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Ex situ conservation of plants in the Red List of Apulia: preliminary results

R. Accogli, S. Marchiori, P. Medagli, F. Ippolito¹

Summary

The Interreg II Italy-Albania Project has allowed to broaden the surface area of the Botanical Garden with a view to propagating and preserving the genetic resources of the Apulian and Albanian flora and studying the re-introduction and diffusion of threatened or endangered wild and cultivated species. The activities carried out by the Botanical Garden of Lecce are performed based on the programme of “Centro Studi per la Protezione e la Conservazione delle Specie Botaniche del Mediterraneo con annesso Giardino Botanico”. Indeed, working for the conservation and preservation of the plant biodiversity implies a scientific and ethical responsibility of the Botanical Garden in order to pursue cultural objectives.

Key words: conservation, environmental education.

1. Introduction

Among the priorities of the Botanical Garden (Lecce) is the conservation of the plant genetic resources of Apulia (Marchiori, 1995).

The Conservation Strategies suggested by the International Union Conservation Nature (IUCN) could not be disregarded and therefore, the programme was worked out based on the guidelines provided in the international plan. The following guidelines were set up:

probing into the knowledge and analyses of biodiversity through regional surveys and investigations into the priority habitats or those of interest for U.E.;

identification and collection of priority species to preserve in a given territory;

propagation and identification of cultural techniques about *ex situ*-conserved species with special regard to those in the regional and national red lists;

computerised management of collections for an easy and immediate

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exchange of information among botanical institutions: adoption of a relational database;

identification of strategies for the *in situ* reintroduction and for the enhancement of local populations;

involvement of mass media and public opinion in order to raise the awareness on the protection of plant and environmental resources (Bedini, 1989).

2. Activities

2.1 Probing into floristic knowledge and biodiversity analysis

The evaluation of the floristic richness of an area is often too slow if compared with the fast degradation of habitats. Therefore, several species disappear and their presence is never assessed (Garbari, 1999). All this requests more detailed investigations into priority habitats or of great interest for the region although compromised by anthropic activities.

To this end, more than 2,000 references were carefully evaluated in order to acquire useful data on the distribution and status of the species.

As for the open field research, specific instruments were purchased (GPS, aerial photogrammetry, geological maps, IGM maps, etc.), whereas appropriate computer systems were chosen for the transfer of habitat data and their processing in the maps.

To date, the following sub-regions have been surveyed in Apulia: N-NE area of Gargano, NW and SE Murge, the area of Gorges, the Ionian Coastline, Salento (Lecce province, partly Brindisi and Taranto provinces).

About 600 areas have been investigated and some habitat data have been reported as for the scientific name and taxonomic class of the species, the geographical position of the area, the status of the habitat and the causes of degradation for the ecological integrity of the observed ecosystem, the exposure and the nature of the geopedological substrate.

2.2 Identification of species to safeguard

Investigations regarded all the 2,075 species which make up the Apulian flora (Marchiori *et al.*, 2000) although they privileged wild and cultivated species whose protection was prioritised based on the IUCN directives (Bedini, 1989).

The species cultivated till the XIX century showed a wide variability made

up of different genotypes since the farmers aimed domesticated and diversified productions; in the XX century, modern varieties were selected from these old varieties since the genetic uniformity ensured the morphological uniformity which, in turn, allowed to standardise cultural practices and to guarantee a higher resistance to plant diseases. About 80-90% of the varieties cultivated over the centuries have been lost in a few decades; an irreversible erosion coupled with a cultural impoverishment since the loss of local knowledge hampers to understand their biology, distribution and quality (Negri and Veronesi, 2000).

Within the framework of conservation and rehabilitation activities old local endangered cultivars have been identified and 60 varieties of *Ficus carica*, 10 varieties of *Citrus* spp., 18 varieties of *Pyrus communis* and 16 cultivars of *Vitis vinifera* have been recovered.

The search and propagation of parental species of edible varieties has been privileged such as: *Beta maritima*, *Cynara cardunculus*, *Aegilops uniaristata*, *Astragalus boeticus*, etc. A special attention was also paid to those wild species which for centuries have populated traditions and beliefs and which are still used in Salento in the form of mixed salads (*Borago officinalis*, *Picris hieracioides*, *Sonchus oleraceus*, *Rumex lapatifolium*, etc.) or agri-food preserves (*Salicornia patula*, *Capparis spinosa*, *Critmum maritimum*, *Chenopodium album*, etc.).

As for wild species, the priority was given to rare and endangered species (included in the Regional and National Red Lists), to those having a known economic interest (fibre, with therapeutical properties, nutritional value, useful for industrial purposes), to the key species for the most important natural ecosystems to be used for the environment preservation.

In Apulia, the species belonging to the Red Lists are 180; more precisely, 72 belong to the national and 108 to the regional one (Conti *et al.*, 1992; 1997); endemic species and those with a phytogeographic interest shall be added. For their identification, bibliographic records were considered, although during the investigations new stations were detected for some of them and their distribution or their status redefined. More than 100 species are grown and propagated in the Botanical Garden; propagation activities are carefully recorded, with all the details for a successful *ex situ* conservation.

Eighty officinal species have already been collected and multiplied in order to strengthen and support a scientific research sector which already exists at the Department of Biology; pharmacological, nutritional or ecological uses are still obscure for several wild species and the shrinkage of the world-wide biodiversity might have a negative economic repercussion.

The Botanical Garden of Lecce has set up protection programmes with a direct participation into projects for the rehabilitation of threatened or impoverished habitats or environments; therefore common woody and herbaceous species are massively multiplied. All the species belonging to the genus *Quercus* have been recovered and multiplied as well as the shrubby species of the Mediterranean maquis and the major plant associations of the hinterland, of the coastline and of wetlands for a total number of about 120 species.

2.3 *Ex situ* conservation

In contrast with cultivated plants, the cultural techniques, diseases and phenological phases are still obscure for most wild species. Therefore, experimental plots are the basic substrate for comparative studies between the environment of the botanical garden and their natural habitat where competitions, pests, and several other factors induce a spontaneous propagation which might differ from that observed *ex situ*.

The conservation of plants and of their reproductive organs (propagules, seeds, callus, tissues or explants, plantlets) is aimed at the conservation of plant biodiversity and at the development of cultural techniques for the survival of the species, their introduction, the rehabilitation of their habitat and ecosystems and of the ecological networks which play a basic role for the human species.

Ex situ conservation shall be geared onto several sectors, each dealing with a specific topic such as: seed bank, micropropagation, nursery activity, *in situ* reintroduction, exchange of information and propagative material with other Boards in charge of conservation.

The seed bank is one of the most valid *ex situ* conservation methods and necessitates a watchful management. The seed bank is a sector of methodical studies which request organisation, accuracy and a very long time; very little is known on the seed germinating power of wild species and on their viability.

It has been estimated that about 20% of the world-wide flora produces recalcitrant seeds, which do not keep their viability when stored, many others do not develop completely and are called "incompetent". Still little is known about the dormancy period of different types of seeds, which is rather long for some types, about the staggered germination power which does not provide immediate results; therefore it is difficult to conduct germination trials specially for endangered species which are still unknown (Bedini, 1989).

The necessity to take propagative material for *ex situ* conservation has led us to identify the best phenological phases for the collection of vegetative (cuttings, suckers, callus, propagules) and reproductive organs (seeds and ripening fruits). This investigation has allowed to extrapolate phenological waves indicative of the territory in that, whatever close stations are, the same species show staggered blooming periods and fruit ripening.

Based on the dates of harvest and accession of samples in the botanical garden, some calendars have been extrapolated concerning flowering, fruit ripening and harvesting; it shall be added that if the material has to be timely harvested because it deteriorates very soon, an early harvest can provide unsuitable or immature seeds.

For most species, both sexual and asexual reproduction has been tested under the most natural conditions in order to compare their *in situ* and *ex situ* diffusion and multiplication.

Given the scant literature on the cultivation of these species, several techniques have been tested for the collection, preparation and conservation of seeds as well as sowing periods and modes. A sowing calendar was set up based on the affinity they have with species or genera of agronomic species or through sowing or propagation techniques in following periods and seasons (Accogli *et al.*, 1999). The following tables report some excerpts from calendars concerning the dates for the collection of seeds and ripe fruits (Tab. 1) and autumn sowing (Tab. 2).

Tab. 1 Harvesting dates of ripening fruits

Genus	Species	Harv. date	Town	Location
<i>Scrophularia</i>	<i>lucida</i>	16-July-98	Lecce	Orto Botanico
<i>Euphorbia</i>	<i>paralias</i>	20-July-98	Ugento	Torre Mozza
<i>Petrorhagia</i>	<i>prolifera</i>	20-July-98	Ugento	Torre Mozza
<i>Spartium</i>	<i>junceum</i>	20-July-98	Nardò	Santa Caterina
<i>Gladiolus</i>	<i>byzantinus</i>	21-July-98	Brindisi	Palude della Contessa
<i>Centaurea</i>	<i>leucadea</i>	6-July-00	Gagliano del C.	Ciolo
<i>Cachrys</i>	<i>libanotis</i>	23-July-98	Tricase	Chiesa nuova
<i>Gladiolus</i>	<i>italicus</i>	23-July-98	Gagliano del C.	Ciolo
<i>Thapsia</i>	<i>garganica</i>	23-July-98	Gagliano del C.	Ciolo
<i>Cachrys</i>	<i>pungens</i>	27-July-98	Tricase	Chiesa nuova
<i>Allium</i>	<i>atroviolaceum</i>	28-July-98	Lecce	Orto Botanico
<i>Cenchrus</i>	<i>echinatus</i>	29-July-98	Otranto	Fontanelle Sud
<i>Vincetoxicum</i>	<i>hirundinaria</i>	30-July-98	Otranto	Punta Palascia
<i>Verbascum</i>	<i>sp.pl.</i>	1-July-99	Castro	S. Antonio
<i>Cynara</i>	<i>cardunculus</i>	2-July-99	Porto Cesareo	La Strea
<i>Asphodelus</i>	<i>albus</i>	4-July-99	Rotonda	Piano di Ruggio
<i>Euphorbia</i>	<i>rigida</i>	4-July-99	Rotonda	Monte Pollino
<i>Genista</i>	<i>sericea</i>	4-July-99	Rotonda	Piano di Ruggio
<i>Sp. pl.</i>		4-July-99	Rotonda	Piano di Ruggio
<i>Anthemis</i>	<i>hydruntina</i>	5-July-99	Cannole	Mass. Torcito
<i>Rhamnus</i>	<i>alaternus</i>	23-July-99	Lecce	Campo Verde S. Cataldo
<i>Morus</i>	<i>nigra</i>	31-July-99	Tricase	Tricase Porto
<i>Helianthemum</i>	<i>jonium</i>	5-July-00	Lecce	Orto Botanico
<i>Lathyrus</i>	<i>sativus</i>	5-July-00	Lecce	Orto Botanico
<i>Centaurea</i>	<i>leucadea</i>	6-July-00	Gagliano del C.	Ciolo
<i>Centaurea</i>	<i>japigica</i>	6-July-00	Castrignano	Capo di Leuca
<i>Centaurea</i>	<i>nobilis</i>	6-July-00	Tricase	Belvedere
<i>Morus</i>	<i>nigra</i>	6-July-00	Tricase	Litoranea
<i>Phlomis</i>	<i>fruticosa</i>	6-July-00	Gagliano del C.	Ciolo
<i>Pistacia</i>	<i>terebinthus</i>	6-July-00	Tricase	Belvedere M. Serra
<i>Centaurea</i>	<i>leucadea</i>	6-July-00	Gagliano del C.	Ciolo
<i>Iris</i>	<i>pseudopumila</i>	9-July-00	Lecce	Orto Botanico
<i>Eryngium</i>	<i>barrelieri</i>	10-July-00	Lecce	Orto Botanico
<i>Leptospermum</i>	<i>laevigatum</i>	11-July-00	Gallipoli	Baia Verde
<i>Prasium</i>	<i>majus</i>	11-July-00	Gallipoli	Baia Verde
<i>Rhamnus</i>	<i>alaternus</i>	11-July-00	Gallipoli	Baia Verde
<i>Sarcopoterium</i>	<i>spinosum</i>	11-July-00	Nardò	Palude del Capitano
<i>Carum</i>	<i>multiflorum</i>	12-July-00	Tricase	Torre Sasso
<i>Cachrys</i>	<i>libanotis</i>	13-July-00	Tricase	Torre Sasso
<i>Acanthus</i>	<i>spinosus</i>	14-July-00	Cutrofiano	Canale Piscopo
<i>Iris</i>	<i>pseudacorus</i>	19-July-00	Lecce	Orto Botanico

Tab. 2 Calendar of autumn sowings

September		October		November		December	
<i>Anthemis arvensis</i>	3556	<i>Acer campestre</i>	3520	<i>Acanthus spinosus</i>	3470	<i>Carduus pycnocephalus</i>	3778
<i>Anthemis hydruntina</i>	3669	<i>Aegiolophila pumila</i>	3877	<i>Aegilops geniculata</i>	1733	<i>Carduus pycnocephalus</i>	3842
<i>Anthemis hydruntina</i>	3814	<i>Allium chamaemoly</i>	2724	<i>Aegilops geniculata</i>	2209	<i>Coronilla valentina</i>	3817
<i>Anthemis</i> sp.	3784	<i>Allium commutatum</i>	3491	<i>Aegilops geniculata</i>	3879	<i>Cynoglossum cheirifolium</i>	3808
<i>Buglossoides arvensis</i>	2163	<i>Allium niger</i>	3615	<i>Aegilops</i> sp.	3337	<i>Cynoglossum cheirifolium</i>	3904
<i>Calendula arvensis</i>	2131	<i>Allium</i> sp.	3332	<i>Aegilops uniaristata</i>	3819	<i>Echinops spinosissimus</i>	3860
<i>Calendula arvensis</i>	3844	<i>Allium</i> sp.	3616	<i>Aurinia leucadea</i>	3752	<i>Echinops spinosissimus</i>	3715
<i>Carum multiflorum</i>	3469	<i>Allium</i> sp.	3897	<i>Aurinia leucadea</i>	3816	<i>Onopordum horridum</i>	3880
<i>Centaurea cfr japigica</i>	3449	<i>Anagyris foetida</i>	3057	<i>Aurinia leucadea</i>	3832		
<i>Centaurea cfr nobilis</i>	3791	<i>Arum apulum</i>	3431	<i>Calicotome infesta</i>	3666		
<i>Centaurea japigica</i>	3445	<i>Arum italicum</i>	3330	<i>Cenchrus cfr echinatus</i>	2227		
<i>Centaurea japigica</i>	3448	<i>Arum italicum</i>	3496	<i>Crupina crupinastrum</i>	3181		
<i>Centaurea japigica</i>	3869	<i>Arum italicum</i>	3671	<i>Crypsis aculeata</i>	3549		
<i>Centaurea leucadea</i>	1376	<i>Arum italicum</i>	3899	<i>Cymbopogon hirtus</i>	3798		
<i>Centaurea leucadea</i>	3344	<i>Asphodeline lutea</i>	3206	<i>Cynosurus echinatus</i>	2011		
<i>Centaurea leucadea</i>	3447	<i>Asphodelus fistulosus</i>	3654	<i>Echinocloa crus-galli</i>	2160		
<i>Centaurea leucadea</i>	3876	<i>Asphodelus fistulosus</i>	3754	<i>Eragrostis megastachya</i>	2133		

Tab. 2 continued

September		October		November		December	
<i>Centaurea melitensis</i>	2610	<i>Asphodelus fistulosus</i>	3828	<i>Eragrostis pilosa</i>	2135		
<i>Centaurea nobilis</i>	3450	<i>Asphodelus fistulosus</i>	3829	<i>Ferula communis</i>	3430		
<i>Centaurea nobilis</i>	3854	<i>Asphodelus sp.</i>	3665	<i>Imperata cylindrica</i>	2159		
<i>Centaurea nobilis</i>	3675	<i>Asphodelus sp.</i>	3755	<i>Iris pseudopumila</i>	795		
<i>Crepis rubra</i>	3673	<i>Cachrys libanotis</i>	3471	<i>Juncus acutus</i>	3540		
<i>Crepis rubra</i>	3837	<i>Capparis spinosa</i>	2048	<i>Juncus sp.</i>	2235		
<i>Crepis vesicaria</i>	3885	<i>Capparis spinosa</i>	3494	<i>Lavatera arborea</i>	3099		
<i>Cyclamen hederifolium</i>	2340	<i>Ceratonia siliqua</i>	3674	<i>Leptospermum laevigatum</i>	3468		
<i>Erodium cicutarium</i>	3820	<i>Cercis siliquastrum</i>	3517	<i>Lygeum spartum</i>	808		
<i>Erodium cicutarium</i>	3823	<i>Chrysanthemum segetum</i>	3849	<i>Ophrys lutea</i>	3351		
<i>Erodium malacoides</i>	3826	<i>Colchicum cupanii</i>	2930	<i>Ophrys lutea</i>	3554		
<i>Erodium malacoides</i>	3847	<i>Crupina crupinastrum</i>	3114	<i>Ophrys tenthredinifera</i>	2731		
<i>Euonymus europaea</i>	2230	<i>Daphne gnidum</i>	2098	<i>Orchis morio</i>	3806		
<i>Euphorbia characias</i>	3783	<i>Daphne laureola</i>	3898	<i>Osyris alba</i>	2204		
<i>Geranium molle</i>	3810	<i>Fraxinus oxycarpa</i>	693	<i>Papaver hybridum</i>	3745		

For the open field propagation and cultivation, the pedoclimatic conditions of the area of origin have been reproduced in the Botanical Garden; in the pots, appropriate substrates have been produced using universal soil mixes in a proportion close to that of the soil horizon where the plant finds its optimum. Collections were kept in the open field and exposed to sun radiation and climatic effects.

On small surface areas, some peculiar environments have been reproduced such as sea dunes, maquis and pseudosteppes for the acclimatisation of the samples and to have mother plants providing propagative material for the study of reproductive and propagative phases.

A preliminary evaluation showed that the total accessions amount to 4,500 for 1,500 sampled taxa. For almost all species both sexual and asexual propagation was tested; 12,500 samples were represented by suckers, cuttings, rhizomes, and 1,420 fruit and seed samples.

Two main sectors may be distinguished in the propagation area: one for the asexual propagation with 10,000 containers varying in shape and size, each of which may contain several propagules, one for the sexual propagation with seed beds and honey-comb trays for more than 100,000 sowing units, each with 1 to 10 seeds.

For technical, logistic reasons, lack of experience or due to adverse climatic conditions, the success rate of propagation activity has differed; however, the total number of living individuals amounts to 150,000 and represents two thirds of the total number of individuals left in the Botanical Garden also for short periods.

The total number of individuals belonging to the Red Lists or of phytogeographic interest is 15,000; the most complete collection concerns Salento entities whereas others are being completed considering that data on the location of these stations are still unclear.

To complete the collections and activities, some sets of pictures illustrate the steps of the biological cycle for the most significant species during their cultivation; for nearly all the species, seeds and fruits are properly stored for further studies. Some *exsiccata* were also made to be included in the *Herbarium Lupiense* of the laboratory of Systemic Botany and Plant Ecology.

2.4 Setting up a relational database

The data acquired for every single species both at the collection time and from bibliographic sources shall be registered in a well organized manner for a rapid access and further corrections or introductions. A correct cataloguing allows to have systematic series of data in a short time starting from a heterogeneous set of information. Should the system be on line, IUCN can have information and add the conservation category to the database of the Botanical Garden and know the richness and the site of the threatened species. Furthermore, computerized programmes enable to store data which are then subdivided into connected categories based on a vertical and horizontal diagram of information packages. For each sample, a data sheet prototype has already been worked out based on the ITF sheet proposed in 1989 by the Commission for the enhancement and diffusion of scientific culture appointed by the Ministry of University and Scientific and Technological Research (Bruno, 1996).

Each data sheet contains a set of fields defining the identification data of the record i.e. the sample code, accession data, scientific name (taxa-related), data on the type and status of the sample and place of origin, the conservation value, bibliographic references and the operations carried out since its very early accession (type of propagative activities, temporary or conclusive classification, treatments, types of substrate used for propagation, pedoclimatic conditions of the natural environment recreated for its cultivation and other data).

In conclusion, a precise and appropriate computerised cataloguing allows to get a wealth of data which can refer to other research or specific information and also to know the biological history of its *ex situ* conservation to compare with the *in situ* one. A data sheet type is herein reported:

Periploca graeca
Asclepiadaceae

STATION DATA:

Accession date : 13/03/00

Region: **Apulia** Place : **Rauccio** Coordinates: **E 18° 10', N 40° 28'**
Town: **Lecce** Habitat: **hygrophilous wood** substrate: **sands and recent marshland silts**
Site of Community Interest

SAMPLE DATA:

Number of samples: 2596 Phenological stage: growth recovery
Sample type: twigs
Twig quantity: 20
Cutting quantity: 138

OPERATIONS CARRIED OUT ON THE SAMPLE:

Date	Category	Type of operation	Object	n. plants	Container	n. pots	Substrat	Position
14/3/00	Propagative	Propagation by cutting	Cutting	138	Pot (7x7x10)	138	mixed	greenhouse
2/04/00	Observation	Death	Cutting	10		10		greenhouse
15/4/00	Maintenance	Weeding	Cutting	128	Pot (7x7x10)	128		greenhouse
27/9/00	Observation	Death	Plants	30	Pot (7x7x10)	30		greenhouse
28/9/00	Maintenance	Re-potting	Plants	98	Pot (19 cm)	98	comp. 2:1	List R

2.5 Strategies of *in situ* reintroduction

At present, only Botanical Gardens are authorised to reintroduce extinct species from their *locus classicus* (Curti, 1994).

In situ conservation implies a rehabilitation and/or enhancement of natural ecosystems which include the above species where they interact with other organisms; furthermore, it envisions an active control of the environment and of the biodiversity status; that is why the scientific instruments Botanical Garden may have access to are exploited by the communities for a correct and constant management of protected areas. This justifies the need for synergic links with the local administrations for the definition and formulation of laws which regulate interventions on the territory (Rinaldi, 1995).

In situ reintroduction has been tested on a species belonging to the National Red List, *Periploca graeca*, which had disappeared from one of the three stations in Salento after a fire. The species reintroduction, after its propagation and cultivation at the Botanical Garden of Lecce, implied the rehabilitation of the habitat and the introduction of some other restructuring species.

The station is located in an area having a high educational value and therefore schools and local administrations have been involved in the environmental rehabilitation (Accogli *et al.*, 2001).

2.6 Activities of environmental education

Botanical Gardens, which were set up so as to back the scientific activity of Universities, are now assigned harder tasks: to support pre-university teaching activities, to involve local institutions for a correct land planning and for the protection of the areas where species live (Curti, 1994); to collaborate with the local ecological associations which promote education projects for the protection of environmental resources; to update and train personnel for the propagation and diffusion of local wild plants (nurserymen or amateurs who exchange propagation material).

Although it is still insufficiently organised, the Botanical Garden of Lecce is successfully involved in extension programmes concerning the local environment. Its participation in several thematic meetings has allowed to disseminate the results achieved and also its reputation as basic contact point of the nature conservation. The Botanical Garden of Lecce actively participates in the drawing up of management programmes for the protected areas of "Rauccio" and "Cesine" and in requalification plans by providing local ecotypes for the environmental rehabilitation.

An appropriate support has also been provided to primary school teachers who had already started up botanical studies with their pupils. The Botanical Garden hosts students from any schools for guided tours and vocational training stages.

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