

# Threatened Valencian Flora:

initiatives for its conservation



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#### Summary

The application of different plant conservation strategies is demanded by the richness of the Valencian flora and by the growing human pressure in this region. The University of Valencia, in collaboration with the local government, assumes an important role in the conservation of endangered flora. The local government has built a network of plant microreserves, and the University of Valencia supports a germplasm bank of rare, endemic and threatened species in the Botanical Garden. The first priority was seed and spore collection and storage, but today the optimisation of the infrastructure has made possible the development of new research lines. Studies to find the best germination conditions for seeds of the stored species are now being carried out. The relation between germination and seed coat structure is studied in collaboration with the anatomy laboratory and the genetic diversity of stored samples is analysed in our molecular biology laboratory.

# Introduction

The Valencian Community covers 2,325,874 ha (4.62% of Spain). The richness of the flora in this territory is closely related to its geographical situation and the high diversity of environments. The altitude ranges from 0 to 1,839 m, the annual mean temperature from 9.0–19.6°C, the annual mean rainfall from 178 to 956 mm (Figure 46.1); there is also a great diversity of soils, types of rocks, bioclimate and orography. The biodiversity is complex, from semi-arid areas with scarce vegetation to humid localities with fragments of Euro-Siberian forests. In addition, influences of different neighbouring territories have an impact on the present composition of our flora.

The Valencian flora includes 3,048 taxa down to the subspecific level (Mateo and Crespo, 1998), 350 of which are endemic (VV.AA., 1998). Knowledge of these taxa is continuously updated with the fieldwork that many botanists carry out in the territory. A catalogue of rare, endemic and threatened species has already been published (VV.AA., 1998). This catalogue, as well as many other publications on the Valencian flora, provides valuable information on the status of natural populations, thereby facilitating more effective species management.

The Mediterranean coast is the only European area with a very high desertification risk according to the World Map of Desertification (FAO, UNEP, UNESCO and WMO, 1977). Widespread deforestation, erosion and soil degradation are bringing about rapid desertification within the Valencian territory. This involves the progressive loss of the natural



Figure 46.1

Geographical location and climatic characteristics of the Valencian Community.

patrimony and agro-forestry production systems. The composition of the flora and the growing human pressures require the implementation of an integrated conservation strategy.

The growing loss of plant diversity in wild habitats has stimulated many botanical gardens to become important conservation centres. Thousands of species are cultivated in botanical gardens; probably more than a quarter of the vascular plants from all over the world are included in their collections (Wyse Jackson, 1999). The botanical gardens have evolved with man's requirements. Nowadays they provide an important tool for environmental education and have become an instrument for *ex situ* conservation of plant species. Botanical gardens play an important role in the conservation of plant species at the global level. Heywood (1992) described the general priorities regarding plant conservation that are contemplated in the Botanical Gardens Conservation Strategy. In principle, the conservation of the endemic flora, especially of the rare or endangered species, should have priority.

# **Conservation Measures**

Legislation, research and education are the basis for the protection of natural habitats in our society. Several institutions are collaborating on both *in situ* and *ex situ* strategies. The Species Conservation Service of the Regional Government (Generalitat Valenciana, Consellería de Medi Ambient) keeps track of the research institutions that are involved in the study of existent biodiversity and the execution of the programmes proposed.

The University of Valencia, through a framework agreement with "Generalitat Valenciana - Consellería de Medi Ambient" (see Box 46.1), has established both *in situ* and *ex situ* measures for the conservation of endangered taxa.



The Botanical Garden of the University is particularly active in the *ex situ* conservation of the flora. Besides its long experience in the study, teaching and conservation of plant species (which dates back to 1802 when the Garden was created), it has overseen, in the last decade, the development of a germplasm bank of wild flora, one of its key conservation projects.

The Valencian Regional Government has introduced a new type of protected area, the floristic micro-reserve (Laguna, 1999; 2001; Laguna *et al.* 2001). These reserves represent an excellent measure for the *in situ* conservation of the vegetation that occurs in small areas of natural habitats that are home to a large number of plant species of high scientific interest. The micro-reserves ensure the protection of more species diversity than other types of protected spaces of greater extent.



Presently, there are 150 micro-reserves extending over 680 ha, protecting 60% of the 350 endemic plants of the Valencian Community. Eighty endemic taxa are encompassed by the CR and EN categories (see Box 46.2) of the IUCN (VV. AA., 1998), 32 of which are enclosed in the micro-reserves. This project is 50% co-financed by the European Community through its Life Programme. Using these micro-reserves, it should be possible to ensure the protection of all threatened taxa of the Valencian Community flora.

The selection of micro-reserves is based on the results of biodiversity studies carried out by specialists at the University and other research institutions. Following designation, research and conservation activities like seed and spore collection are concentrated on these.

The germplasm bank is a base collection for long-term storage of wild Valencian flora, and runs two different conservation programmes, one for seeds and one for Pteridophyte (fern) spores.

### 1. The Seed Bank

The seed collection programme is restricted to the Valencian Community. Rare, endemic and threatened plants are given priority. The conservation of the genetic diversity from throughout the species range within the territory is intended. In some cases, the seeds conserved in the seed bank could be used to compensate natural or man-made disasters, which may occur in the natural reserves. Presently, 1,167 accessions are stored in the seed bank of the Botanical Garden of Valencia. The seed collection is planned to conserve as much genetic diversity as possible, so few seeds from many plants are sampled from each locality. In this way, the natural regeneration capacity of the populations is not harmed. Hawkes (1992) states that ideally 50 seeds from 50 plants should be collected, and then mixed in one sample. Generally this recommendation is followed, but when the species are rare or endemics with few very small populations, a few seeds are sampled from the available plants, and the process repeated in subsequent years.

The samples are immediately identified, then cleaned, dried, encapsulated in airtight tubes (Gómez-Campo, 2001), labelled, sealed with paraffin wax and stored at a low temperature of about -25°C. Most of the species produce seeds exhibiting orthodox storage behaviour. Desiccation involves reducing the seed moisture content to 5% using silica gel, prior to storage. The viability of the material stored in the seed bank is periodically checked through germination tests.

### 2. The Fern Spore Bank

Ferns represent another threatened group of plants that are very sensitive to environmental changes. Consequently, creation of fern spore banks for biological studies, biodiversity conservation, and regeneration of the threatened ecosystems is important.

In the region, there are 49 native fern species and four introduced taxa. About 51% of the Valencian pteridoflora is threatened to some degree. According to the IUCN categories, seven taxa are considered VU, seven CR, seven EN and four possible EW (Ibars *et al.*, 1999).

In recent decades, various studies have been carried out on the Valencian pteridoflora, analysing their distribution, status and the evolution of present populations. Of particular note are several initiatives developed with help from the local government, such as multiplication techniques and reintroduction in the genus *Marsilea* L. (Estrelles *et al.*, 2001a; 2001b).

The fern spore bank, initially financed by the University, is included within the germplasm bank of the Valencian Botanical Garden. Germination and loss of viability of spores under different storage conditions are studied. The samples are filtered through a sieve with 0.074 mm pores, then placed in closed bottles under three different storage conditions: room temperature, 4°C, and -20°C. A germination test is performed on each accession for checking the initial viability of the spores. Monthly tests are carried out under all storage conditions until the sixth month of storage, and then they are repeated after one year, 18 months, two years, three years, etc. The germination medium of Dyer (1979) is used. Spores are sown in two petri dishes for every storage condition and germination checked daily thereby allowing rate and final percentage of germinated spores to be assessed.

# **Optimisation of the Germplasm Protocols**

Species of special interest for conservation are subjected to more detailed studies, such as an analyses of optimal germination temperature and light conditions, pre-treatments for breaking the dormancy, etc. Understanding the biology of the species, particularly germination requirements and seedling establishment, are essential for taxa conservation. The germplasm bank has a special interest in developing different research lines on conservation techniques and germination for Valencian species.

### 1. Germination

Ongoing germination studies at our laboratory include some threatened taxa such as:

*Oxytropis jabalambrensis* (Pau) Podlech. This is classified in the red list of Spanish vascular flora (VV.AA., 2000) with the EN category of IUCN. It has a very low rate of germination under normal conditions, but after a pre-treatment of scarification and washing, almost 100% of seeds germinate on the first day (Figure 46.2A).

*Sideritis chamaedrifolia* Cav. is in the VU category of IUCN (VV.AA., 2000), that grows on sandy soils. Its germination also depends on temperature and light conditions. Optimal germination rates occur at 20°C in complete darkness. Figure 46.4 shows the comparison of its pattern of germination with other *Labiatae* species (Figure 46.2B).

Thelypteris palustris (A. Gray) Schott is considered to be a CR species (Ibars *et al.*, 1999), which grows on waterlogged or very damp soils, near the water. Figure 46.2C shows the differences in the viability of its spores stored in different conditions.

### 2. Anatomy

Collaboration is taking place with the Anatomy Department of the Botanical Garden on studies of the seed coat structure that in some species influences the variation in germination responses. This is the case with *Silene diclinis* (Lag.) Laínz, which has the EN category in the Red List of the Spanish vascular flora (VV. AA., 2000). Studies on the relation between the structure of the testa (Figures 46.3A and B) and germination (Figure 46.2D) are in process.

### 3. Genetic Variability

In addition, methods for detecting genetic variability within the germplasm bank collections are being established. The preservation of genetic diversity is a fundamental goal of conservation efforts. Various molecular markers have



**Figure 46.2** Germination pattern in various species studied: A. Effect of different pretreatments on *Oxytropis jabalambrensis*; B. Effect of different temperatures on germination in *Labiatae* species: *Teucrium lepicephalum* (TI), *Teucrium homotrichum* (Th), *Sideritis pungens* (Sp) and *Sideritis chamaedrifolia* (Sc); C. Effect of different temperatures on spores at harvest moisture content of *Thelypteris palustris*: room temperature (a), 4°C (b) and -20°C (c); D. Effect of different testa structure on germination in a seed lot of *Silene diclinis* (alternate conditions of 25/15°C and photoperiod 14h light 10h dark). (see also Figures 46.3A and 46.3B). Seeds with different testa structure can be distinguished by their colour.



Figure 46.3 A. and B. Cross sections through testa in seeds of Silene diclinis; C. Pattern of bands obtained by RAPDs in different samples of *Leucojum valentinum* and indicates genetic variation.

been used in a variety of ways to determine this diversity (Weising *et al.*, 1995). The Random Amplified Polymorphic DNA (RAPD) method has been chosen for its well-known advantages. These include a lack of requirement for previous knowledge of the genomes, the need for only limited DNA amounts, efficiency, and limited expense. RAPD analysis can be used to assess the genetic variability, both among and within populations. The application of this method shortens the time required to gather comprehensive information on the populations under consideration. Results from a study on *Leucojum valentinum* Pau are presented in Figure 46.3C. This species is included in the red list of Spanish vascular flora within the VU category (VV.AA., 2000). Its populations are very scarce and consist only of a few individuals. The plant material for this study was randomly selected from all accessions stored in our germplasm bank.

# **Education and Conservation**

Finally, the germplasm bank is involved in public awareness activities. Conservation, ultimately, relies on environmental education. This comprises formal education (preschool, primary, secondary and university students), non-formal education (remaining population), and especially that aimed at professionals whose activities impact on the environment. It is important to transmit the concept that plant biodiversity conservation entails the conservation of resources for people's benefit around the entire world. Educational values are changing and environmental education gets into all the aspects of our daily life. This change of attitude is directed at the creation of a more sustainable future. Important for this change is careful presentation to the public, of data and results obtained in the research centres. Botanical gardens are ideal centres for the development of educational programmes on these topics. Nearly 150 million people visit the 1,600 botanical gardens of the world every year. For many of them, and in particular for those who live in urban areas, botanical gardens may represent the only opportunity to come closer to nature and to learn about plants. The germplasm bank of the University of Valencia helps to create public awareness about plant conservation. Exhibitions and conferences on conservation and the importance of the botanical world are organised by the centre. The topics vary from the endemic flora of the region to transgenics. A programme of courses for the training of secondary school teachers was started several years ago.

Through the bank's activities, it is intended to develop the image of the botanical gardens of the twenty first century as protectors of the environment, researchers in conservation topics, and organisations that are responsible for education and popularisation of environmental science.

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