regarding germination, the forb group showed the highest average value (39.9%), and the average value of graminoid group was only moderately lower (38.5%) (*Table 1*). Significantly the lowest GP mean (12.5%) belonged to the woody plant group (two-sample t-test,  $P < 0.001$ ).

**Table 1.** Thousand-seed weight means [g] and germination percentage means [%] of the simplified morphological life form groups. Notation:  $N_t$  – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval. Letters “a” and “b” written as superscript indicate significantly different ( $P < 0.001$ ) groups based on the Kruskal-Wallis test (with Tukey-Kramer significant difference criterion).

Life form group	Thousand-seed weight			Germination percentage		
	$N_t$	Mean [g]	CI95%	$N_t$	Mean [%]	CI95%
woody	39	56.9 <sup>a</sup>	37.9 - 88.2	28	12.5 <sup>a</sup>	6.6 - 25.8
forb	635	3.5 <sup>b</sup>	3.0 - 4.3	597	39.9 <sup>b</sup>	37.3 - 42.6
graminoid	132	2.6 <sup>b</sup>	1.9 - 4.0	119	38.5 <sup>b</sup>	32.1 - 45.5

Further investigating herbs regarding plant life span – perennial, biennial and annual groups – we found the GP mean of the annual group (48.9%,  $N_t=159$ , where  $N_t$  is the number of taxa used as sample) to be significantly higher ( $P < 0.001$ ) than that of the perennial group (36.6%,  $N_t=503$ ), but the GP mean of either group above did not differ significantly from that of the biennial group (41.3%,  $N_t=54$ ) based on the two-sample t-test. However, the groups did not differ significantly regarding their TSW means (annual: 2.7 g,  $N_t=162$ ; biennial: 3.3 g,  $N_t=54$ ; perennial: 3.6 g,  $N_t=551$ ) based on the two-sample t-test.

The woody plant, forb and graminoid morphological life form groups were investigated further by dividing them into perennial, biennial and annual groups (*Table 2*). The groups showed the following decreasing order of magnitude regarding their TSW means: woody perennials, annual graminoids (5.4 g), perennial forbs (4.0 g), biennial forbs (3.3 g), annual forbs (2.4 g) and perennial graminoids (2.1 g). The two-sample t-test confirmed that the TSW mean of the perennial forb group was significantly higher than those of the annual forb ( $P < 0.01$ ) and perennial graminoid groups ( $P < 0.01$ ) (*Table 2*). Regarding the GP means of the different groups, we found the following decreasing order of magnitude: annual graminoids (59.4%), annual forbs (47.5%), biennial forbs (41.3%), perennial forbs (37.1%),

perennial graminoids (34.6%) and woody perennials. The two-sample t-test confirmed that the GP mean of the annual graminoid group was significantly higher than those of the perennial graminoid ( $P < 0.05$ ) and perennial forb groups ( $P < 0.05$ ). Furthermore, the GP mean of the annual forb group was significantly higher than those of the perennial forb ( $P < 0.005$ ) and perennial graminoid groups ( $P < 0.005$ ) based on the two-sample t-test (Table 2).

**Table 2.** Thousand-seed weight means [g] and germination percentage means [%] of the simplified life form groups, and differences between means based on the two-sample t-test. Notation: wp – woody perennial, pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid,  $N_t$  – number of samples (where sample means taxon, sensu species and subspecies),  $P$  – significance level, ns – not significant difference. Abbreviation “tsw” written as subscript refer to thousand-seed weight, abbreviation “gp” written as subscript refer to germination percentage.

Life form group		<div><div>wp</div><div>pf</div><div>bf</div><div>af</div><div>pg</div><div>ag</div></div>						
		Thousand-seed weight						
N <sub>t</sub>		39	438	54	143	113	19	
Mean [g]		56.9	4.0	3.3	2.4	2.1	5.4	
[%]								
wp	Germination percentage	28	12.5	-	P <sub>tsw</sub> <0.001	P <sub>tsw</sub> <0.001	P <sub>tsw</sub> <0.001	P <sub>tsw</sub> <0.001
pf		403	37.1	P <sub>gp</sub> <0.001	-	ns <sub>tsw</sub>	P <sub>tsw</sub> <0.01	P <sub>tsw</sub> <0.01
bf		54	41.3	P <sub>gp</sub> <0.001	ns <sub>gp</sub>	-	ns <sub>tsw</sub>	ns <sub>tsw</sub>
af		140	47.5	P <sub>gp</sub> <0.001	P <sub>gp</sub> <0.005	ns <sub>gp</sub>	-	ns <sub>tsw</sub>
pg		100	34.6	P <sub>gp</sub> <0.001	ns <sub>gp</sub>	ns <sub>gp</sub>	P <sub>gp</sub> <0.005	-
ag		19	59.4	P <sub>gp</sub> <0.001	P <sub>gp</sub> <0.05	ns <sub>gp</sub>	ns <sub>gp</sub>	P <sub>gp</sub> <0.05
								-

### Analysis of SBTs

We analysed the different SBT groups in terms of seed weight and germination capacity, respectively. Since the TSW mean and GP mean of the woody plant group differed significantly from those of the other groups (see above), they could have hidden the relations among the SBT groups, so woody plants were excluded from the analyses first. Based on the Kruskal-Wallis test, the ruderal group differed significantly from the stress tolerant group ( $P < 0.05$ ), but it did not differ significantly from the competitor group, furthermore, there was no significant difference between the stress tolerant and competitor groups regarding their seed weights (Table 3). Regarding germination, the ruderal group differed significantly from both the stress tolerant ( $P < 0.01$ ) and competitor groups ( $P < 0.01$ ), while significant difference between the two last groups was not detectable based on the Kruskal-Wallis test (Table 3).

The groups showed the following decreasing order of magnitude regarding their TSW means: stress tolerants (3.9 g), ruderals (2.8 g) and competitors (2.6 g) (Table 3). The two-sample t-test confirmed that the TSW mean of the ruderal group was

significantly lower than that of the stress tolerant group ( $P < 0.05$ ). Regarding germination, the ruderal group had the highest average value (45.3%), which was followed by the stress tolerant (36.6%) and competitor groups (31.1%) (Table 3). The two-sample t-test confirmed that the GP mean of the ruderal group differed significantly from those of the other two groups ( $P < 0.005$ ). Furthermore, the histogram based on GP values showed that germinations closer to 100% were more frequent for taxa of the ruderal group, than for taxa of the competitor and stress tolerant groups.

**Table 3.** Thousand-seed weight means [g] and germination percentage means [%] of the social behaviour type groups based on the herbs belonging to them. Notation:  $N_t$  – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval. Letters “a” and “b” written as superscript indicate significantly different (thousand-seed weight:  $P < 0.05$ , germination percentage:  $P < 0.01$ ) groups based on the Kruskal-Wallis test (with Tukey-Kramer significant difference criterion).

Social behaviour type group	Thousand-seed weight			Germination percentage		
	$N_t$	Mean [g]	CI95%	$N_t$	Mean [%]	CI95%
competitor	72	2.6 <sup>ab</sup>	1.8 - 4.2	66	31.1 <sup>a</sup>	24.0 - 40.3
stress tolerant	394	3.9 <sup>a</sup>	3.2 - 5.1	357	36.6 <sup>a</sup>	33.0 - 40.0
ruderal	301	2.8 <sup>b</sup>	2.3 - 3.6	293	45.3 <sup>b</sup>	41.6 - 49.2

Table 4 and 5 show the detailed analyses of TSWs and GPs of the SBT groups within groups specified by life forms such as perennial, biennial and annual forbs, and perennial and annual graminoids (analysis of woody perennials by SBT groups was omitted due to small number of samples). The relative magnitude of the TSW means and GP means of competitors, stress tolerants and ruderals within the different life form groups showed different order compared to our former findings in many cases (Table 4). However, if groups with reliable number of samples ( $N_t \geq 20$ ) were only considered, significant differences were detected mainly among the SBT groups mentioned above (Table 5). Within the group of perennial forbs, the TSW mean of stress tolerants (4.4 g) was significantly higher (two-sample t-test,  $P < 0.005$ ) than that of ruderals (2.6 g). Within the group of perennial graminoids, the TSW mean of stress tolerants (2.7 g) was also significantly higher (two-sample t-test,  $P < 0.05$ ) than that of ruderals (1.0 g). Within the group of annual forbs, such relation regarding TSW means was not detectable between ruderals and stress tolerants. Within the group of perennial forbs, the GP mean of ruderals (42.8%) was significantly higher (two-sample t-test,  $P < 0.05$ ) than that of stress tolerants (35.4%). Within the group of annual forbs, the GP mean of ruderals (48.2%) was also higher than that of stress tolerants (44.3%), although this difference was not significant based on the two-sample t-test. The differences between competitors and stress tolerants and between competitors and ruderals either regarding TSW means or GP means were not significant within any life form groups.

**Table 4.** Thousand-seed weight means [g] and germination percentage means [%] of the combined groups of simplified life form and social behaviour type groups. Notation:  $N_t$  – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval, pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid.

Combined group	Thousand-seed weight			Germination percentage		
	$N_t$	Mean [g]	CI95%	$N_t$	Mean [%]	CI95%
pf competitor	12	5.2	3.2 - 9.0	11	25.7	8.1 - 52.9
pf stress tolerant	317	4.4	3.4 - 5.9	284	35.4	31.4 - 39.1
pf ruderal	109	2.6	1.8 - 3.8	108	42.8	36.7 - 49.1
bf stress tolerant	19	1.8	0.5 - 6.3	19	46.4	33.1 - 59.7
bf ruderal	35	4.1	2.7 - 7.3	35	38.4	28.7 - 48.4
af stress tolerant	24	1.6	0.9 - 2.9	24	44.3	31.2 - 59.1
af ruderal	119	2.5	1.7 - 3.7	116	48.2	42.0 - 54.7
pg competitor	59	2.2	1.3 - 3.9	54	32.6	24.0 - 42.0
pg stress tolerant	34	2.7	1.5 - 5.2	29	35.9	25.2 - 52.1
pg ruderal	20	1.0	0.6 - 1.5	17	38.4	23.5 - 55.1
ag competitor	1	0.2	- - -	1	13.0	- - -
ag ruderal	18	5.7	2.6 - 12.2	18	61.9	44.4 - 79.8

**Table 5.** Comparison of the combined groups of simplified life form and social behaviour type groups by the two-sample t-test regarding respectively their thousand-seed weight means [g] and germination percentage means [%]. Notation: pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid, ns – not significant, nt – not tested (due to small number of samples).

Combined group	Significance level	
	Thousand-seed weight	Germination percentage
pf competitor vs. pf stress tolerant	nt	nt
pf competitor vs. pf ruderal	nt	nt
pf stress tolerant vs. pf ruderal	P<0.005	P<0.05
bf stress tolerant vs. bf ruderal	nt	nt
af stress tolerant vs. af ruderal	ns	ns
pg competitor vs. pg stress tolerant	ns	ns
pg competitor vs. pg ruderal	ns	nt
pg stress tolerant vs. pg ruderal	P<0.05	nt
ag competitor vs. ag ruderal	nt	nt

## Discussion

Genebank seed collections have multiple benefits: they store genetic material for conservation and for research, and their data can provide valuable scientific information. In this paper the HUSEED<sup>wild</sup>, a database relying on investigations of seed collection established within the frame of Pannon Seed Bank project (LIFE08 NAT/H/000288), and its ecological applicability were presented through analytical examples.

### *The HUSEED<sup>wild</sup>*

The HUSEED<sup>wild</sup>, which is available on the <http://huseed.nodik.hu:8243/en/wild> website, reports regional data on TSWs of 806 taxa and on GPs of 744 taxa based on direct, original measurements (*Electronic Appendix 1*). Thus, our data cover nearly 40% of the Pannonian flora. The presented data, as original and regional data, are valuable on their own. At the same time, the value of the database is increased by the fact that we published several TSW (20 taxa) and GP (228 taxa) data of the European flora for the first time (*Electronic Appendix 1*). Our database contains mainly native species of Hungary, within which the easy-to-collect *Compositae*, *Poaceae* and *Leguminosae* families dominate, but it contains several hard-to-collect taxa as well.

In accordance with our expectations, seed weight data were in the  $10^{-6}$ - $10^4$  g range which was predicted by Harper (1977). In some cases, we found diverse seed weights within the same species. This phenomenon can be found in other databases as well. The reason for this phenomenon might be for example weather fluctuations or in the case of species with wide ecological tolerance the adaptation to different environmental conditions.

During germination evaluation, we took into account that most wild plant species are characterised by some form of dormancy (e.g. ENSCONET, 2009b; Baskin and Baskin, 2014). Matured seeds of most wild plant species do not reach their ability to germinate at the same time in order to avoid competition for resources (Grubb, 1988). Given this knowledge, we considered the germination of most species successful, although considerable proportion of their seeds did not show germination willingness. Successful germination results also meant that we properly adapted the applied germination methods to the species of the local flora.

Seeds, which did not complete germination, were most likely deep dormant, or less likely enforced dormant (insofar the selected germination method was not optimal despite our efforts).

Germination percentages often differed widely between different accessions of the same species. The differences may be caused by variability in proportion of dormant seeds and seeds able to germinate among different populations of species or rather among different individuals of populations (Milberg et al., 1996; Baloch et al., 2001; ENSCONET 2009b; Baskin and Baskin, 2014). Furthermore, the well-known seasonal changes in dormancy/non-dormancy cycle (Baskin et al., 2003; Baskin and Baskin, 2014; Garcia et al., 2014) can also cause the above mentioned phenomenon.

Some of the investigated taxa did not show willingness to germinate at all. Since these taxa proved to be viable by tetrazolium test, failure of germination was interpreted as a reproductive strategy. Presumably, these taxa were characterised by deeper dormancy level, which we could not break by scarification, soaking and warm/cold stratification either.

### ***Application of TSW and GP data in analysing plant strategies***

Here, we considered a difference significant with a significance level of  $P < 0.05$  (for the exact P-values, see the “Results” section).

#### ***Analysis of life forms***

We found correlations between the seed weights and the life forms of species. This is in accordance with literature (e.g. Salisbury, 1942; Fenner, 1985; Westoby et al., 1996; Csontos, 1998; Csontos et al., 2007; Moles et al., 2007; Tautenhahn et al., 2008; Török et al., 2013). According to the expectations (e.g. Salisbury, 1942; Fenner, 1985; Westoby et al., 1996; Csontos et al., 2007) the TSW mean of the woody plant group was significantly higher than those of the groups of herbs (*Table 1*). The higher seed weight of woody plants has ecological importance in providing advantages during emergence and establishment of seedlings under canopy light conditions. The larger (heavier) seeds are less dependent on light for germination than smaller (lighter) ones and there is a greater chance of establishment due to their reserved resources (e.g. Leishman and Westoby, 1994; Saverimuttu and Westoby, 1996; Milberg et al., 2000; Burmeier et al., 2010; Pivatto et al., 2014). The heavier seeds of species which develop in shady environment were supported by Csontos (1998) based on comparing relative light demand of 193 Pannonian flora elements. First, we did not detect significant differences among the herbaceous perennial, biennial and annual groups, which corresponded to the results of Csontos et al. (2007). However, the detailed analyses showed more complex differences. Corresponding to Török et al. (2013), we found the following decreasing order of the groups regarding their TSW means: annual graminoids, perennial forbs, biennial forbs, annual forbs and perennial graminoids (*Table 2*). Within the forb group, the TSW mean of perennials compared to that of annuals proved to be significantly higher, which corresponded to our expectations based on r/K selection theory (Grime, 1977). Within the graminoid group, we found the opposite of it, which contradicted our expectations based on r/K selection (Grime, 1977). Although, we could not confirm the significance of the difference statistically, TSW of the annual graminoids was higher than that of the perennial graminoids on average, and this relation was in accordance with the results of Török et al. (2013) and furthermore with the results of Csontos and Kalapos (2012). We presume, that the lighter seeds of perennial graminoids compared to annual graminoids are a result of the fact that most species in this group produce both seeds and clonal offspring (Kleyer et al., 2008), thus the energy budget they allocate for reproduction has to be shared between two sorts, and this may lead to smaller seeds.

We found correlations between the germination attributes and the life forms of species, which confirm, among others, the assumptions of Csontos and Kalapos (2006). Corresponding to the results of Grime et al. (1981), we found the woody plant group to have the significantly lowest GP mean (*Table 1*). Tendency for dormancy of woody plants is well-known from literature (Baskin and Baskin, 2014), which explains their low willingness to germinate. For the herbs, we found that GP mean of the annual group was significantly higher than that of the perennial group, however germination failure was not negligible even in the case of the annual group (on average ca 50%). The significantly higher GP mean of the annuals compared to that of the perennials was confirmed within both the graminoid and forb groups (*Table 2*). Corresponding with our

expectations based on *r/K* selection (Grime, 1977), our results lead to the conclusion that a relatively faster response to the environmental conditions that are optimal for germination is in the interest of annuals which have faster life cycle compared to perennials. This is because early germination means competitive advantage in utilising limited nutrients of the environment in contrast to delayed germination, and this plays a more important role in the survival of annuals than that of perennials (Abraham et al., 2009). This is supported by the results of Grime et al. (1981), that germination rate of annuals is higher than that of perennials.

### *Analysis of SBTs*

We found correlations between the seed weights and the SBTs of species. Based on the maximised reproductive rates of ruderals and the limited reproductive rates of stress tolerants (Grime, 1977, 1979), and based on the negative correlation between the seed weight and seed number (e.g. Shipley and Dion, 1992; Moles et al., 2004), we presumed a huge number of small seeds for ruderals, and a smaller number of larger seeds for stress tolerants. Our results confirmed the expectations: the TSW mean of the herbaceous ruderal group proved to be significantly lower than that of the herbaceous stress tolerant group (*Table 3*). Such relation regarding TSW means was detected between the ruderals and stress tolerants within both the perennial forb and perennial graminoid groups (*Table 4-5*). Our results are in accordance with the proven fact that ruderal species usually have long-lived (persistent) seed bank in the soil (e.g. Harper, 1977; Thompson and Grime, 1979; Fenner, 1985; Thompson, 1992; Bakker et al., 1996; Thompson et al., 1997; Bekker et al., 1998; Matus et al., 2005; Bossuyt and Honnay, 2008; Török et al., 2009) and this persistent seed bank assumes small seed size. Latter correlation between soil seed bank persistence and seed weight (persistent seed bank – small seed size) is proved in most flora types (e.g. Thompson and Grime, 1979; Thompson et al., 1993, Bakker et al., 1996; Bekker et al., 1998; Hodgkinson et al., 1998; Funes et al., 1999; Thompson et al., 2001; Cerabolini et al., 2003; Peco et al., 2003; Zhao et al., 2011), including in the Pannonian flora (Csontos, 1998, 2001), however, it was not detected for the flora of Australia (Leishman and Westoby, 1998) and New Zealand (Moles et al., 2000).

Regarding germination, the herbaceous ruderal group showed significantly higher GP mean than the herbaceous stress tolerant and herbaceous competitor groups (*Table 3*). The significant difference between the ruderals and stress tolerants also was confirmed within the group of perennial forbs (*Table 5*). Although the ruderal group showed greater germination willingness compared to the other SBT groups, it is important that even its germination did not exceed the 45% average value. Presumed cause of low average GP may be the high frequency of “risk-spreading” survival strategy (Grubb, 1988) within the group. Species characterised by such strategy maintain persistent seed bank in the soil with the help of numerous dormant seeds, and even under optimal conditions their germination is partially delayed. However, germinations of species in the ruderal group came closer to 100% more often than germinations of species in the other groups. The presumed cause of their great germination willingness may be the “disturbance-broken” strategy, which is another typical strategy for ruderals (Grubb, 1988). Species with this strategy build up their persistent soil seed bank by their seeds becoming enforced dormant due to fast burial, and they start to germinate from this explosively under favourable environmental conditions caused by disturbance, which makes their fast colonisation possible.

### ***Ecological uses of our data***

The knowledge of seed weight and germination data, and optimal conditions for germination can be directly used in applied and theoretical fields of plant ecology. This information has particular importance, even by species respectively, for example during restoration works (Török, P. et al., 2016): in view of seed weight and germination capacity of species, necessary amount of seeds in case of sowing can be designed in order to achieve the expected number of individuals. Knowledge of optimal germination conditions of species is also necessary for *ex situ* propagations during restoration works. Seed weight and germination capacity of species can be used in invasion and migration ecology as well. In the light of seed weights, dispersal capacity of seeds (Bekker and Bakker, 2003) and seed longevity in soil (e.g. Thompson and Grime, 1979; Thompson et al., 1993, Bakker et al., 1996; Bekker et al., 1998; Hodkinson et al., 1998; Funes et al., 1999; Thompson et al., 2001; Cerabolini et al., 2003; Peco et al., 2003; Zhao et al., 2011) can be estimated and this information can help to predict the probability of spontaneous regeneration of native species.

The data are useful not only on their own, but can be used as a dataset. Our seed trait dataset, which includes data of 806 taxa, can provide input data to large scale ecological analyses as well. It can be used for revealing correlations between the seed traits and other plant traits or environmental factors, and in the light of these correlations, hard traits can be estimated. In this paper, the applicability of our seed trait dataset was exemplified through analysing correlations between (i) seed traits and life forms and (ii) seed traits and SBTs. The results of these analyses can be utilised among others in community, conservation and restoration ecology.

The HUSEED<sup>wild</sup>, which is available online, is being extended and updated continuously, and due to its standardised data it can be suitable for integration into other larger European online databases (e.g. SID), which allows even more widespread accessibility and applicability.

**Acknowledgements.** The Pannon Seed Bank project (LIFE08 NAT/H/000288) was completed with the financial contribution of EU LIFE+ fund and the Hungarian Ministry of Agriculture. The authors are thankful to the colleagues of associated beneficiary institutes and national park directorates, and to the following botanical experts for their help in seed collection: Baji, B., Balogh, L., Békássy, G., Békefi, N., Böhm, É. I., Böszörményi, A., Bujdosó, L., Czibula, Gy., Czucz, B., Cservenka, J., Deák, J. Á., Galambos, I., Illyés, Z., Juhász, M., Kádár, F., Kecskés, F., Kohári, Gy., Kopor, Z., Kóródi, B., Makra, O., Mészáros, A., Nagy, E., Nagy, J., Nagy, L., Németh, A., Papp, L. jr., Penksza, K., Sass-Gyarmati, A., Simon, P., Sinigla, M., Szabó, G., Szabó, M., Szitár, K., Tatár, S., Tóth, Z., Török, P., Vókó, L., Zsigmond, V.

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## ELECTRONIC APPENDIX

**Electronic Appendix 1.** *The extract of the online HUSEED<sup>wild</sup> (available at: <http://huseed.nodik.hu:8243/en/wild>) concerning the single thousand-seed weight data [g], germination percentage data [%] and the applied germination methods of the 806 taxa (sensu species and subspecies) accessible in it on 1 June 2015.*