

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

INVESTIGATION OF GERMINATION ABILITY ON CROP-WILD RELATIVES

DOI: 10.54598/001640

Erzsebet Peti

Gödöllő

2021

The doctoral school:					
Name:	Doctoral School of Environmental Sciences				
Discipline:	Environmental Sciences				
Head of school:	Dr. Erika Csákiné Michéli, DSc				
	professor				
	Hungarian University of Agriculture and Life Sciences				
	Institute of Environmental Sciences				
Supervisor:	Dr. Dezső Surányi				
	university professor, Doctor of Science				
	MATE, Institute of Horticultural Science, Research Centre for Fruit Growing, Cegléd				
Co-supervisor:	Dr. Ferenc Gyulai				
	university professor, Doctor of Science				
	Hungarian University of Agriculture and Life Sciences				
	Institute of Environmental Sciences				
Approval of the Head of Sc Supervisor approval Dr. Dezső Surányi	 Phool				
Supervisor approval Dr. Ferenc Gyulai	•••				

1. BACKGROUND OF THE WORK AND OBJECTIVES

Seed is suitable for the ex situ (off-site) conservation of plant genetic resources (Guerrant et al. 2014). Seeds are mostly conserved in genebanks (seed banks) (Smith et al. 2003, Lima et al. 2014, Groot et al. 2015). Plant seeds require special storage conditions, and continuous monitoring of their status is necessary in order to get more reliable information about their viability. Investigation of viability is essential during managing seed collections, and it is an effective way of fast determination of possible problems during storage (Godefroid et al. 2010, van Treuren et al. 2013). Necessary knowledge in germination character of native plants is still incomplete contrary to reasonably investigated weeds. In particular, it is appropriate to investigate effects of cooled conditions on plant species, and on their viability. Limited information is available on viability of stored seeds under laboratory and in parallel, under field conditions (Kiran Babu et al. 2018). Reasons for reduced productivity of rare species, number of produced seeds, germination capacity and natural reproductivity rate of seeds are slightly known (Kricsfalusy és Kommendar 1990). These are very important information during not only regeneration of stored accessions, but application of them for nature conservation purposes (Merritt et al. 2003), which is a primary aim in the case of stored materials.

In my thesis I investigated the germination ability of seeds of 23 wild herbaceous plants from 5 different families under laboratory and field conditions. Dried seeds are stored in genbank under cooled conditions. Investigated accessions have already been stored for 1-5 years in 2017.

Main aims of the investigation:

- 1. Comparing germination ability of selected species under different genbank conditions (under 0 °C and -20 °C);
 - 2. Investigation of viability under laboratory and field conditions;
- 3. Analysing germination data before and after storing, and defining the possible reasons of significant differences (germination influencing factors).

2. MATERIALS AND METHODS

2.1. Aspects of selecting investigated species

41 accessions of 23 species from 5 families were investigated, which were stored at least one year at the beginning of the research. Following aspects were taken into consideration during selecting species for investigation:

- I selected those families, which are represented by many species with more accessions in Pannon Seedbank, so at the beginning of the investigation at least one-year stored accession is available.
- I chose species with ortodox or presumably ortodox storage behaviour, otherwise I was not able to investigate germination ability under cooled conditions. Storage behaviour of 5 species was not available, thus my investigation may provide a clearer picture about it.
- Sufficient seed amount (min. 5000 pieces) during selecting species was an important aspect in order that enough seeds will be available in 0 °C and -20 °C storage cabinets for experiments without endangering long-term conservation of species.
- Nature conservation importance of species (based on nature conservation categories by Simon (Simon 1988)) was also taken into consideration. So mainly natural species and some species indication degraded areas are involved in in the research. One protected species per family and two protected species from *Caryophyllaceae* and *Fabaceae* were also chosen. Selected species are mostly grassland composing plant species and there are some mono- and dicotyledonous, dominant and accessorial plant species too.

2.2. Seed collecting

Accessions of investigated species were collected in Pannon Biogeographical Region in the frame of Pannon Seedbank Project, where I worked as project manager between 2010 and 2014. Seed collecting was conducted according to quality and quantity aspects of protocol of European Native Seed Conservation Network (ESCONET 2009a). Accessions were collected by the colleagues of National Biodiversity and Gene Conservation Centre (Tápiószele), Centre for Ecological Research Hungarian Academy of Science Centre for Excellence (Vácrátót) and national park directorates and botanical experts of other institutes. Random,grid and line transect sampling were applied depending on the size of population. Taxonomic identification of species was based on "Új Magyar Füvészkönyv" (Király 2009). During the project great attention was paid on larger-scale sampling of genom of populations, thus

collecting mixed seed samples (further on, I refer to seed samples that represent individual populations as "accessions"). Metadata of the collections [e.g. location, date, sampling method, habitat type according to Bölöni et al. (2011), etc.] were thoroughly documented. Figure 1. shows the location of collected accessions.

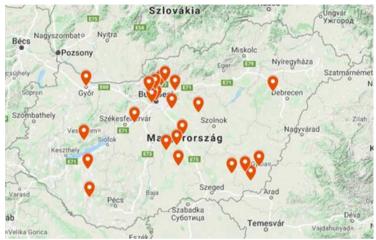


Figure1: Location of collecting of investigated accessions Source of map: Google

Further details of seed collecting protocol are available in Peti et al. (2015) and Török K. et al. (2016).

We paid particular attention to collecting high-quality (ripe, healthy) accessions with proper seed amounts which are suitable for long-term gene conservation. Based on ENSCONET (2009a) specifications, acceptable seed amount was defined as 5000 pieces of seed (Zsigmond 2011).

2.3. Processing and germination of seed accessions

During cleaning, foreign matters, other plant remnants, pests, infected, immature and empty seeds were eliminated from accessions according to recommendations of methodological literature [Rao et al. (2006), ENSCONET (2009b)]. Seed weight measurements were carried out with accessions cleared from non-propagule fractions according to the seed weight measurement protocol (http 4) of Pannon Seedbank. Corresponding with standards of international databases (e.g LEDA (Kleyer et al., 2008); Hintze et al., 2013), air-dry weights of propagules 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 accession and taxon. Seed weight was expressed as thousand-seed weight (g).

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. 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. Depending on the species, scarification, soaking or warm and/or cold stratification were used as a pretreatment to break non-deep dormancy.

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 accession and taxon. Germination capacity was expressed as germination percentage (GP) [%].

2.4. Drying and storing

After seed weight measurements and germination tests, moisture content of the seeds were reduced to 3-7%. Seeds were dried in drying rooms operated in 16 ± 1 °C and 15-20% relative humidity [ENSCONET (2009b), FAO (2013)]. Dried accessions were stored in sealed, three-layer aluminium foil bags. The cold rooms operate on 0 °C (active store) and on -20 °C (base store) according to standard.

2.5. Water activity measurement

Water activity (aw) content of seeds is a ratio, which is proportional to moisture content of seeds, which is defined as the ratio of steam tension (P) of free water in samples and parcial pressure (Po) of relative humidity of space above clear water. Aw value was measured with Testo 650 equipment. Followings were investigated:

- Effects of 0 and -20 $^{\circ}$ C storing temperature on water content of species in 2016 and 2017.
- Effects of 0 and -20 $^{\circ}$ C storing temperature on water content of species in three successive years compared with control values.
- Investigation of connection between germination value and water activity.

2.6. Production in greenhouse and field sowing

Plantings in greenhouse started in May in 2015, with four replicates, where 4*25 pieces of seeds were used per replicate, in 10*10 cm size plastic potteries. Percentage of germinated seeds within a year was determined.

Accessions were sown in field conditions in potteries filled with neutral soil, and potteries were sunk in the soil. Figure 2. shows the arrangement of the experiment.

Accession were sown 5 replicates (5 seeds per pottery, totally 5 potteries/accessions, with 5*5, totally 25 seeds) in the following dates:

- end of August 2015
- beginning of February 2016
- mid-March in 2016
- end of March 2016
- beginning of August 2016

In the case of those species where enough seed amount was available, there were 3 sowing dates [August 2015 (28. 08. 2015.- 01.09.), February 2016 (02.-03. 02. 2016.) and at the end of March], where seed amount was more restricted, only two sowing dates were applied [March 2016 (22.03.2016) and August (08.-10.08.2016)].



Figure 2. Field experiment after replantation in May 2016 (own photo)

3. RESULTS

3.1. Water content measurements

Storing temperature of 0 and -20 °C have significant effects on water content in 2015 and 2016, storing temperature of -20 °C reduced more considerably the water content of the accessions. On the contrary, in 2017 storing temperature has no significant effects on water content of samples.

Storing temperature of -20 °C provides better preservation of water content of investigated seeds, as water content increased after storing at 0 °C compared to control and storing temperature of -20 °C.

There was no close correlation between germination ability and water content of seeds.

3.2. Germination under laboratory conditions

Germination was successful in most cases, however many seeds are not able to germinate. Successful germination means that the applied germination method was successfully adapted for Hungarian native species. Deep dormancy most likely caused failed germination, while enforced dormancy less likely caused it.

Germination percentages varied widely between different accessions of the same taxa. One reason may be the variability in the ratio in dormant and non-dormant seeds within different populations of each species, moreover different species of populations (Milberg et al. 1996; Baloch et al. 2001; ENSCONET 2009b; Baskin & Baskin 2014). Possible explanations for germination results may be the different storing time of accessions, changes of accessions during storing, effects of not appropriate storage (management) technique or not proper quality of collected seeds. This was particularly confirmed in the case of the *Lamiaceae* family, where more species (e.g. *Phlomis tuberosa*, *Salvia nemorosa*) and accessions showed very weak germination results under all conditions.

Experience gained from laboratory germination, it may be considered that permanently extremely low temperature is more effective in dormancy breaking than temperature around $0\,^{\circ}\text{C}$.

3.2.1. Effects of storing temperature of 0 °C on seed germination

Figure 3 shows that germination results are gradually reduced in each year. The highest reduction was last year, when average result before storing declined from 64 % to 22%.

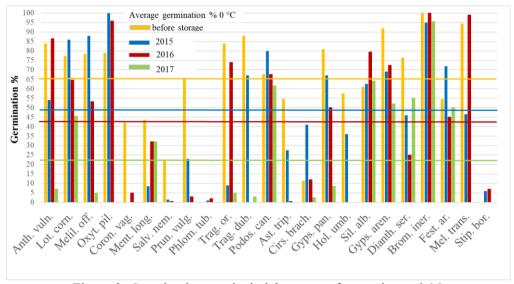


Figure 3: Germination results in laboratory after storing at 0 °C

Last year of investigation, after storage under temperature of 0 °C average germination results reduced significantly in the case of 17 out of 23 investigated species compared with results before storage. Changes were not significant in the case of 5 species (*Podospermum canum*, *Festuca arundinacea*, *Silene alba*, *Mentha longifolia*, *Phlomis tuberosa*).

3.2.2. Effects of storing temperature of -20 °C on seed germination

Germination results moderately reduced in 2015 and 2017 compared with 64% average result obtained before storing, while in 2016 average result exceeded initial results. Analysing the results of each species it may be considered that all species - except *Oxytropis pilosa* - germinated even in the last year too, and the number of species which germinated hectic every year also reduced (Figure 4).

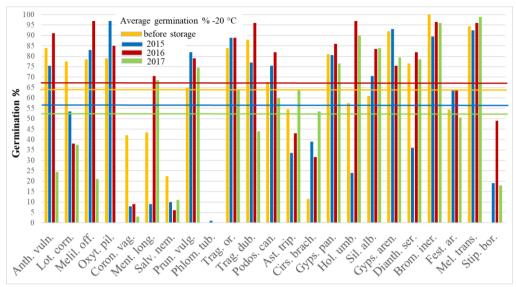


Figure 4: Germination results in laboratory after storing at -20 °C

In the case of 8 species (Aster tripolium, Cirsium brachycephalum, Melica transsilvanica, Holosteum umbellatum, Silene alba, Prunella vulgaris, Phlomis tuberosa and Mentha longifolia) results exceeds the initial values after storing temperature of -20 °C.

3.3. Germination in greenhouse

In most cases germination results in greenhouse exceeded field results, these rather approached laboratory values, which was benefited greatly by the verified conditions. Furthermore seeds germinated faster (after one month), germination was not so long-continued than in the field.

Within *Fabacae* family, seeds of *Lotus corniculatus* germinated most effectively, but germination averages on both temperatures - similarly to other species - were significantly lower than results of control and other years. Accessions of *Oxytropis pilosa* and *Coronilla vaginalis* germinated very weakly (<10%).

Phlomis tuberosa and Salvia nemorosa from the Lamiaceae family also germinated very slightly in the greenhouse (however Phlomis tuberosa germinated hardly in each year, but it showed best results in greenhouse). Accession of Prunella vulgaris stored at temperature of 0°C showed weak results, but accession from temperature of -20°C germinated above 80%.

In the case of species from the *Asteraceae* family, accessions from lower temperature germinated better all the while.

In the *Caryophyllaceae* family, accessions of *Holosteum umbellatum* from temperature of -20°C showed 50% average results, while accessions from temperature of 0°C hardly germinated. By contrast, accessions of *Gypsophila*

arenaria and Dianthus serotinus stored at temperature of 0°C germinated, but accessions stored at lower temperature didn't germinate at all.

Stipa borysthenica from the Poaceae family didn't germinate at all, whilst accessions of Melica transsilvanica from temperature of -20°C showed 70% average results, but accessions from temperature of 0°C didn't germinate at all.

3.4. Field germination

Germination of seeds sowed in spring was faster and more synchronized than those sowed in autumn. Most species germinated in April and May, and they reached the maximum germination. Out of species sowed in February *Melilotus officinalis*, *Tragopogon* species and *Podospermum canum* germinated at first. Out of species sowed in March *Silene alba*, *Holosteum umbellatum*, *Tragopogon* species and *Podospermum canum* germinated at first.

In most cases germination starts during in April, May and June, however accessions of some species started to germinate only in spring next year such as *Salvia nemorosa*, *Lotus corniculatus*, *Festuca arundinacea*, *Anthyllis vulneraria* and *Coronilla vaginalis*. *Dianthus serotinus* showed the most long-continued germination, where seedlings appeared even in autumn and next April.

In the case of sowing in August, germination started in August, but it was slow. Some species germinated in September, but others began to germinate only in October or next spring. *Salvia nemorosa*, *Bromus inermis*, *Festuca arundinacea* and *Anthyllis vulneraria* germinated at first.

Storage temperature seemed effective for dormancy breaking, so species sown at the end of winter and spring started to germinate within 1-2 month, but species sown in summer even started to germinate in September. Results show that grass species can be propagated effectively by seeds, *Bromus inermis* and *Festuca arundinacea* also confirmed it during the field experiment.

Table 1 shows that *Tragopogon orientalis*, *Tragopogon dubius*, *Cirsium brachycephalum*, *Holosteum umbellatum*, *Dianthus serotinus*, *Oxytropis pilosa*, *Coronilla vaginalis* and *Salvia nemorosa* can be sewed effectively both in autumn and spring too.

Table 1. Comparative data of germination time of plants (-0%; +0-25%, ++25-50%, +++50-75%)

		+++50-75%) germination			
species	storage	autumn	spring		
	temp.				
Anthyllis vulneraria		+	+		
-	-20 °C	+++	+		
Lotus corniculatus	0 °C	+	+		
	-20 °C	++	+		
Melilotus officinalis	0 °C	++	+		
33	-20 °C	++	+		
Oxytropis pilosa	0 °C	+	+		
2 1 1	-20 °C	+	+		
Coronilla vaginalis	0 °C	+	+		
0	-20 °C	+	+		
Mentha longifolia	0 °C	-	+		
	-20 °C	-	+		
Salvia nemorosa	0 °C	-	-		
Saivia nemorosa	-20 °C	+	+		
Prunella vulgaris	0 °C	-	-		
Trinetta vingaris	-20 °C	+	-		
	0 °C	-	-		
Phlomis tuberosa	-20 °C	-	-		
Tragopogon orientalis	0 °C	++	++		
Tragopogon orientalis	-20 °C	+	+++		
Tuga an a san dubing	0 °C	+++	+		
Tragopogon dubius	-20 °C	-	+++		
Podospermum canum	0 °C	++	++		
1 oaospermum canam	-20 °C	+++	++		
Aster tripolium	0 °C	-	-		
Aster tripottum	-20 °C	-	-		
Cincinus Innochrange	0 °C	+	+		
Cirsium brachycephalum	-20 °C	+	+		
C 1:1 : 1 :	0 °C	+	+		
Gypsophila paniculata	-20 °C	+	++		
TT 1 . 1 11 .	0 °C	++	+		
Holosteum umbellatum	-20 °C	++	++		
G:1 11	0 °C	+	+		
Silene alba	-20 °C	++	+		
C 1:1	0 °C	+	+		
Gypsophila arenaria	-20 °C	++	+		
D: 1	0 °C	+	+		
Dianthus serotinus	-20 °C	+	+		
D	0 °C	++	+++		
Bromus inermis	-20 °C	++	+++		

an a stag	storage	germination			
species	temp.	autumn	spring		
E-to	0 °C	+	+		
Festuca arundinacea	-20°C	+	++		
Melica transsilvanica	0 °C	-	-		
	-20 °C	-	+		
Cian hamathania	0 °C	+	+		
Stipa borysthenica	-20 °C	++	+		

Phylogenetically older families (e.g. *Fabaceae*, *Lamiaceae*) lost gradually their viability, while younger families (e.g. *Poaceae*) remained viable longer, they were able to germinate a high proportion last year of the investigation. Younger families germinated moderately in the laboratory, but older families showed more varied and hectic results (Table 2). However this may help their long-term survival too, as their seeds won't germinate at once under favourable conditions, but their germination is prolonged, which provides long term survival of species.

Mortality among seedlings was not significant after one year, most of them produced seeds in the field.

Table 2: Average germination rates (%) within 5 investigated families

	Average germination %						
Family name	Before	0 °C			-20 °C		
	storage	2015	2016	2017	2015	2016	2017
Fabaceae (5)	75,1	79,4	67,2	12,8	66,1	70,1	18,8
Poaceae (4)	83,0	61,9	70,7	48,5	72,9	80,3	72,7
Caryophyllaceae							
(5)	73,6	55,8	47,7	35,9	59,9	85,1	81,7
Asteraceae (5)	54,9	47,4	29,3	17,0	60,9	62,4	57,9
Lamiaceae (4)	37,4	9,6	10,4	9,1	29,0	44,4	44,0

3.5. Seed weight investigations

Based on the measured average thousand seed weights, following species had smallest (<0,5 g) seed weights: *Holosteum umbellatum*, *Mentha longifolia*, *Gypsophila arenaria*, *Melica transsilvanica* and *Aster tripolium*.

Podospermum canum, a Bromus inermis, Tragopogon orientalis, Tragopogon dubius and Stipa borysthenica had the largest average thousand seed weight (3,4-11 g).

According to social behaviour types (SBT) competitors (2,89 g) and stress-tolerants (2 g) have the largest average seed weights, they were followed by ruderals (1,95 g).

3.6. New scientific results

• Determination of storage behaviour of 5 species (Coronilla vaginalis, Gypsophila arenaria, Phlomis tuberosa, Stipa borysthenica, Tragopogon orientalis).

Taking into account the Alpin-Balcan flora character of *Coronilla vaginalis*, it can be predicted that species is short-term persistent (viability remains more than one year, but until maximum 5 year, Thompson 1993), which may cause fast decline of their germination ability. Recalcitrant and transient storage behaviour of the species is excludable, as it germinated in last year in minimal scale, so the species is presumably ortodox.

Ortodox storage behaviour is predicted in the case of *Gypsophila arenaria* based on the results of the current research, as under laboratory conditions accessions germinated well (above 50 and 70%) after two or three years-long storage. The same could be said about *Stipa borysthenica* which showed poor results, but the species was able to germinate after three-years-long storage under laboratory and field conditions. Ortodox storage behaviour was confirmed in the case of *Tragopogon orientalis*, as in last year of investigation accessions stored under -20°C germinated above 60% after 5 year-long storing in the laboratory, and above 50% in the field. *Phlomis tuberosa* germinated poorly before and after storage too under all temperature, bad quality and reduced viability of seeds may also cause poor results. Presumably the species is rather ortodox (or probably intermedier) and not recalcitrant.

• In the case of 10 species (*Tragopogon orientalis*, *Aster tripolium*, *Cirsium brachycephalum*, *Bromus inermis*, *Melica transsilvanica*, *Stipa borysthenica*, *Silene alba*, *Gypsophila arenaria*, *Coronilla vaginalis*, *Phlomis tuberosa*) laboratory germination protocol was not available in concerning databases (RBGK 2016), so I made suggestions for the possible protocols.

Data for germination ability of seeds of Central European native plant species is still very limited, data available only in three databases (HUSEED wild — Peti et al. 2017, Kiss et al. 2018, RBGK 2016). Germination information of Hungarian native plant species are still missing from most international plant trait databases (LEDA — Kleyer et al. 2008, Hintze et al. 2013). So my investigations added new data for germination of native species.

Germination data of Pannonian flora are limited, especially investigating them
under natural conditions (Kiss et al. 2018). Thus my thesis consists of novel
results concerning propagation of selected species under field conditions and
their germination ability, in particular in view of nature conservation
utilisation of species stored in genbank. It follows that knowledge of
germination ability of seeds of selected species is essential for using seeds for

restoration purposes as germination may vary (sometimes considerably) year by year. Furthermore only few authors (e.g. Mándy 1974) investigated links between germination under laboratory and field conditions, but especially concerning cultivated species.

4. CONCLUSIONS AND SUGGESTIONS

Laboratory germinations serve as good control for detecting viability, because these give a relatively fast and exact picture about germination ability of accessions, but laboratory methods sometimes overestimate the actual viability. Laboratory, greenhouse and field results show also that laboratory results do not always reflect the real extent of germination on field, as field conditions are not always optimal for germination of seeds. Laboratory germination results were considerably higher than field results. Germination of wild plant species often can be weak and fluctuating, which makes their usage difficult in habitat reconstructions, where changing local conditions increase further uncertainty of investigation. Estimation of field germination results is often can be difficult in the case of species with low germination ability, so this may be subject of further investigations.

In the case of cultivated plants, the regeneration standard in the laboratory is 85%, but in the case of wild plant species this value is hardly obtained, thus lower standards (70% or even lower) are anyway necessary for germination of wild plants under laboratory conditions (FAO 2013).

According to the laboratory results, permanently extremely low temperature is more effective in breaking seed dormancy than 0°C.

My results are also important in the restoration aspect too, as it was confirmed that in most cases the viability of seeds is not reduced considerably after some years of storage, so storage of seeds may help in surviving weak seed productivity years and the lack of seeds due to shortage of the national seed market (Merritt és Dixon 2011).

In the case of some species dry storage may break dormancy, but for other species storage may have the opposite effect, it may induce dormancy. So reduced, or even zero germination values do not always mean the loss of germination ability, as these may refer to induced dormancy. If the germination is reduced directly after storing, it is assumed that long term storage caused a similar effect by seeds being stored without light where fluctuation in daily and seasonal temperature was ceased. Germination results may be improved due to dormancy braking.

Other reasons for reduced germination results may be risk-spreading germination strategy (Grubb 1988), which is particularly typical within the ruderal group. Species with this strategy maintain persistent soil seed bank by several dormant seeds and under optimal conditions they germinate only to a smaller extent but consistently. However within ruderal group, number of taxon which germinate around 100 % (eg. *Tragopogon* species, *Silene alba*, *Holosteum umbellatum*, *Melilotus officinalis*) is common. Possible reason for this increased germination willingness is the "disturbance-broken" strategy (Grubb 1988) which is typical within the ruderal group. 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 explosively under favourable environmental

conditions caused by disturbance, which makes their fast colonisation and survival possible.

Results also draw attention to importance of starting regeneration of accessions with reduced viability, based on the results of the field experiment.

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 for example during restoration works (Török, P. et al., 2016): in view of seed weight and germination capacity of species, necessary amount of seeds for 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. But according to our results we see a chance for renewing on an ecological basis the difficult system of weeds and cultivated plants.

Field results show that *Poaceae* species propagated successfully by seeds, which is very important in habitat restoration view, as some of them (like grassland composing, competitor species e.g. Festuca pseudovina, F. rupicola, F. pratensis, F. arundinacea, Poa pratensis, P. angustifolia, Bromus inermis - Deák and Kapocsi 2010) must establish in the first phase of restoration, and then they necessary to propagate. Additional planting of rarer, accompanying elements (e.g. Fabaceae species such as Trifolium spp., Lotus corniculatus, Lathyrus spp., Vicia spp.) may serve as supplementation of seed sowing. Before using genbank accessions, multiplication of them is necessary anyway. Methods of multiplication are less developed for wild plant species. Results of this current research propagation character support the of species some accompanying elements, furthermore can give suggestions for which species can be involved more successfully in restoration projects due to their easier propagation (e.g. Silene alba, Festuca arundinacea, Bromus inermis, Tragopogon orientalis, Podospermum canum, Dianthus serotinus, Gypsophila arenaria, Anthyllis vulneraria, Lotus corniculatus), and which species requires further investigations (e.g. Mentha longifolia, Coronilla vaginalis, Aster tripolium).

We can conclude from the results of the experiment that in the case of most species sowing time is not the primary limiting factor for successful development, but temperature at sowing time is more important as species can germinate in a wide range of sowing dates. Most species can germinate a relatively wide range of temperatures and can even be two germination periods in the same year, as I found seedlings both in spring and at the end of summer. But there were some species that could germinate only in autumn such as *Prunella vulgaris*, but *Melica transsilvanica*, *Gypsophila paniculata* and *Mentha longifolia* germinated only in spring.

In many cases, accessions stored under lower temperature germinated better, than those stored at higher temperature. This shows that reduction of permanently cold winter periods may weaken germination ability. Partly lack of proper cold effect may hamper germination of those species which require cold effect for their germination (e.g. T3, T4 annual weeds). On the other hand those species which can germinate autumn as well, but they germinate mostly rather in spring, they may germinate greater numbers also in autumn due to warmer autumn and milder winter. This may be a potential possibility for survival of certain species. However it means further problems for elimination of certain weed species.

So temperature is a key element for germination, but under natural conditions light, humidity and surrounding species are also important. After all, it can be decisively determined during climate change that most of the species (especially recalcitrant and dying sensitive species) are not able to respond with appropriate flexibility to higher temperatures and drought. This may be a substantial risk during reintroductions/ habitat restorations, as it may significantly reduce the germination and survival rate of reintroduced species. Dry periods may reduce the number and size of seeds, which may increase the number and ratio of bad quality seeds.

5. PUBLICATIONS RELATED TO THE DISSERTATION

Peer-reviewed articles with impact factor

1. **Peti, E.** Schellenberger, J., Németh, G., Málnási Csizmadia, G., Oláh, I., Török, K., Czóbel, Sz., Baktay, B. (2017): Presentation of the HUSEED^{wild} – a seed weight and germination database of the Pannonian flora – through analysing life forms and social behaviour types. — Applied Ecology and Environmental Research 15(1): 225 – 244. (Print ISSN: 1589 1623, Online ISSN: 1785 0037, DOI: http://dx.doi.org/10.15666/aeer/1501_225244). (Impact Factor 2015: 0,500)

<u>Articles in peer-reviewed journals – foreign language</u>

- 2. Török, K., Szilágyi, K., Halász, K., Zsigmond, V., Kósa, G., Rédei, T., **Peti, E.**, Schellenberger, J., Tóth, Z., Szitár, K. (2016): Seed collection data encompassing half of the vascular flora of the Pannonian ecoregion stored by the Pannon Seed Bank. Acta Botanica Hungarica 58 (3-4), p. 435–445, (Print ISSN: 0236-6495, Online ISSN:1588-2578 DOI: https://doi.org/10.1556/ABot.58.2016.3-4.13).
- Török, P., Tóth, E., Tóth, K., Valkó, O., Deák, B., Kelbert, B., Bálint, P., Radócz, Sz., Kelemen, A., Sonkoly, J., Miglécz, T., Matus, G., Takács, A., Molnár, V. A., Süveges, K., Papp, L., Papp, L. jr., Tóth, Z., Baktay, B., Málnási Csizmadia, G., Oláh, I., Peti, E., Schellenberger, J., Szalkovszki, O, Kiss, R, Tóthmérész, B.(2016): New measurements of thousand-seed weights of species in the Pannonian Flora. Acta Botanica Hungarica 58(1-2). p.187-198. (Print ISSN: 0236-6495, Online ISSN: 1588-2578, DOI: http://dx.doi.org/10.1556/034.58.2016.1-2.10).

Articles in peer-reviewed journals – Hungarian language

- 4. Peti, E., Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Török, K., Halász, K., Baktay, B. (2015): A Pannon Magbank program (2010-2014) maggyűjtési, tárolási, előzetes életképesség vizsgálati eredményei és módszerei [Seed collevting and storing results and preliminary seed viability results and methods of Pannon Seed Bank project (2010-2014)] Természetvédelmi Közlemények 21: p. 215.-231. (ISSN 1216-4585)
- 5. Ponicsánné Gyovai, Á., Kollár, Zs., **Peti E.**, Horváth, B., Oláh, I., Szalkovszki, O., Baktay, B. (2013): Tájfajták a Zempléni-hegységben A 2013-2014-es gyűjtőút program első állomásának tapasztalatai./ Landraces in the Zemplén mountains The first target area of the collection mission program 2013-2014. Tájökológiai lapok 11 (2) p. 367-371. (ISSN 1589-4673)

Full-text document, study in journals

 Peti, E., Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Baktay, B., Török, K., Halász, K. (2015): Éltető gyűjtemény – A Pannon Magbank, TermészetBúvár 70(6): p.11.-13. (ISSN 0866-1510)

Conference proceedings- Hungarian (full-text)

7. Holly, L., Málnási Csizmadia, G., **Peti, E.**, Szilágyi, K., Szitár, K., Török, K. (2012): Előzetes gyűjtési, tisztítási, csíráztatási és visszatelepítési eredmények, tapasztalatok és problémák a Pannon Magbank projekthez kapcsolódóan. XXIX. Vándorgyűlés (Magyar Biológiai Társaság), Budapest, Magyarország 2012.10.19., Előadások és poszterek összefoglalói pp. 27-30. (ISBN 978-963-87343-6-5)

Conference proceedings – Hungarian (abstract)

- 8. **Peti, E.** (2021): A Fabaceae és a Caryophyllaceae családok 10 fajának csírázási képessége és alkalmazhatóságuk vizsgálata élőhely rekonstrukciós kísérletekben. XXVII. Növénynemesítési Tudományos Napok. Összefoglaló kötet. Martonvásár, 2021.március 24-25., p. 59.
- 9. Peti, E. (2017): A csíraképesség változása az Asteraceae és a Poaceae családokhoz tartozó néhány vadon élő faj különböző hőmérsékleti körülmények között tárolt magmintáinak esetében. XXX. Vándorgyűlés 2017.02.17-18. (Magyar Biológiai Társaság), Budapest. Program és összefoglalók pp. 56-57. (ISBN 978-963-87343-8-9)
- 10. Peti, E. (2017): A csíraképesség változása a Caryophyllaceae, Fabaceae és Lamiaceae családokhoz tartozó néhány vadon élő faj különböző hőmérsékleti körülmények között tárolt magmintáinál. Botanikai Közlemények 104 (2), p. 257. (ISSN 0006-8144)
- 11. **Peti, E**., Málnási Csizmadia, G., Holly, L., Tóth, Z., Szilágyi, K., Szitár, K., Szmorad, F. (2012): A Pannon Magbank szerepe Magyarország természetes növényi változatosságának ex-situ megőrzésében. In: Bartha, S., Mázsa, K (eds.) "9. Magyar Ökológus Kongresszus" Keszthely, Magyarország, 2012.09.05.-07., Programfüzet, Előadások és poszterek összefoglalói, p. 86. (ISBN 978-963-8391-54-4)
- 12. Balogh, L., Halász, K., Oláh, I., **Peti, E.** (2015): A Pannon Magbank Projekt (2010–2014) és eddigi eredményei. In: Puskás, J. (ed.) X. Regionális Természettudományi Konferencia. NYME SEK Természettudományi Kar, Szombathely, 2015. 01.28. Program és az előadások összefoglalói, pp. 10–11.
- 13. **Peti E.**, Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Baktay, B., Török, K., Halász K., (2015): A Pannon Magbank program előzetes eredményeinek összefoglalása In: "I. Budai Campus Diákszervezeti Konferencia", Budapest, Magyarország, 2015.03.27., pp. 3.

<u>Conference proceedings – Foreign language (abstract)</u>

14. Kiss, R., Tóthmérész, B., Tóth, K., Miglécz T., Valkó O., Kelemen, A., Deák, B., Sonkoly, J., Tóth, E., Radócz, Sz., Matus, G., Takács, A., Molnár V., A. Ruprecht, E., Horváth, O., Kelbert, B., Bálint, P., Balogh, N., Süveges, K., Hüse, B., Papp, L., ifj. Papp, L., Tóth, Z., Baktay, B., Málnási Csizmadia, G., Oláh, I., Peti, E., Schellenberger, J., Szalkovszki, O., Török, P. (2016): New database of thousand-seed weights of species in the Pannonian Flora. [A pannon flóra új magtömeg adatbázisa – In: Barina, Z., Buczkó, K., Lőkös, L., Papp, B., Pifkó, D., Szurdoki, E. (eds.) "11th International Conference "advances in research ont he flora and vegetaion of the

- Carpato.Pannonian Region", Budapest, Hungary, 2016. február 12–14., Book of abstracts p.169-170. (ISBN 978-963-9877-25-2).
- 15. **Peti, E.,** Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Török, K., Halász, K., Baktay, B. (2016): Seed biology and morphology investigations on Pannon Seed Bank collection and possible applicability of the results. [A Pannon Magbank gyűjteményének magbiológiai és morfológiai vizsgálatai és azok felhasználási lehetőségei.] In: Barina, Z., Buczkó, K., Lőkös, L., Papp, B., Pifkó, D., Szurdoki, E. (eds.) "11th International Conference "advances in research ont he flora and vegetaion of the Carpato.Pannonian Region", Budapest, Hungary, 2016. február 12–14., Book of abstracts pp.209-210. (ISBN 978-963-9877-25-2).
- 16. Peti, E., Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Veres, E., Baktay, B.(2015): Ex-situ conservation and investigation of some Rosaceae species in Pannon Seedbank. [Rózsafélék (Rosaceae) néhány fajának ex situ megőrzése és vizsgálata a Pannon Magbankban.] In: Kerényi-Nagy, V., Szirmai, O., Helyes, L., Penksza, K., Neményi, A. (eds.) "1st Rose. and hawthornconference in Carpathian Basin" international conference, Gödöllő, Hungary, 2015.05.30., Proceedings-Book pp. 244-246. (ISBN: 978-963-269-479-5)
- 17. **Peti, E.**, Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Baktay, B., Török, K., Halász, K. (2014): Through collecting to storages: summary of the practical experiences related to Pannon Seedbank project [A gyűjtéstől a hűtőtárolókig: a pannon magbank program gyakorlati tapasztalatainak összefoglalása.]- In: Zimmerman, Z., Szabó, G. (eds.) "II. Sustainable development in the Carpathian Basin" international conference, Budapest, Hungary, 2014.12.11-12. Book of abstracts, pp. 43-44. (ISBN 978-963-269-455-9)
- 18. **Peti, E.**, Málnási Csizmadia, G., Oláh, I., Schellenberger, J., Halász, K., Török, K., Baktay, B., (2014): Results of the Pannon Seedbank project. [A Pannon Magbank LIFE+ Program eredményei.] In: Zimmermann, Z., Szabó, G. (eds.) "II. Sustainable development in the Carpathian Basin" international conference, Budapest, Hungray, 2014.12.11.-12., Book of abstracts, pp. 122-123. (ISBN 978-963-269-455-9)
- 19. Schellenberger, J., **Peti, E.**, Szirmai, O., Barczi, A., Czóbel, Sz. (2014): Research of vegetation and soil seed bank in a marsh and xero-mesic grassland int he North Hungarian Mountains (NE Hungary) [Vegetáció és talajmagkészlet vizsgálatok egy északi-középhegységi nedves és xero-mezofil gyepen.] In: Zimmerman, Z., Szabó, G. (eds.) "II. Sustainable development in the Carpathian Basin" international conference, Budapest, Hungary, 2014.12.11-12. Book of abstracts, pp. 129-130. (ISBN 978-963-269-455-9)
- 20. Peti, E., Málnási Csizmadia, G. Oláh, I. (2013): Collecting, cleaning and germination results, experiences and problems related to Pannon Seedbank project [Gyűjtési, tisztítási, csíráztatási eredmények, tapasztalatok és problémák a Pannon Magbank projekthez kapcsolódóan.] In: Zimmermann, Z., Szabó, G. (eds.) "VIII. Carpathian Basin Biological Symposium I. Sustainable development in the Carpathian Basin" international conference, Budapest, Hungary, 2013. 11.21.-23. Book of abstracts, pp. 60.-62. (ISBN 978-963-269-387-3)
- 21. Ponicsánné, Gyovai Á., Kollár, Zs., **Peti, E.**, Horváth, B., Oláh, I., Szalkovszki, O., Baktay, B. (2013): Landraces in the Zemplén mountains The first target area of the collection mission program 2013-2014 [Tájfajták a Zempléni-hegységben A 2013-2014-es gyűjtőút program első állomásának tapasztalatai] In: Zimmermann, Z., Szabó, G. (eds.) "VIII. Carpathian Basin Biological Symposium I. Sustainable

- development in the Carpathian Basin" international conference, Budapest, Hungary, 2013. 11.21.-23. Book of abstracts, pp. 63.-64. (ISBN 978-963-269-387-3)
- 22. Holly, L., Hock, Zs., Fidlóczky, Zs., Peti, E., Simon, A., Kutta, G., Szmorad, F. (2012): A Pannon Magbank szerepe Magyarország természetes növényi változatosságának ex-situ megőrzésében [Possible role of the Pannon Seed Bank in improving the collaboration among ex situ, in situ and on farm conservation efforts for the maintenance of diversity in the Hungarian natural and cultivated flora]. In: Molnár V., A. (ed.) Kitaibelia- 17 (1), "Az Aktuális Flóra- és Vegetációkutatás a Kárpát-medencében IX. című konferencia előadásainak és posztereinek összefoglalói". Gödöllő, Magyarország, 2012.02.24.-26. pp. 132 (ISSN 1219-9672)
- 23. Zsigmond, V., Halász, K., Török, K., Kecskés, F., Kósa, G., Rédei, T., Szilágyi, K., Peti, E. (2015): Collecting results of the Pannonian Seed Bank Project (PSBP).- In: "Genetic resources conservation scientific and social challenges" international conference, Karpacz, Lengyelország, 25. június, 2015. p. 46.
- 24. **Peti, E.**, Málnási Csizmadia, G., Oláh I., Schellenberger J., Halász K., Török K., (2015): Summary of the results of Pannon seedbank project with particular regard to the conservation of orchid species. In: Pénzes-Kónya et al. (eds.) "Planta Europa Wild Orchid Conservation Workshop", Eger, Magyarország, 2015.06. 1-6. European collaboration for native orchid conservation, pp. 13. DOI: http://dx.doi.org/10.13140/RG.2.1.1785.0961
- 25. **Peti, E.**, Szilágyi, K., Szitár, K., Málnási, Csizmadia G., Holly, L., Szmorad, F. (2012): Possible role of the Pannon Seed Bank in improving the collaboration among ex-situ, in situ and on farm conservation efforts for the maintenance of diveristy in the hungarian natural and cultivated flora. In: BIT's 1st Annual World Congress of Biodiversity. Xi'an, China, 2012.04.25.-28., Posters and papers. pp. 149.

Consultant work -submitted thesis

Horváth Adél (2016): A génbankok szerepe a spontán fajok megőrzésében Magyarországon

6. REFERENCES

- BALOCH, H.A., DITOMMASO, A., WATSON, A.K. (2001): Intrapopulation variation in Abutilon theophrasti seed mass and its relationship to seed germinability. Seed Science and Research, 11: 335-343.
- BASKIN, C.C., BASKIN, J.M. (2014): Seeds: ecology, biogeography, and evolution of dormancy and germination. 2nd edn. Elsevier, San Diego, London, Waltham. 1600 p., pp. 145-166.
- BÖLÖNI, J., MOLNÁR, ZS., KUN, A. (szerk.) (2011): Magyarország élőhelyei. A hazai vegetációtípusok leírása és határozója. ÁNÉR 2011. MTA Ökológiai és Botanikai Kutatóintézete, Vácrátót, 441 p.
- ENSCONET (2009a): ENSCONET Seed Collecting Manual for Wild Species. Royal Botanic Gardens, Kew (UK) és Universidad Politécnica de Madrid (Spain), 32 p. http:// ensconet.maich.gr/PDF/Collecting_protocol_English.pdf. Keresőprogram: Google. Kulcsszavak: seed collecting manual. Lekérdezés időpontja: 2020.10.30.
- ENSCONET (2009b): ENSCONET Curation protocols and recommendations. [ebook] European Native Seed Conservation Network (ENSCONET), Royal Botanic Gardens, Kew. 42. p.
 - https://www.luomus.fi/sites/default/files/files/curation_protocol_english.pdf. Keresőprogram: Google. Kulcsszavak: seed curation protocol. Lekérdezés időpontja: 2020.10.30.
- FAO (2013): Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rome, 166 p., pp. 31.
- GODEFROID, S., VAN DE VYVER, A., VANDERBORGHT, T. (2010): Germination capacity and viability of threatened species collections in seed banks. Biodiversity Conservation, 19: 1365–1383.
- GROOT, S. P. C., GROOT, L., DE KODDE, J., VAN TREUREN, R. (2015): Prolonging the longevity of ex situ conserved seeds by storage under anoxia. Plant Genetic Resources, 13(1): 18–26.
- GUERRANT, O., HAVENS, K., VITT, P. (2014): Sampling for effective ex situ plant conservation. International Journal of Plant Sciences, 175 (1): 11.
- HINTZE, C., HEYDEL, F., HOPPE, C., CUNZE, S., KÖNIG, A., TACKENBERG, O. (2013): D3: the Dispersal and Diaspore Database Baseline data and statistics on seed dispersal. Perspectives in Plant Ecology, Evolution and Systematics, 15 (3): 180-192.
- ISTA (2012). International Rules for Seed Testing, Bassersdorf: International Seed Testing Association.
- KIRÁLY, G. (szerk.) (2009): Új magyar füvészkönyv. Magyarország hajtásos növényei. Határozókulcsok. Ábrák. Aggteleki Nemzeti Park Igazgatóság, 616 p, 675 p.
- KIRAN BABU, P., RADHAMANI, J.(J), R., ARAVIND, J., TYAGI, R.K. (2018): (2018): Field Performance of 30-year-old Soybean Germplasm Conserved in Indian National Genebank. Plant Genetic Resources, 31 (2): 152-163.
- KISS, R., SONKOLY, J., TÖRÖK, P., TÓTHMÉRÉSZ, B., DEÁK, B., TÓTH, K., LUKÁCS, K., GODÓ, L., KELEMEN, A., MIGLÉCZ, T., RADÓCZ, SZ., TÓTH, E., BALOGH, N., VALKÓ, O. (2018): Germination capacity of 75 herbaceous species of the pannonian flora and implications for restoration. Acta Botanica Hungarica, 60: 357–368.
- KLEYER, M., BEKKER, R. M., KNEVEL, I. C., BAKKER, J. P., THOMPSON, K., SONNENSCHEIN, M., POSCHLOD, P., VAN GROENENDAEL, J. M., KLIMES,

- L., KLIMESOVÁ, J., KLOTZ, S., RUSCH, G. M., HERMY, M., ADRIAENS, D., BOEDELTJE, G., BOSSUYT, B., DANNEMANN, A., ENDELS, P., GÖTZENBERGER, L., HODGSON, J. G., JACKEL, A.-K., KÜHN, I., KUNZMANN, D., OZINGA, W. A., RÖMERMANN, C., STADLER, M., SCHLEGELMILCH, J., STEENDAM, H. J., TACKENBERG, O., WILMANN, B., CORNELISSEN, J. H. C., ERIKSSON, O., GARNIER, E., PECO, B. (2008): The LEDA Traitbase: a database of life-history traits of Northwest European flora. Journal of Ecology 96: 1266-1274. Integrant online database is available at: http://www.unioldenburg.de/en/landeco/research/projects/LEDA [accessed 25 March 2016]
- LIMA, M. DE JR., HONG, T. D., ARRUDA, Y. M. B. C., MENDES, A. M. S., ELLIS, R. H. (2014): Classification of seed storage behaviour of 67 Amazonian tree species. Seed Science and Technology, 42: 363–392.
- MÁNDY, GY. (1974): A bő termés biológiai alapjai. Mezőgazdasági Kiadó, Budapest. IN: SZABÓ, L. GY., (szerk.) (1980): A magbiológia alapjai. Akadémiai Kiadó, Budapest, 391 p., pp. 306.
- MERRITT, D., SENARATNA, T., TOUCHELL, D., DIXON, K., SIVASITHAMPARAM, K. (2003): Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity. Seed Science Research, 13(2): 155-165.
- MILBERG, P., ANDERSSON, L., ELFVERSON, C., REGNÉR, S. (1996): Germination characteristics of seeds differing in seed mass. Seed Science Research, 6: 191-197.
- PETI, E., MÁLNÁSI CSIZMADIA, G., OLÁH, I., SCHELLENBERGER, J., TÖRÖK, K., HALÁSZ, K., BAKTAY, B. (2015): Seed collecting and storing results and preliminary seed viability results and methods of Pannon Seed Bank project (2010-2014). Természetvédelmi Közlemények, 21: 215-231.
- PETI, E., SCHELLENBERGER, J., NÉMETH, G., MÁLNÁSI CSIZMADIA, G., OLÁH, I., TÖRÖK, K., CZÓBEL, SZ. ÉS BAKTAY, B. (2017): Presentation of the HUSEEDwild –a seed weight and germination database of the Pannonian flora through analysing life forms and social behaviour types. Applied Ecology and Environmental Research, 15: 225–244.
- RAO, N. K., HANSON, J., DULLOO, M. E., GHOSH, K., NOVELL, D., LARINDE, M. (2006): Manual of seed handling in genebanks. Handbooks for genebanks No. 8. Bioversity International, Rome, p. 47., pp. 20-28.
- RBGK (2016): Seed Information Database (SID) 7.1. [online database] Royal Botanic Gardens, Kew (RBGK). http://data.kew.org/sid/ Keresőprogram: Google. Kulcsszavak: seed information database. Utolsó lekérdezés időpontja: 2020.10.29.
- SIMON T. (1988): A hazai edényes flóra természetvédelmi értékének becslése. Abstracta Botanica, (12): 1–23.
- SMITH, R. D., DICKIE, J. B., LININGTON, S. H., PRITCHARD, H. W., PROBERT, R. J. (eds.) (2003): Seed Conservation: Turning Science Into Practice. Royal Botanic Gardens, Kew, UK, 1023 p. 981-992. p.
- THOMPSON, K. (1993): Seed persistence in soil. In: HENDRY, G. A. F., GRIME, J. P. (eds.): Methods in comparative plant ecology. Champman and Hall, London, 252 p., pp. 199–202.
- TÖRÖK, K., SZILÁGYI, K., HALÁSZ, K., ZSIGMOND, V., KÓSA, G., RÉDEI, T., PETI, E., SCHELLENBERGER, J., TÓTH, Z., SZITÁR, K. (2016): Seed collection

- data encompassing half of the vascular flora of the Pannonian ecoregion stored by the Pannon Seed Bank. Acta Botanica Hungarica, 58(3-4): 435-445.
- VAN TREUREN, R., DE GROOT, E. C., VAN HINTUM, T. J. L. (2013): Preservation of seed viability during 25 years of storage under standard genebank conditions. Genetic Resources and Crop Evolution, 60(4): 1407-1421.
- ZSIGMOND, V. (szerk.) (2011): Maggyűjtési Útmutató. Kézirat, 16 p. http://http://botkert.hu/sites/botkert.hu/files/PMB_Maggyujtesi-utmutato.pdf)
 Keresőprogram: Google. Kulcsszavak: maggyűjtési útmutató. Lekérdezés időpontja: 2020.10.30.

http4: http://www.pannonmagbank.hu/pmbmagyar/wp-content/uploads/2016/10/PMBmagtarolasiprotokollprogressreportbaSCH2015.pdf. Internet search engine: Google. Key words: Pannon Seedbank seed storage protocol. Date of accession: 2019.01.19.