

In situ and ex situ studies to improve the conservation status of critically endangered endemic plants.

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Gadoria falukei and *Cistus heterophyllus* subsp. *carthaginensis* are two of the most critically endangered species of Spain. *G. falukei* is an autogamous species that present only one population with less than 100 individuals. *C. heterophyllus* subsp. *carthaginensis* presents autoincompatibility and there are two natural populations, one in Murcia with high levels of hybridization with *Cistus albidus* and one in Valencia with only one individual.

Gadoria falukei Güemes & Mota (Plantaginaceae) is a monotypic genus endemic of Sierra de Gádor (Andalusia, Spain). It was described in 2017, and in the first census were found only 24 plants. The species was declared as Critically endangered following the IUCN categories.

Cistus heterophyllus subsp. *carthaginensis* (Pau) M.B. Crespo & Mateo (Cistaceae) is an exclusive endemism of the SE of the Iberian Peninsula. There are only two natural populations, one in Valencia (with only one individual) and other in Murcia (10-15 individuals) with some degree of hybridization with *Cistus albidus*.

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Aims:

Improve the knowledge of the species conducting *in situ* and *ex situ* studies at the Botanical Garden of University of Valencia:

- Annual censuses of the natural population and exploration of several caves in the Sierra de Gádor to look for new populations, even with drones.
- Seed production.
- Studies of the genetic diversity of the species.
- With *ex situ* plants were conducted experiments of:
 - Breeding system (tests of autogamy, xenogamy, geitonogamy and control)
 - Seed germination (to obtain the optimum conditions)

Results:

During prospectations were found two new areas with *G. falukei* at the adjacent caves, and the species increased the number of known individuals. In 2021 were counted 96 individuals.

In the tables 1 and 2 are represented the results of seed production after different pollinations treatments and the genetic diversity of natural population.

Every plant produce numerous fruits and seeds. In the field fruits produce a mean of 110.51 ± 19.28 (69-143) seeds with a mean weight of 2.39 ± 0.03 mg (10 seeds) and on *ex situ* cultivated plants fruits present a mean of 100.22 ± 29.07 (47-126) seeds with a mean of 2.45 ± 0.30 mg (10 seeds).

The genetic diversity was tested on 15 individuals distributed around the population and there was not observed any variability with the markers used.

Seeds presented high levels of germinability and the optimum conditions to germinate it are 20°C and 12h light/12h darkness. In these conditions we obtained 95% of germination and T50 of 9 days.

Table 1. Seed production after different pollination treatments

Treatment	Nº seeds	Seed weight (10 seeds)
Autogamy	80	2.64
Xenogamy	58	2.81
Geitonogamy	60	2.20
Control	78	2.21



Table 2. Genetic diversity on natural *G. falukei* population.

Molecular markers		Differences
Localization	Fragment	
Nuclear	ITS	0
	trnLc — trnf	0
	trnQ — rps16	0
Chloroplastidial (cpDNA)	rpL32 — trnL	0
	Total differences found	0

The presence of only one population, with less than 100 individuals, the lack of genetic variability found and the presence of herbivorous on the natural population are the most important threats. *G. falukei* should be included on the endangered flora catalogue of Spain and a recovery and conservation plan might be needed. Further studies are needed in order to better understand the species as well as looking for new populations with new genotypes and suitable areas to conduct translocations.

G. falukei present few individuals but we started to observe new plants, instead, even if in Valencia there are hundreds of *C. heterophyllus* subsp. *carthaginensis* distributed on different translocations we cannot speak of improving of the conservation status, as there are not new generations reproducing by themselves. We need to continue to study these species to observe its evolution and improve the survivorship of the new plants.

Fundación Biodiversidad funded this research.

Aims:

Improve the knowledge of the reproductive biology of the species by experiments conducted on three translocated populations, one in Valencia (Cañada Fría) and two in Murcia (C1 and P4):

- Census of Cañada Fría population.
- Fruit and seed production.
- Seed characterization.
- Breeding system.
- Pollinator census.

Results:

In Cañada Fría there are 256 adult plants that produce a mean of 69 flowers per plant (18-187), but only 54,53% of the plants produce mature fruits, with a mean of 7.23 ± 10.52 (1-64) per plant.

In the tables 3 and 4 are represented the results of fruit and seed production on the three studied populations.

C. heterophyllus subsp. *carthaginensis* behave in a different way for the two locations. The Murcian ones produce 67% of the fruits, with a mean of 36 seeds and have observed recruitment of seedlings. Instead, the Valencian one (originated from the only one natural plant) produce only 15% of fruits with a mean of 20 seeds. Only 6 seedlings were observed, but none of them survived.

Were conducted six pollination treatments and only 10% of them produced fruits, being the xenogamy treatment the one that produced higher fruit set (19,74%).

The main pollinators observed were Himenopters, being *Apis mellifera* the most abundant. During the study were observed several pollen thieves.

Table 3. Fruit and seed production on the studied populations

Population	Fruit production (%)	Seed number
Cañada Fría (Valencia)	15.27	20.10 ± 8.79
C1 (Murcia)	68.07	36.15 ± 15.92
P4 (Murcia)	66.24	36.92 ± 18.47



Table 4. Results of the pollination treatments on the 3 studied populations

Population	Treatment	Nº flowers	Fruit set (%)	Mean seeds/fruit	Seed weight (10 seeds)
Cañada Fría	Control	64	12.50	18.38	0.0124
	Supplementary pollination	62	6.45	19.50	0.0170
	Autogamy	56	7.14	17.25	0.0150
	Spontaneous autogamy	35	5.70	2.00	-
	Xenogamy	76	19.74	34.50	0.0193
C1	Geitonogamy	63	3.17	6.00	-
	Control	15	80.00	67.10	0.0119
P4	Control	25	84.00	48.36	0.0112
	Spontaneous autogamy	22	18.18	79.45	0.0130

The efforts carried out to date have allowed to increase the number of individuals of the species to hundreds, but the reproductive success of these in the studied population is still very low. It is important to continue to conduct reproductive studies to understand and try to reverse the reproductive limitation of the taxa.

We consider that the data from Murcia can be due to the presence of higher genetic diversity and maybe by the hybridization of the species with *Cistus albidus*.