

## GERMINATION OF *ERYSIMUM PIENINICUM* AND *ERYSIMUM ODORATUM* SEEDS AFTER VARIOUS STORAGE CONDITIONS

Péter CSONTOS<sup>1</sup>, Anna RUCINSKA<sup>2</sup>, Jerzy T. PUCHALSKI<sup>2</sup>

<sup>1</sup> Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Herman O. út 15, Budapest, H-1022, Hungary, e-mail: cspeter@rissac.hu

<sup>2</sup> Botanical Garden, Center for Biological Diversity Conservation of the Polish Academy of Sciences, Prawdziwka 2, 02-973 Warszawa 76, Poland

**Keywords:** cold treatment, germination, liquid nitrogen, seed storage, protected species, endemic species, wallflower.

### Abstract

*Erysimum pieninicum* and *E. odoratum* have similar morphology and habitat preference, however biogeography of the two species is contrasting. *E. pieninicum* is a narrow endemic, expressing a very limited distribution in SE Poland, whereas *E. odoratum* is rather frequent in Hungary and the surrounding countries. The aim of this paper is to investigate the germination behaviour of the two species and to highlight if the differences in the germination of the two species could be related with their distribution patterns.

Prior to germination tests, seeds of the two species were subjected to three different storage conditions: 1) room temperature (RT); 2) room temperature followed by 40 days at +4 °C before germination test (CT); 3) eight years storage in liquid nitrogen (LN) (for *E. pieninicum* only). After surface sterilisation of the seeds germination tests in Petri-dishes were initiated on 5th March 2009, and were terminated after 25 days.

The studied *Erysimum* species germinated very fast showing a germination pattern of “*Erysimum wittmannii* type” sensu CZARNECKA and WŁADYKA (2007). *E. odoratum* expressed a relatively low rate of total germination percentage (around 20%) that does not correspond previous reports. The very high germination rate of *E. pieninicum* (around 97% and 99%) found in RT and CT treatments, respectively, agrees with results of CZARNECKA and WŁADYKA. Cold treatment did not influence germination of the studied *Erysimum* species. In case of the LN treatment *E. pieninicum* retained a high rate of germination (81.65%).

Considering the results of the present study and also the published results of other researchers, germination behaviour of *E. pieninicum* does not seem to be a limiting factor for the distribution of this species. According to their seed biological characteristics (preserved viability in seed gene banks for long time and sudden germination without the need of dormancy breaking treatments) *E. pieninicum* and *E. odoratum* are promising candidates for nature conservation and habitat restoration projects.

### Introduction

*Erysimum* L. is a relatively species rich genus counting about 58 species in the flora of Europe (BALL 2002), of which eight species are present in Hungary (SIMON 2000) and nine (incl. 3 ephemerophytes) are found in Poland (MIREK et al. 1995). The ecological amplitude of the genus is wide, ranging from agricultural weeds like *E. repandum* Höjer to the narrow endemic *E. pieninicum* (Zapał.) Pawł. (PIEKOŚ-MIRKOWA and MIREK 2003).

According to their morphology annual herbs and partially wooded sub-shrubs are the extremities. One of the typical growth forms within *Erysimum* is the erect tall-herb biannual or perennial form. Within this group there are some closely related species of which we selected *Erysimum pieninicum* and *E. odoratum* Ehrh. Their ecology is very similar, both species prefers calcareous bedrocks, where they often grow in cracked rocky walls, fissures of cliffs or under semi-closed canopy of bush-forests on steep slopes of hilly landscapes.

In spite of their very similar morphology and habitat preference, biogeography of the two species is contrasting. *Erysimum pieninicum* is a narrow endemic, expressing a very limited distribution in SE Poland (PIEKOŚ-MIRKOWA and MIREK 2003), whereas *E. odoratum* is rather frequent in the Central Hungarian Mountain Range and the surrounding countries including also Poland (SOÓ 1968, BARINA 2009). In accordance with its rarity *E. pieninicum* received attention of researchers and was also included in genetic conservation programmes in Poland (CZARNECKA et al. 2006, CZARNECKA and WŁADYKA 2007, MACIEJEWSKA-RUTKOWSKA et al. 2007, PUCHALSKI and GAWRYS 2007), whereas *E. odoratum* was much less studied (MICHALKOVA 2000, CSONTOS and SIMKÓ 2008). Nevertheless, the reasons of the contrasting distribution success of the two species are not yet understood. Differences in their reproductive success can be considered among the potential causes. Therefore, the aim of this paper is to investigate the germination behaviour of the two species. Under this aim the following questions were posed: What are the germination characteristics of the two species? Are there any differences in the germination of the two species? Can these differences be related with the distribution patterns? How the germination capacity of *E. pieninicum* seeds is retained during long-term storage, i.e. can we rely in liquid nitrogen storage for species conservation?

### Materials and Methods

Seeds of *E. odoratum* were collected at full-ripened stage, in the Budai Mts., Hungary (47° 31' 15" N, 18° 58' 35" E, alt.: 242 m) on 7<sup>th</sup> September 2008. The sampled population was formed about 500 individuals of which seeds about 50 specimens were harvested.

Seeds of *E. pieninicum* were harvested in the Botanical Garden of the Polish Academy of Sciences at Powsin (52° 06' 26" N, 21° 05' 48" E, alt.: 120 m), on 1<sup>st</sup> September, 2008, for experiments B1 and B2, and at Czorsztyn Castle in Pieniny National Park, Poland (49° 26' 32" N, 20° 25' 45" E, alt.: 500–550 m) on 8<sup>th</sup> August, 2001, for experiment B3 (liquid nitrogen treatment). About 10 individuals were harvested in the Botanical Garden, whereas 50 individuals at the Czorsztyn Castle.

Using these seeds five different germination experiments were initiated (Table 1). Prior to germination tests all seed lots were subjected to surface sterilisation for 10 minutes in 5% NaOCl solution. After the surface sterilisation seeds were washed in distilled water.

Germination tests were initiated on 5<sup>th</sup> March 2009, and were terminated after 25 days. Germinated and non-germinated seeds were counted at 3–4 days intervals; numbers of seeds or seedlings with fungal infections were also registered.

Table 1. Basic parameters of the germination experiments of the studied *Erysimum* species

Code	Species name	Storage conditions prior to germination tests	Nr of seeds	Germination medium	Germination environment
RT-o	<i>Erysimum odoratum</i>	room temperature (RT)	288	1% agar in Petri-dishes	natural daylight regime at 22°C
CT-o	<i>Erysimum odoratum</i>	RT, then 40 days at +4 °C before germination test	225	1% agar in Petri-dishes	natural daylight regime at 22°C

Table 1, continued

RT-p	<i>Erysimum pieninicum</i>	room temperature (RT)	206	1% agar in Petri-dishes	natural daylight regime at 22°C
CT-p	<i>Erysimum pieninicum</i>	RT, then 40 days at +4 °C before germination test	196	1% agar in Petri-dishes	natural daylight regime at 22°C
LN-p	<i>Erysimum pieninicum</i>	liquid nitrogen, -193 °C for 8 years	218	1% agar in Petri-dishes	natural daylight regime at 22°C

## Results

Germination of the seed samples took place within 18 days, no further germination was observed during the last week of exposition (Table 2). Germination was especially intensive during the first few days of the experiment. By the fourth day 85.17%, 85.72% and 81.46% of the final germination was observed for treatments RT-o, CT-o and LN-p, respectively. Two samples of *E. pieninicum* (RT-p and CT-p) had to be terminated much earlier because of serious fungal infection after the fourth day of the experiment. Consequently, the ratio of 4th-day and “final-day” germination was not possible to calculate for these samples, however, their germination was almost completed by the fourth day, and the seedlings transplanted to flower pots recovered soon from fungal infection and developed into healthy young plants.

Table 2. Germination rates of the studied *Erysimum* species after various seed storage conditions  
RT= room temperature, CT= cold treatment, LN= liquid nitrogen, o= *Erysimum odoratum*,  
p= *Erysimum pieninicum*.

Species name	Treatment	Germination percentages on the inspection days (Number of days elapsed)					
		9 March (4)	12 March (7)	16 March (11)	19 March (14)	23 March (18)	30 March (25)
<i>E. odoratum</i>	RT-o	15.97	16.32	16.67	18.40	18.75	18.75
<i>E. odoratum</i>	CT-o	18.67	19.56	20.00	20.00	21.78	21.78
<i>E. pieninicum</i>	RT-p	98.54	-	-	-	-	-
<i>E. pieninicum</i>	CT-p	96.94	-	-	-	-	-
<i>E. pieninicum</i>	LN-p	66.51	67.43	68.81	73.85	81.65	81.65

Final germination rate of *E. odoratum* samples were around 20% (Table 2). Cold-treated samples (CT-o) resulted almost the same number of seedlings as the one kept at room temperature (RT-o) and also the germination speed of the two samples were very similar.

*Erysimum pieninicum* samples resulted much higher germination percentages than that of *E. odoratum* samples (Table 2). Germination rate of the one-year-old seeds was extreme high: 98.54% and 96.94% for RT-p and CT-p samples, respectively. The sample

kept in liquid nitrogen for 8 years (LN-p) produced somewhat less number of seedlings (81.65%), but - similarly to the other two *E. pinienicum* samples - it was still significantly higher than the germination rate of *E. odoratum* samples. Within the *E. pinienicum* treatments the LN-p sample showed somewhat lower initial germination rate (observed on the 4th day) than that of the other two samples.

## Discussion

The studied *Erysimum* species germinated very fast approaching their maximum values of germination strength within very short time, thus showing a germination pattern of “*Erysimum wittmannii* type” sensu CZARNECKA and WŁADYKA (2007). *E. odoratum* is newly classified into this group whereas our results for *E. pinienicum* reinforce earlier observations (CZARNECKA and WŁADYKA 2007).

Based on the sudden germination of both species and considering reports on potential persistence of their seeds in the soil (cf. CSONTOS and SIMKÓ 2008 for *E. odoratum*, and CZARNECKA and WŁADYKA 2007 for *E. pinienicum*) their soil seed banks seem to follow the disturbance broken strategy sensu GRUBB (1988).

*Erysimum odoratum* expressed a relatively low rate of germination (around 20%) that does not correspond previous reports. PÉREZ-GARCÍA et al. (2007) found 100% initial germination for this species, whereas 78–88% was reported by CSONTOS and SIMKÓ (2008). The latter study concerns the same *E. odoratum* population as used in the present study but refers to a different sampling year. Maternal effect on seed germinability and its interrelations with climatic and habitat factors are known from literature. In the present case more detailed experiments should be planned to detect the effect of climatic years on the seed production and viability of *E. odoratum*.

Cold treatment did not affect the germination rate of *E. odoratum*, and it corresponds to previous results (CSONTOS and SIMKÓ 2008).

The very high germination rate of *E. pinienicum* found in this study agrees with results of CZARNECKA and WŁADYKA (2007). Regarding the effect of cold treatment on germination – although we have only results of the 4<sup>th</sup> day – it seems that seeds of *E. pinienicum* do not require cold treatment for quick and mass germination. KARLSSON and MILBERG (2002) identified an increased germination of *E. cheiranthoides* L., an annual weed in Sweden, after cold stratification and concluded that this strategy contributes to avoid autumn germination what could be lethal for this weed considering the long Swedish winter. CSONTOS and SIMKÓ (2008) found the lack of such dormancy in *E. odoratum* as an adaptation to the mild winter on the Hungarian habitats of this species. The habitat of *E. pinienicum* in southern Poland is in between Swedish and Hungarian conditions, however its germination strategy is very close to that of *E. odoratum*. Considering the results of the present study and also the published results of other researchers, germination behavior of *E. pinienicum* does not seem to be a limiting factor for the distribution of this species.

*E. pinienicum* retained a high rate of germination (81.65%) in liquid nitrogen. For some other *Erysimum* species very long survival (38–39 years) in liquid nitrogen were documented (PÉREZ-GARCÍA et al. 2007) with varying rate of germination (*E. cheiri* (L.) Crantz, 97%; *E. odoratum*, 98%; *E. repandum*, 100% and *E. scoparium* (Brouss. ex Willd.) Wettst., 21%). As opposed to liquid nitrogen storage, seeds of *E. odoratum* were stored in

paper bags at room temperature for 6 years and it resulted very low germination rate (3.5–4.0%; CSONTOS and SIMKÓ 2008). From these records it is obvious that seed longevity of *Erysimum* species is much enhanced by liquid nitrogen storage. Without professional seed storage techniques their viability decline after few years, although a short term survival (for 1 or 2 years) is still possible under traditional storage circumstances, since CSONTOS and SIMKÓ (2008) reported 68.5% and 63.8% germination of *E. odoratum* seeds after 18 and 20 months storage at room temperature.

According to their seed biological characteristics (preserved viability in seed gene banks for long time and sudden germination without the need of dormancy breaking treatments) *E. pieninicum* and *E. odoratum* are promising candidates for nature conservation and habitat restoration projects.

#### Acknowledgements

We are grateful to Dr. ZOLTÁN BARINA (Hungarian Natural History Museum, Budapest) and Dr. ANDRÁS VOJTKÓ (Eszterházy Károly College, Eger) for discussions. Many thanks are due to Mrs. IWONA WRÓBEL (Pieniny National Park, Poland) for collecting seeds of *E. pieninicum* in 2001. This work was supported by the Hungarian Academy of Sciences and Polish Academy of Sciences within the frame of Bilateral Research Cooperation Program.

#### References

- BALL P. W. 2002: *Erysimum* L. - In: TUTIN, T. G. et al. (eds.) Flora Europaea 1., pp: 325–335.
- BARINA Z. 2009: Brassicaceae (Cruciferae) – Keresztesvirágúak családja, pp: 168–192, in: KIRÁLY G. (ed.) Új magyar fűvészkönyv. Magyarország hajtásos növényei. Határozókulcsok. [New Hungarian Herbal. The Vascular Plants of Hungary. Identification key.] Aggteleki Nemzeti Park Igazgatóság, Jósvalfő. (in Hungarian)
- CSONTOS P., SIMKÓ H. 2008: A magyar repcsény (*Erysimum odoratum* Ehrh.) csírázásbiológiájának vizsgálata. Tájékológiai Lapok–Journal of Landscape Ecology 6(3): 247–253. (in Hungarian)
- CZARNECKA B., WLADYKA M. 2007: Ecological meaning of seed size and shape for seed persistence and germinability in some mountain plants from the collection of the Botanical Garden in Lublin. Bulletin of Botanical Gardens, Museums & Collections 16: 3–10.
- CZARNECKA B., WLADYKA M., FRANSZCZAK-BYĆ M. 2006: Flowering phenology of some mountain plants in the collection of the Botanical Garden in Lublin. Bulletin of Botanical Gardens, Museums & Collections 15: 79–86.
- GRUBB P. J. 1988: The uncoupling of disturbance and recruitment, two kinds of seed bank, and persistence of plant populations at the regional and local scales. Ann. Zool. Fennici 25: 23–36.
- KARLSSON L. M., MILBERG P. 2002: Stratification responses in the late-germinating summer annual weed *Erysimum cheiranthoides*. Journal of Applied Botany–Angewandte Botanik 76(5–6): 172–175.
- MACIEJEWSKA-RUTKOWSKA I., BEDNORZ L., FUJIKI T. 2007: SEM observations of pollen grains, fruits and seeds of the Pieniny Mountains (South Poland) endemic species *Erysimum pieninicum* (Zapal.) Pawl. (Brassicaceae). Acta Soc. Bot. Pol. 76(2): 127–132.
- MICHALKOVA E. 2000: Chromosome numbers of *Erysimum odoratum* (Brassicaceae) in Slovakia. Biologia 55(4): 381–385.
- MIREK Z., PIEKOŚ-MIRKOWA H., ZAJAC A., ZAJAC M. 1995: Vascular plants of Poland: a checklist. (Polish botanical studies, guidebook series; no. 15). W. Szafer Institute of Botany, Polish Academy of Sciences, Krakow, 303pp. (1 computer disk – ISBN 8385444386)
- PÉREZ-GARCÍA F., GONZÁLEZ-BENITO M. E., GÓMEZ-CAMPO C. 2007: High viability recorded in ultra-dry seeds of 37 species of Brassicaceae after almost 40 years of storage. Seed Science and Technology 35(1): 143–153.
- PIEKOŚ-MIRKOWA H., MIREK Z. 2003: Endemic taxa of vascular plants in the Polish Carpathians. Acta Soc. Bot. Pol. 72(3): 235–242.

- PUCHALSKI J. T., GAWRYS W. 2007: Collection of protected law and endangered plants and species protected by Bern Convention in Polish botanical gardens. Bulletin of Botanical Gardens, Museums & Collections 16: 47–184. (in Polish)
- SIMON T. 2000: A magyarországi edényes flóra határozója. Harasztok - virágos növények (4., átdolgozott kiadás). Nemzeti Tankönyvkiadó, Budapest, 976 pp. (in Hungarian)
- Soó R. 1968: Synopsis Systematico-Geobotanica Florae Vegetationisque Hungariae III. Akadémiai Kiadó, Budapest, 506+51 pp. (in Hungarian)

AZ *ERYSIMUM PIENINICUM* ÉS AZ *ERYSIMUM ODORATUM* MAGJAINAK  
CSÍRÁZÓKÉPESSÉGE KÜLÖNBÖZŐ TÁROLÁSI VISZONYOKAT KÖVETŐEN

CSONTOS P.<sup>1</sup>, A. RUCINSKA<sup>2</sup> és J. T. PUCHALSKI<sup>2</sup>

<sup>1</sup> Magyar Tudományos Akadémia Talajtani és Agrokémiai Kutatóintézete,  
Budapest, Herman O. út 15, H-1022, E-mail: cspeter@rissac.hu

<sup>2</sup> Botanical Garden, Center for Biological Diversity Conservation of the Polish Academy of Sciences,  
Prawdziwka 2, 02-973 Warszawa 76, Poland

**Kulcsszavak:** bennszülött faj, csírázás, folyékony nitrogén, hidegkezelés, magok tárolása, repcsény, védett faj.

**Összefoglalás:** Az *Erysimum pieninicum* és az *E. odoratum* alaktanilag, rendszertanilag és élőhely-preferenciáikat tekintve is egymáshoz közelálló fajok, ugyanakkor biogeográfiai elterjedésük jelentősen különböző. Az *E. pieninicum* egy nagyon szűk elterjedésű endemikus faj, amely csak Lengyelország DK-i részén fordul elő, míg az *E. odoratum* nem különösebben ritka Magyarországon és több környező országból is ismerjük előfordulásait. Jelen dolgozatunk célja a két faj csírázási tulajdonságainak vizsgálata volt, valamint annak elemzése, hogy a csírázásukban mutatkozó különbségek kapcsolatba hozhatók-e az elterjedésükben megfigyelt különbséggel.

A csíráztatásokat megelőzően a magtétéleket háromféle kezelésben részesítettük: 1) tárolás szobahőmérsékleten (RT); 2) tárolás szobahőmérsékleten, majd 40 napos hidegkezelés +4 °C-on közvetlenül a csíráztatást megelőzően (CT); 3) nyolc év tárolás folyékony nitrogénben (LN) (csak az *E. pieninicum* esetében). Ezután a magok felületét sterilizáltuk, majd azokat 1%-os agarral töltött Petri-csészékben csíráztattuk 25 napon át, 22 °C-os állandó hőmérsékleten, természetes megvilágítás mellett.

Mindkét *Erysimum* faj nagyon gyors csírázásának bizonyult és megfigyeléseink alapján CZARNECKA és WŁADYKA (2007) rendszerében az “*Erysimum wittmannii*-típusú” csírázási csoportba sorolhatóak. Az *E. odoratum* minták a maximális csírázási érték tekintetében viszonylag alacsony (20% körüli) eredményt értek el, ami elmarad a korábban publikált irodalmi adatoktól. Az *E. pieninicum* igen magas csírázási eredményei (mintegy 97% és 99% az RT ill. CT kezeléseket esetén) megfelelnek a CZARNECKA és WŁADYKA által közölt eredményeknek. A hidegkezelés (CT) a vizsgált fajok csírázási eredményeit nem befolyásolta. A folyékony nitrogénben tárolt *E. pieninicum* magok jól megőrizték életképességüket, és nyolc év után 81,65 százalékos eredménnyel csíráztak.

Tekintetbe véve a jelen dolgozatban közölt eredményeket, valamint más szerzők által publikált adatokat, az *E. pieninicum* csírázási viselkedése vélhetően érdemben nem hozható összefüggésbe a faj szűk földrajzi elterjedtségével. Az *E. pieninicum* és az *E. odoratum* magbiológiai tulajdonságai (génbanki viszonyok között a magok hosszan megőrződő életképessége, valamint a gyors, hidegkezelést sem igénylő csírázása) arra utalnak, hogy mindkét faj jó eséllyel sikeresen bevonható természetvédelmi és élőhely-restaurációs programokba.