First phase of conservation translocations of the Cartagena's rockrose in the Valencian Community, Spain

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Introduction

Cistus heterophyllus Desf. is an Iberian-North African rockrose species. The populations of the Iberian Peninsula are differentiated as subsp. *carthaginensis* (Pau) M.B. Crespo & Mateo, "Cartagena's Rockrose", which is one of the more threatened plants in Europe. This subspecies only lives in the regions of Valencian Community, where only a unique pure specimen in wild has been found and in Murcia where around 40 specimens, apparently showing traits of hybridization with the close relative white rockrose (*Cistus albidus* L.). The two Iberian populations are over 300 km far from each other. The Cartagena's rockrose has been categorized Critically Endangered (CR) by the Spanish Red List of Threatened Vascular Flora (Moreno, 2008). It is strictly protected with the maximum level of the Spanish laws (Imperiled of Extinction). It is also protected at the same level in the Valencian Community and Murcia throughout their regional laws, and both regions have passed a recovery plan. This plant is a calcicolous shrub up to 0.8 m tall living in semi-arid (rainfall amount between 200 - 350 mm/year) or dry-semiarid (350 - 450 mm/year) areas. The rockroses use to be self-incompatible for pollination and further seed production, and only scarce fruits containing very few seeds can be collected every year.

Flowering close-up of *Cistus heterophyllus subsp. carthaginensis* © Emilio Laguna

Goals

- Goal 1: Producing *ex-situ* new plants through vegetative propagation techniques, as a safety

measure (preventing the lack of sexual reproduction) and achieving a vegetative orchard.
- Goal 2: Obtaining seeds *ex-situ* from two or more generations. Getting an *ex-situ* pool of genetically diverse plants ('biodiverse plants').
- Goal 3: Making plantations able to produce new individuals.
- Goal 4: Obtaining at least one population with long-lived parental plants under 'suitable' ecological conditions, similar to those of the site where the unique wild specimen is found.

Success Indicators

- Indicator 1: A safety pool of at least 50 - 100 plants produced through vegetative methods i.e. *in-vitro*, cuttings to set up a plant orchard.
- Indicator 2: New plants grown *ex-situ* from seeds, taken from the original native wild individual, and/or the plant orchard.
- Indicator 3: One or more new populations established and self-maintained in 'suitable' ecological conditions similar to the native population.

Project Summary

Feasibility: Full recovery of the species in the Valencian Community region which holds the Northernmost population, with only one wild specimen should be expected for a very long-term, so its rescue has been planned following several phases. The first one includes the obtaining of new specimens and first experimental plantations in natural areas. The conservation of the Murcian population, bigger than the Valencian ones but apparently hybridized (Jiménez *et al.*, 2007; Aguilella *et al*., 2010) should be implemented in further phases. It will depend on the propagation success of the Valencian plant material which could be used to dilute the effect of hybridization noticed in the Murcian plants. The provision of new Valencian specimens has been performed through three different ways: 1) clonal, *in-vitro* specimens; 2) clonal plants grown after cuttings collected from the wild specimen; and 3) seeds from outstanding fruiting episodes, produced by the unique wild specimen or its clonal descendants obtained via 1 or 2 (Escribá *et al.*, 2007). At least the new plants obtained via 1 and 2, have shown a strong incompatibility to produce seeds in nursery, even after artificial pollination. The feasibility of the recovery in future phases will depend on several factors, for which there is no sufficient certainty: 1) the progressive obtaining of new generations of Cartagena's rockrose specimens from seeds - where a lower incompatibility could be expected; 2) the maintenance of enough vigour and/or the reduction of negative expected effects of endogamy; 3) the finding of available sites without *Cistus albidus* and the positive performance of *C. heterophyllus* subsp. *carthaginensis* after its implantation.

Implementation: The first phase consisted of 1) obtaining new Valencian clonal individuals, 2) to test the plantation of individuals using clonal Valencian plants in order to obtain a field protocol, to be used in the future using plants obtained from seeds and 3) the establishment of a seed orchard pool of Valencian 'pure' plants, not introgreded by *C. albidus*. Clonal *in-vitro* propagation was achieved in 1990 - 1991 (Arregui *et al.*, 1993) and more than 200 individuals have been produced. *In -vitro* plants have been the unique way to save the species from the extinction

throughout 1991 - 2011. It has been recently demonstrated - unpublished data that *in-vitro* plants could carry a little mutation in their rDNA chomosomic region. Its codability is unknown, but no apparent morphological changes have been noticed on the *in-vitro* plants, compared to the wild specimen. Clonal propagation using hormonated cuttings, were unsuccessfully attempted between 1987 and 2011. After good rainfall seasons in 2011 - 2012, enough plant material in good condition was collected from the unique wild plant and more than 50% of cuttings rooted, producing an initial pool of a dozen of new individuals. Their lack of chromosomic alterations has been tested.

Searching for new seedlings in the Plant Micro-reserve 'Tancat de Portaceli' © Emilio Laguna

An outstanding episode of seed production was recorded in the wild specimen in 2013, collecting up to 50 seeds. Twenty-five new individuals grown *ex-situ* have started to flower in 2015, and a few fruits with new seeds are being currently collected, in order to start a future second generation. Additionally, artificial crosses in isolation chambers have been made since 2013, using Valencian, Murcian and African plants, show a remarkable hybrid vigor (i.e. crossing the two subspecies) has been noticed. Up to seven plantations of 150 individuals were made from 1997 to 2010 on several kinds of soils, plant communities and altitudes. The plantations were made depending on the *ex-situ* plant production.

Post-plantation monitoring: Most part of the seven plantations failed and the planted individuals died without recruited new plants. The majority of those sites suited the theoretical good conditions similar to the site of the native individual. However a plantation of 25 *in-vitro* specimens made in 1997 in the Plant Micro-Reserve (PMR) 'Tancat de Portaceli' (Serra, province of Valencia) produced new individuals from 2011. The PMR was not a theoretical good site, because of its tree cover provided by Aleppo pine (*Pinus halepensis*) and the presence of *Cistus albidus*. In 2012 - 2013, 40% of the individuals planted in 1997 still survived, and four newly recruited plants of *C. heterophyllus* were found. The death of the remainder 60% was mainly caused by the strong competition of bigger shrubs such as *Pistacia lentiscus*. The new plants of *C. heterophyllus* had no external effects of hybridization, but they could carry the chromosomic alteration. As a expected bad result, several hybrid young plants were also found in the same PMR. Both individuals of *C. albidus* and hybrids were removed from the PMR in

2013 - 2014. In despite of these results, recent attempts to create a new population without Aleppo pine cover, in 'suitable' conditions have failed. The main reason was the lack of enough support through artificial watering.

Major difficulties faced

- The optimal new individuals should be grown from seeds, and the species is a self-incompatible taxon for seed production. Only rare failures of the incompatibility mating systems can provide new seeds.
- The *in-vitro* produced plants can be maintained as a safety measure for the species conservation. A slight chromosomal difference from the unique wild plant has been recently found, but no morphological differences have been noticed.
- The best site where the implanted plants survived is a bad one to ensure the long-term self-maintenance of a new population without artificial help, due to the risk of hybridization with *Cistus albidus* and the strong competition caused by other local shrubs.
- Although a first generation from seeds has been obtained, the new individuals come from a unique mother plant, so future effects of endogamy could be expected for a long time.

Major lessons learned

- The species is close to be genetically exhausted, so a first genetic recovery *ex -situ* (self-crossing for several generations) will be needed, in order to obtain enough seeds and new plants for the future translocations.
- The *in-vitro* plants can survive and produce new descendants *in-situ*. As an emergency alternative for the recovery program, *in-vitro* plants could be used in a future as a last resort, if the genetic rescue using seeds will fail.
- The recruitment of new seedlings, coming from *in-vitro* parentals, has only been noticed long time after the plantations ~15 years. Therefore a true recovery of the Cartagena's rockrose may last some decades.
- Apparently 'best' conditions for plant growth i.e. sites with deeper soil, tree cover, etc. could ensure the survival and successful reproduction of the Cartagena's rockrose, but the species must face the risk of hybridization, and increased competition caused by other shrubs. The maintenance of those new populations force the managers to remove the white rockrose and their hybrids, as well to reduce competition i.e. pruning or removing competitor shrubs, removing recruited pine seedlings, etc..

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
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Reason(s) for success/failure:

- No successful plantation where at least a big part of the planted individuals had survived for some years has been achieved on the 'suitable' sites for this subspecies. The main reason was the lack of irrigation support.

- The Cartagena's rockrose can survive if planted on sites where the artificial irrigation is not needed due to a higher rainfall amount, bigger plant cover, etc. but these sites hold white rockrose amid strong competition by bigger shrubs.

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