DENDROBIOLOGY
2004, vol. 52, 33–37

Hanna Malinowska, Magdalena Janyszek, Marzena Szczepaniak

Propagation of leather leaf *Chamaedaphne calyculata* (L.) Moench from seeds and shoot cuttings

Received: 7 October 2004, Accepted: 25 October 2004

Abstract: Flowering and fruit setting was analyzed in specimens of *Chamedaphne calyculata* (L.) Moench growing in a natural stand in the "Sicienko" reserve in the Drawa National Park. Seed production, seed viability and shoot rooting was investigated.
It was shown that *Chamaedaphne calyculata* has abundant flowers but sets few fruits. Numerous seeds (even up to 87) were found in fruits; however, the percentage of developed seeds was low and ranged from 17% to 45%. The viability of developed seeds was similarly low (maximum 29%). The performed germination test showed a positive effect of stratification on the breaking of seed dormancy. Obtaining seedlings from seeds sown *in vitro* on agar medium was a considerable success.
Shoots cut perpendicularly to the shoot axis, with a 1-cm incision on the side and treated with a rooting agent, rooted 100%. Shoots which were not treated with a rooting agent, irrespective of their having been incised or not, rooted 78%.

Additional key words: flowering, seed production, germination, rooting, seedling

Addresses: H. Malinowska, M. Szczepaniak, Department of Seed Science, Technology and Horticultural Nursery, August Cieszkowski Agricultural University of Poznań,
Szamotulska 28, 62-081 Baranowo, Poland, e-mail: hamal@owl.au.poznan.pl.
M. Janyszek, Department of Botany, August Cieszkowski Agricultural University of Poznań,
Wojska Polskiego 71C, 60-625 Poznań, Poland, e-mail: carexmag@owl.au.poznan.pl.

Introduction

Leather leaf *Chamaedaphne calyculata* (L.) Moench is one of the rarest vascular plant species in Poland. It is a wintergreen dwarf shrub of the *Ericaceae* family (Fig. 1), growing on peat bogs. This species was found primarily in Siberia and in the northern part of North America. In Europe the range of distribution covers northern Scandinavia, while farther to the south this taxon is found only in individual stands and only in the nearest vicinity of the largest glaciation limit (Kulczyński 1923). In Poland 13 stands of *Chamaedaphne calyculata* were found, but only 7 have been confirmed in recent years (Zając and Zając 2001). The Polish stand of this species extending farthest to the west of Poland is the "Sicienko" (53°11' N; 16°01' E) reserve found in the Drawa National Park (Kujawa-Pawlaczyk 1996).

Leather leaf is a species under full legal protection. The limited number of existing stands makes this taxon considerably threatened by extinction as a result of accidental destruction of biocenoses, in which it is found. For this reason it is of paramount importance to determine the possibility of propagating this species for the purpose of potential metaplantation or *ex situ* protection.

Fig. 1. Flowering plants of *Chamaedaphne calyculata* (L.) Moench in the nature reserve "Sicienko" (Phot. H. Malinowska)

The aim of this study was to determine the characteristic of flowering and fruiting and seed production of leather leaf in a natural stand and to investigate the possibility of propagating this species from seeds and from apical shoot seedlings under cultivated conditions.

Material and methods

Observations of self-sowing in leather leaf under natural conditions were conducted on a peat bog with the area of 2.11 ha at the "Sicienko" nature reserve in the Drawa National Park. Measurements were taken on four, randomly selected, 100 m^2 square plots.

In March a total of 30 plants were selected at random for observations from each plot. In order to determine the percentage of developed flowers, flowers and flower buds were counted separately in one plot. Due to the considerable time span of the observations, flower buds and developed flowers were calculated jointly on the other three plots.

The observation of fruits was performed when they were still green and closed. In order to determine the number of seeds per fruit a total of 10 well-developed ripe closed fruits were selected from each plot. Seeds were collected in October. After collection they were counted and classified into two groups: undeveloped and developed. Among the developed seeds convex and flat seeds were distinguished. Only developed seeds were taken to the experiment.

Seed viability was investigated with the use of the tetrazolium test, using the phenomenon of the reaction of 2,3,5-triphenyltetrazolium chloride (or hydrochloride) under the influence of active dehydrogenases contained in living seed tissues. As a result of the reaction 2,3,5-triphenylformazone hydrochloride is formed and the colour of the tissue containing active enzymes changes to red. Developed seeds were used for the tetrazolium test. Whole seeds were macerated in water for 18 hours. Next they were one-sided incised so as not to damage the germ and placed in a tetrazolium solution for 18–24 h at the temperature of 30°C. After that, they were washed in water and cut along the symmetry plane to see the germ color. Stained germ and endosperm indicated viable seeds.

In case of seed germination tests, in order to break seed dormancy, they were subjected to stratification (on wet blotting paper at 3.5°C for 20 days) or potassium nitrate treatment. Only convex seeds were used in the experiment. Three samples of 200 seeds each were divided into batches of 50 in four replications. Seeds on Petri dishes and wet blotting paper were placed in germination apparatus at the temperature of 24–26°C, using a 12-hour lighting regime. The control sample (not stratified) was sown on blotting paper moistened with distilled water. The pre-cooled sample was kept in a refrigerator at the temperature of 3.5°C for 20 days, after which it was transferred to a germination apparatus. Blotting paper was moistened with distilled water. The sample treated with potassium nitrate was placed in germination apparatuses on blotting paper soaked with 0.2% KNO_3 solution. The experiment was conducted in accordance with the recommendations of Ellis et al. (1985) for *Ericaceae* family species. Observation time was 10 days.

Rooting of shoots was investigated using 64 seedlings, collected in the third decade of August. Seedlings were cut under the node, perpendicularly to the shoot axis. Some shoots were incised on the side for 1 cm, exposing the cambium. The experiment included three combinations: shoots without the incision, incised shoots and incised shoots treated with a rooting agent A (P.P.H. Himal Łódź). The first combination consisted of two, whereas the second and third – of three replications. A replication included 8 shoots. An average shoot was 7–8 cm long. The substratum was a mixture of peat and sand in a 2:1 ratio, pH 4.7. Shoots were planted in such medium in plastic boxes. Rooting was conducted in an unheated greenhouse under an additional foil covering with the height of 60 cm. They were sprayed throughout the whole rooting period. During the rooting period the substratum was not watered. Seedlings wintered in an unheated greenhouse under foil covering. Eight months after planting the number of rooted shoots, the length of the root system, the length of growth since the planting and the number of new branches were observed.

A total of 60 developed – convex seeds were used to obtain seedlings. Seeds were disinfected with 10% Clorox solution, by soaking for 15 minutes. After the stratification, they were placed on agar medium in small flasks, 5 seeds in each. The experiment was prepared according to Anderson (1980).

For the collected numerical data, the variance analysis was conducted and the results were tested by using Duncan-test.

Results

Observations on flowering of *Chamaedaphne calyculata* showed abundant blooming. The number of flowers on a single specimen ranged from 1 to 128. On average there were from 14 to 26 flowers per plant (Table 1). However, it turned out that the number of fruits set from these flowers was very small. On average, depending on the plot, there were from 0.3 to 3.2 fruits/plant, and the number of fruits on one plant ranged from 0 to 29 (Table 2).

Significant differences were observed in seeds number in fruits on individual plots. On average, depending on the plot, there were from 55 to 75 seeds per fruit. In order to conduct observations on the quality of formed seeds, they were divided into two groups. The first was composed of developed seeds, among which convex seeds, i.e. those with a protuberance where the endosperm and germ were forming, and flat seeds were distinguished. The other group consisted of undeveloped seeds, which were much smaller than the developed ones. A significant difference was observed between the plots in the number of convex seeds. The number of such seeds per fruit ranged – depending on the plot – from 0–6 to 2–14 (Table 3). Statistical computations for the number of flat seeds did not show significant differences.

Both convex and flat seeds were viable (Table 4). Observed differences in viability for both types of seeds were not statistically significant.

Differences were observed in the germination of seeds subjected to different environmental conditions. Stratified seeds germinated on average in 40%, whereas seeds subjected to KNO_3 action on average in 17% (Table 5). However, statistical analysis did not show significant differences in this respect.

Rooting of leather leaf shoots varied in results, depending on the method of shoot preparation. Only 6% of control seedlings rooted (1 seedling out of 16 being rooted). Seedlings incised laterally with the application of a rooting agent rooted in 100%, while those with no rooting agent applied on average rooted

Table 1. Characteristics of flowering of *Chamaedaphne calyculata* (L.) Moench; 30 of plants were observed on each plot

No. of plot	Total number of flower buds on the observed plants	Minimal and maximal number of flower buds on single plant	Average number of flower buds on single plant
1	770	3–71	25.6
2	464	1–70	15.5
3	512	1–63	17.1
4	434	1–128	14.5

Table 2. Characteristics of fruit forming by *Chamaedaphne calyculata* (L.) Moench; 30 of plants were observed on each plot

No. of plot	Percentage of the formed fruits	Total number of formed fruits	Minimal and maximal number of formed fruits on single plant	Average number of formed fruits on single plant
1	12.5	96	0–13	3.2
2	2.2	10	0–3	0.3
3	14.1	72	0–29	2.4
4	7.9	34	0–29	1.0

Table 3. Number of developed convex and flat seeds in the fruits of *Chamaedaphne calyculata* (L.) Moench; 10 fruits were observed on each plot

No. of plot	Minimal and maximal number of seeds in single fruit	Average number of seeds in fruits	Minimal and maximal number of:		Average number of:		Percentage [%] of:	
			convex seeds	flat seeds	convex seeds	flat seeds	convex seeds	flat seeds
1	64–78	69.8	0–11	5–32	4.8	12.7	7	18
2	19–64	54.8	2–14	0–22	5.3	13.0	10	36
3	67–87	75.2	2–9	1–15	4.5	7.6	6	11
4	34–78	64.1	0–6	4–22	1.4	11.2	2	17

Table 4. Seed viability of *Chamaedaphne calyculata* (L.) Moench according to the tetrazolium test (TTC); four fruits were studied for each plot

No. of plot	Convex seeds		Flat seeds	
	Number of seeds	TTC [%]	Number of seeds	TTC [%]
1	20	29	48	8
2	30	9	57	4
3	26	27	41	12
4	4	25	40	4
Average	20	22.5	47	7

Table 5. Germination of seeds of *Chamaedaphne calyculata* (L.) Moench.

Environmental conditions	Germination in %				
	Replication I	Replication II	Replication III	Replication IV	Average
Control sample	16	44	16	0	19
Subjected to KNO_3	28	22	18	0	17
Stratified seeds	48	38	8	68	40

Table 6. The impact of rooting agent on the rooting percentage of shoots of *Chamaedaphne calyculata* (L.) Moench

Rooting of shoots					
Without rooting agent [%]			With rooting agent [%]		
Replication I	Replication II	Replication III	Replication I	Replication II	Replication III
87	75	50	100	100	100
Average percentage of rooted: 78			Average percentage of rooted: 100		

Table 7. The impact of rooting agent on the growth of seedlings of *Chamaedaphne calyculata* (L.) Moench

Rooting agent	Average length of root system [cm]	Average length of previous year growth [cm]	Average number of previous year growths
–	3.14	0.7	0.2
+	9.52	0.81	0.2

in 78% (Table 6). Observed differences were statistically significant.

The rooting agent had a significant effect on the length of the root system in seedlings. With the application of the preparation the root system was on average 9.5 cm long, whereas without it – 3.1 cm long (Table 7). The rooting agent did not have a significant effect on the number and total length of previous year growths in seedlings.

The attempt to obtain seedlings *in vitro* from seeds collected from a natural stand was successful. A total of 50% seeds germinated after 10 days, and after the next 10 days seedlings were already approx. 1 cm long.

Discussion and conclusions

Observations showed that leather leaf flowers abundantly but does not set many fruits. It is consistent with the conclusion by Tylżanowski (1975), who found the same phenomenon at a stand in the Bory Tucholskie Forests. It was also observed that on the plot, which received the least light, the smallest number of flowers was found. These observations confirmed the opinion of Kloss (1992) that open space with considerable access to light resulted in leather leaf flowering more abundantly than in shaded positions.

Laboratory investigations showed that many seeds were found in fruits. However, the percentage of developed seeds was very small and the probability of seedling formation was also lowered by low seed viability.

Laboratory seed germination tests using stratification and KNO_3 treatment made it possible to observe that a higher seed germination index was found for stratified seeds. Aparicio (1995), while investigating another *Ericaceae* family species – *Erica andevalensis,* found that quantitative germination indexes depended on the fact whether seeds were subjected to stratification. This opinion was confirmed in this study.

Peat bog, where leather leaf grows, is characterized by low nutrient content (Podbielkowski 1975). High moisture content and strong moss growth are conducive to the rooting of recumbent leather leaf shoots (Stecki 1954, Kobendza 1966). However, such conditions are unfavourable for seeds, which to germinate require not only moisture but also light. Light is one of the factors interrupting seed dormancy (Ellis et al. 1985). In spite of that, assuming that seeds would germinate under unfavourable conditions and seedlings were formed, the probability of their winter surviving would be small, since seedlings are susceptible to frost and need to be protected against it (Bartels 1982, Hrynkiewicz-Sudnik et al. 1990). Seedlings with the application of a rooting agent rooted considerably better than those not treated with this type of preparation.

Seedling incision considerably increased the number of rooted seedlings. It turned out that the third decade of August is a good time for rooting. It is consistent with the recommendations by Krüssmann (1954) and Hrynkiewicz-Sudnik et al. (1990).

In this study it was attempted to obtain seedlings from seeds sown on agar medium. Seeds germinated and already after 10 days seedlings were approx. 1 cm long. The production of seedlings is a considerable success, as Ferchmin (1992) claimed that it had never been done in Poland before. Thus, it is the first written successful attempt to produce *Chamaedaphne calyculata* seedlings in Poland.

References

Anderson W.C. 1980. Tissue culture propagation of red and black raspberries, *Rubus idaeus* and *R. occidentalis*. Acta Horticulturae, 112: 13–20.

Aparicio A. 1995. Seed germination of *Erica andevalensis* Cabezudo and Rivera (*Ericaceae*), an endangered edaphic endemic in southwestern Spain. Seed Sciences & Technology, 23: 705–713.

Bartels A. 1982. Rozmnażanie drzew i krzewów ozdobnych. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa.

Ellis R.H., Hong T.D., Roberts E.H. 1985. Handbook of seed technology for gene banks. Vol II. Compendium of specific germination information and test recommendations. Depart. of Agricult. and Hort., University of Reading, UK. Intemational Board for Plant Genetic Resources, Rome.

Ferchmin M. 1992. Rozmarynek. Puszcza Kampinoska. Wydawnictwo Kampinoskiego Parku Narodowego.

Hrynkiewicz-Sudnik J., Sękowski B., Wilczkiewicz N. 1990. Rozmnażanie drzew i krzewów liściastych. Państwowe Wydawnictwo Naukowe, Warszawa.

Kloss M. 1992. Chamedafne północna w Puszczy Kampinoskiej. Las Polski 11: 11.

Kobendza J. 1966. Puszcza Kampinoska. Wiedza Powszechna, Warszawa.

Krüssmann G.1954: Die Baumschule. Paul Parey in Berlin und Hamburg.

Kujawa-Pawlaczyk J. 1996. Roślinność rezerwatu „Sicienko" na terenie Drawieńskiego Parku Narodowego. Mscr. of Ph.D. thesis. Department of Plant Taxonomy. Adam Mickiewicz University, Poznań.

Kulczyński S. 1923. Borealny i arktyczno-górski element we florze Europy Środkowej. Polska Akademia Umiejętności, Kraków.

Podbielkowski Z. 1975. Roślinność kuli ziemskiej. Wydawnictwo szkolne i Pedagogiczne, Warszawa.

Stecki K. 1954. Modrzewnica północna – *Andromeda* (*Chamaedaphne*) *calyculata* (L.) Moench. Chrońmy Przyrodę Ojczystą 1: 25–33.

Tylżanowski T. 1975. Chamedafne północna – *Chamaedaphne calyculata* w Borach Tucholskich. Chrońmy Przyrodę Ojczystą 31: 51–52.

Zając A., Zając M. 2001. Distribution atlas of vascular plants in Poland. Institute of Botany, Polish Academy of Sciences, Kraków.