Ex situ Conservation of European Threatened Plants in Western Crete, Greece (CRETAPLANT project, EU-LIFE)

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Introduction

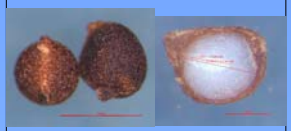
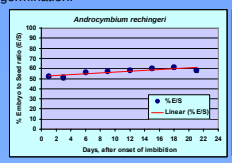
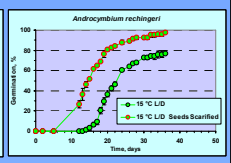
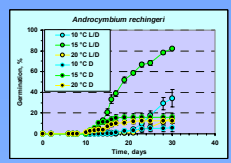
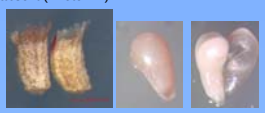
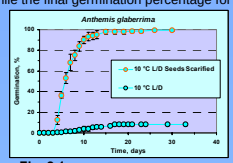
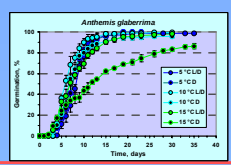
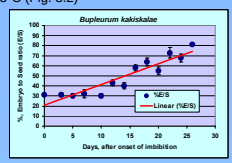
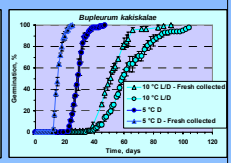
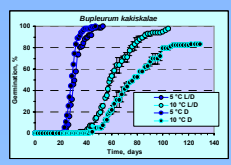
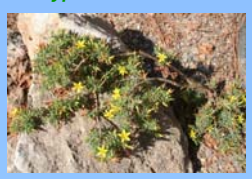
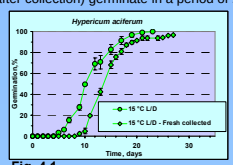
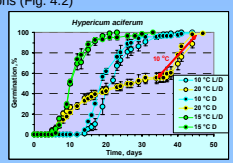
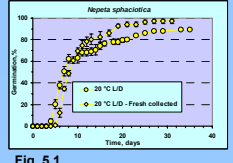
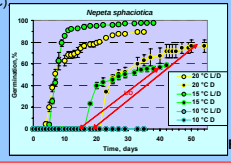
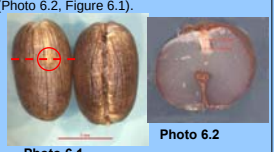
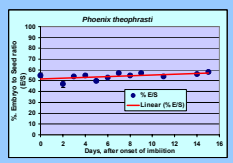
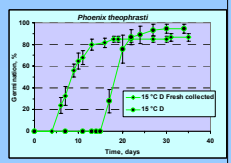
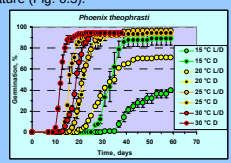
Through the LIFE–Nature Programme, the European Union finances activities for the protection and conservation of plants and habitats of Community importance ('Habitat Directive' 92/43 EEC). The main objective of the LIFE-Nature project 'CRETAPLANT - A Pilot network of Plant Microreserves in Western Crete' is the in situ conservation of 6 threatened plants namely: 1. * *Androcymbium rechingeri* Greuter, 2. * *Anthemis glaberrima* (Rech. f.) Greuter, 3. * *Bupleurum kakiskalae* Greuter, 4. * *Cephalanthera cucullata* Boiss. & Heldr., 5. * *Hypericum aciferum* (Greuter) N.K.B. Robson, 6. * *Nepeta sphaciotica* P. H. Davis.and 1 priority habitat type 9370, * Palm groves of *Phoenix* through the establishment of a network of microreserves (conservation areas of small extent, less than 20 ha each).

The goal of the present work (Action D2 of CRETAPLANT – Ex-situ conservation) is to complement in situ conservation by (a) collecting and storing a significant number of seedlots of priority plants in the Seed Bank of MAICh, (b) preparing protocols of seed handling, storage and germination and (c) elaborating techniques for growing seedlings and outplanting in the field (both in botanical gardens and plant microreserves).

Methods

Seeds of most plant species were collected during the years 2005-2006. Seeds were collected from the natural populations of the above native *taxa*. Special care was given to the collection of the rare and threatened species in order to preserve the survival of their natural populations. Most of the germination experiments were done after one year of storage in the drying room (temperature 15-20 ºC - 15 % R.H.). The seed storage behaviour was studied after the storage of the seeds in the cold room (-20 ºC) for more than 3 months (Hong and Ellis, 1996). The embryo morphology was based on the revised Martin's classification system (Baskin & Baskin, 2007). For germination experiments seeds were sown on agar gel and incubated in growth chambers with temperature and light control, at various constant temperature (5, 10, 15 & 20 ºC) and under a daily photoperiod (12/12 h, light/dark). For germination experiments in darkness, seeds were incubated within light-proof, metal containers in the same cabinets, and the seeds were counted in a dark room with green safelight.

Results

Species	Seed and Embryo morphology	Seed Storage Behaviour	Dormancy	Germination
1. *Androcymbium rechingeri* 	Seeds subspherical, granulate (Photo1.1) Embryo central axile, linear **fully developed (E/S > 0.5)**, in hard endosperm (Photo 1.2, Figure 1.1)  Photo 1.1 Photo 1.2	Orthodox	*Androcymbium rechingeri* seems to have a kind of mechanical dormancy. Higher values of final germination percentage and rate of germination have been recorded for scarified seeds (by abrasion of seed coat with sandpaper) than for intact seeds (Fig. 1.2). Warm stratification has not any effect on germination.  Fig. 1.1  Fig. 1.2	The scarified seeds of *Androcymbium rechingeri* germinate within a narrow range of cool temperatures (10-15 °C). The germination is promoted by light (Fig. 1.3).  Fig. 1.3
2.Anthemis glaberrima 	Achenes 1-1,25 mm (excluding corona), absolutely ribbed, cylindrical turbinate; corona up 0,5 mm (Photo 2.1). Embryo **spatulate fully developed ,** endosperm is absent (Photo 2.2).  Photo 2.1 Photo 2.2 Photo 2.3	Orthodox	*Anthemis glaberrima* seems to have a kind of mechanical dormancy and germinates under laboratory conditions after the removal of the seed covering structures, while the final germination percentage for "intact" seeds is very low (Fig. 2.1)  Fig. 2.1	After the removal of seed covering structures, *Anthemis glaberrima* germinates under cool temperatures (5-15°C), irrespectively of light conditions (Fig. 2.2).  Fig. 2.2
3.Bupleurum kakiskalae 	Mericarps are elliptical to oblongate, (3) 4 – 5 (6) mm, plane to ± concave on inner face (Photo 3.1). The seeds have an oily endosperm and a small, **underdeveloped, linear embryo** (E/S < 0.5)(Photo 3.2).  Photo 3.1 Photo 3.2	Orthodox	*Bupleurum kakiskalae* has morphological dormancy and embryo growth is required before germination occurs. Growth of underdeveloped embryos takes place at low temperatures and the Embryo/Seed (E/S) ratio increases from ~0.3 to 0.8 before germination (Fig. 3.1). "Fresh" collected mature seeds (1 to 2 days after collection) germinate within a period of 20 days at 5ºC (Fig. 3.2)  Fig. 3.1  Fig. 3.2	*Bupleurum kakiskalae* germinates only at low temperatures (5ºC and 10ºC). The germination is promoted by light at 10 °C (Fig. 3.3). Cold stratification has not any effect on germination at temperatures 15°C and 20°C.  Fig. 3.3
4.Hypericum aciferum 	Seeds 1.5 -1.7 mm long, elongate, subcylindrical, slightly carinate, with a fleshy caruncle (Photo 4.1) Embryo **linear fully developed**, endosperm absent (Photo 4.2)  Photo 4.1 Photo 4.2 Photo 4.3	Orthodox	*Hypericum aciferum* seeds are non-dormant. "Fresh" collected mature seeds (1 to 2 days after collection) germinate in a period of 20 days at 15ºC(Fig. 4.1)  Fig. 4.1	*Hypericum aciferum* seeds germinate under cool temperatures, with optimum at 15°C, irrespectively of light conditions (Fig. 4.2)  Fig. 4.2
5.Nepeta sphaciotica 	Nutlets oblong-ovoid, rugose 2.0-2.3 x 1.0-1.2mm, tuberculate (Photo 5.1) Embryo **investing** (Photo 5.2 & 5.3)  Photo 5.1 Photo 5.2 Photo 5.3	Orthodox	The final percentage of germination was more than 90% of "fresh" collected mature seeds of *Nepeta sphaciotica* in a 20 days-period, at 20ºC(Fig. 5.1)  Fig. 5.1	Germination of *Nepeta sphaciotica* (an alpine plant at a habitat covered by snow for 6-7 months) is manifested only in the light and within a narrow temperature range (15-20ºC).  Fig. 5.2
6.Phoenix theophrasti 	Seeds with rounded apices, 11-13 X 6-7 mm, a small depression in the testa marks the exact position of embryo (Photo 6.1). Embryo is in lateral position, **linear fully developed** (E/S >0.5), endosperm homogenous hard (Photo 6.2, Figure 6.1).  Photo 6.1 Photo 6.2	Orthodox	*Phoenix theophrasti* seeds are non-dormant. The germination rate of "fresh" collected mature seeds was higher than that of dry seeds (Fig. 6.2)  Fig. 6.1  Fig. 6.2	*Phoenix theophrasti* seeds require relatively high temperature for germination (20-30ºC). The germination rate is lower under light conditions, compared with the rate observed under dark conditions, at the same temperature (Fig. 6.3).  Fig. 6.3

References

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Bascin C.C.and Baskin, J.M (2007) A revision of Martin's classification system, with particular reference to his dwarf-seed type. Seed Science Research 10: 25-35.

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