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Recovery plans for *Delphinium bolosii* and *Thymus loscosii*: results from three-year studies and conclusions

Abstract

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Delphinium bolosii (*Ranunculaceae*) and *Thymus loscosii* (*Lamiaceae*) are the only two species in Catalan flora listed in the Spanish Catálogo Nacional de Especies Amenazadas (CNEA) as 'at risk of extinction' (BOE 1990). In the framework of an agreement with the Department of Environment of the Generalitat de Catalunya (DMA, Catalan Autonomous Government), three-year studies have been carried out in order to obtain the scientific bases necessary to prepare the conservation strategy for those species. The survey included pollination ecology and genetic diversity as well as chorological and demographic aspects. We also reevaluated their categories using the new IUCN criteria (2001). The results obtained showed that the low level of threat to the gynodioecious species *Thymus loscosii* (LR, 'lower risk' according to IUCN), does not require a strict recovery plan because it has a sufficient number of individuals, proper biological and reproductive functioning and a lack of direct threats, at least in Catalonia. However, some populations may be locally threatened and the species might become rare in some territories. Therefore we simply recommend a management plan and the long-time monitoring of some selected populations. On the other hand, *Delphinium bolosii* is EN ('endangered') given the low number of populations and individuals (three known populations, one of them extinct, and less than 3,500 individuals), low genetic diversity and threats to its habitat (tourism and agricultural activities). For this reason it is necessary to implement a recovery plan that consider both the conservation of the two existing populations and a reintroduction plan for the extinct one.

Introduction

A first step in the determination of the conservation status of a given threatened species and subsequently in the preparation of a conservation strategy for it, is the development of a basic study, integrating demographic, reproductive and genetic data (Oostermeijer 1996; Oostermeijer & al. 2003). However, although a great number of conservation studies were focused in the significance of genetics for population viability and the loss of genetic variation in small and isolated populations (Frankel & Soulé 1981; Schonewald-Cox & al. 1983; Barret & Kohn 1991; Young & al. 1996), for some time various authors have claimed

that demographic factors are of more immediate importance than genetics (Lande 1988).
Delphinium bolosii and *Thymus loscosii* are listed in the Spanish *Catálogo Nacional de Especies Amenazadas* (BOE 1990) as being 'at risk of extinction' (*en peligro de extinción*) (codes FL/52 and FL/40, respectively). This qualification implies some generic restrictions (including collection) and the need for preparing a Recovery Plan (according to article 31.2). Since the two species are in the one case totally and in the other partially distributed in Catalonia, the Department of the Environment of Catalonia (the DMA, pertaining to the autonomous government) sponsored, during the period 2000-2002, the basic scientific studies and the preparation of a plan for the Autonomous Community, both of which are presented below.
In both species the basic study was similar, although adapted to their particular characteristics. This study included chorological, demographic, reproductive and genetic diversity aspects. In order to ascertain the present geographic distribution in the territory studied, chorological prospecting in the field based on information from bibliographic compilation data and herbarium vouchers was carried out to confirm the presence of the species, work which was especially intense in the case of *T. loscosii*. In located populations we estimated their size and looked for possible adjacent populations. We also searched for the existence of hybrids in *T. loscosii*. Demographic monitoring was done to analyze the population dynamics and to identify the critical phases. Pollination ecology studies consisted of the following issues: dependence on pollinators for the reproduction of the species, insect visitors and main pollinator vectors (visitation rates and behavior), efficiency of pollination services in terms of stigmatic pollen loads, and reproductive success as fruit set and seed set in open-pollinated conditions. We also addressed specific questions such as the effects of gynodioecy on pollination and reproduction in the case of *T. loscosii* or the effects of population size on pollination mechanisms in *D. bolosii*. We assessed genetic diversity through allozyme electrophoresis performed in horizontal starch gels. To estimate the levels of genetic variation, we computed the following statistics: *P*, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; *A*, the mean number of alleles per locus; H_o, the observed heterozygosity; and H_e, the expected panmictic heterozygosity. The mean fixation index (*F*) for all polymorphic loci in each population was also computed to compare genotype proportions with those to be expected under Hardy-Weinberg equilibrium. Genetic divergence among populations was calculated with the fixation index (F_{ST}) or alternatively with the genetic differentiation coefficient (G_{ST}), although both parameters are considered equivalent (Nei 1975).

Delphinium bolosii

Delphinium bolosii C. Blanché & Molero (*Ranunculaceae*) is a very rare endemic, originally described in 1983 (Blanché & Molero 1983) and known only in three populations in Catalonia, one of them extinct (last record was in 1912, described under a different name). It is a rhizomatous, tall perennial larkspur (up to 1.5-2.0 m), producing rosettes of dissected leaves and simple racemes of up to (15) 40 (100) flowers. *D. bolosii* is a dysploid diploid (2n = 18), a trait very rare in the genus (Bosch 1999). It belongs to a group of plants with Eastern Mediterranean affinities, which arrived on the Iberian Peninsula in the

Messinian period (Blanché 1991). It is closely related to *D. fissum* Waldst. & Kit. (euploid diploid with $2n = 16$), which extends to the western Iberian Peninsula, but always in small and scattered populations, and to the recently described *D. mansanetianum* Pitarch, Peris & Sanchís (Pitarch 2002).
Conservation status: besides the Spanish Catalogue category (BOE 1990), it is also listed as 'endangered' (EN) both in a study of threatened flora of Catalonia (Sáez & al. 1998) and in the Red List of Vascular Spanish Flora (Aizpuru & al. 2000).

Demography
The total number of individuals of *D. bolosii*, including both juveniles (i.e. vegetative rosettes) and reproductives (i.e. plants with flowering raceme), ranged from 3200-3400 individuals over three consecutive years (Table 1). However, the actual number should be higher because some individuals may remain several years in the soil as dormant rhizomes and emerge when they accumulate enough resources to sprout successfully. The rate of rosettes that flower each season is around 10-24 % of those individuals, differing between populations (10-12 in Ulldemolins vs. 17-32 % in Rubió de Baix), indicating certain local components that should be considered in a reintroduction plan. Though this number seems low, it is sufficient to produce enough seeds to ensure survival (Table 2).
A demographic monitoring of a smaller sample of individuals (80-150 per population) has been carried out to study the demographic flow between the different life-span phenophases and to learn about the dynamics of the species. We have to take into account that demographic data from a period of three years are insufficient and also fragmentary because of the difficulty in determining certain parameters such as the percentage of rhizomes that

Table 1. Total number of individuals and number of reproductives in the two known populations of *D. bolosii*, over three consecutive years.

Year	Ulldemolins (Tarragona)		Rubió de Baix (Lleida)		Total species
	Total	Reproductives	Total	Reproductives	
2001	1368	139	1850	319	3218
2002	965	115	2426	793	3391
2003	1239	113	2156	481	3395

Table 2. Total fruit and seed production (%) in *D. bolosii* over three consecutive years.

	2001		2002		2003	
	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds
PRI	38.8	46.7	50.5	55.5	35.1	31.9
LNO	54.8	69.5	40.6	54.2	61.6	50.6

Table 3. Estimation of *T. loscosii* individuals in Catalonia in 2002.

UTM 10×10 km squares	Individuals
31TCF07	275441
31TCF08	63900
31TCF17	8963
31TCF27	1522142
31TCF28	25675
31TCF38	30414
31TCF49	3625
31TCF68	100
TOTAL:	1930260

remain latent each year (it is not possible to dig them up without affecting the population), the number of rosettes that emerged from those rhizomes or seedling mortality rates. The results obtained indicate that the most critical phase is the germination and seedling establishment (2-4 % of seeds). Values found in the field were notably lower than ones obtained under greenhouse conditions (20 %), although an underestimate of field values may occur since dispersal mechanisms can carry seeds far away from their progenitors. The longevity of individuals and rootstock bank seems to maintain the populations at a stable average size according to data from previous estimations (Blanché & Molero 1983; Bosch & al. 1998).

Reproductive biology of D. bolosii

D. bolosii has a complex floral morphology and, despite its apparent pollinator specialization for insects with robust bodies and/or with long proboscis that enable them to reach the nectar accumulated in the spur, it attracts a broad spectrum of visitors, basically Hymenoptera (bumblebees and halictid bees), Lepidoptera (*Macroglossum stellatarum* and a variety of diurnal species) and Diptera (Syrphidae and Bombylidae) (Bosch & al. 1998; Bosch & al. 2003). However, this difficult access to the nectar reward promotes nectar robber behavior, which has a notable incidence (in some flowering seasons up to 80 % of flowers presented holes in the spur), although with marked yearly fluctuations (Bosch & al. 1998). The effectiveness of pollination services measured as pollen quantity deposited on the stigmas showed that *D. bolosii* stigmas received large amounts of pollen (on average 360 pollen grains per flower for all species), and 4 % of this pollen was heterospecific. In one population some competition with *Rubus canescens* pollen for pollinator services was detected. Under open-pollinated conditions fruit set range from 39-60 % and seed set from 30-55 % (Table 2). An insect exclusion test showed some dependence on insect pollination, although spontaneous self-pollination is given; around 20 % of seeds are produced in bagged flowers. This result denotes the self-compatible character of *D. bolosii* and indicates that its proterandric mechanisms are not effective in preventing self-pollination.

Germination rates under greenhouse conditions are relatively low and ranged from 0-21 %. Seeds exhibit a dormancy period of around three months.
In addition to sexual reproduction, *D. bolosii* also displays vegetative multiplication by rhizome fragmentation which would make possible a rootstock bank strategy.

Genetic diversity studies of D. bolosii
Low levels of genetic diversity were found in the two populations of *D. bolosii* studied (Table 4) which agree with the lack of variability expected for endemic species (*P* = 26.3%, *A* = 1.39, and *He* = 0.063; Hamrick & Godt 1990). Low levels of diversity found in endemics are due mainly to their small population sizes, isolation of populations and adaptation to a uniform habitat (Barrett & Kohn 1991; Ellstrand & Elam 1993). Nevertheless, levels of genetic diversity are also greatly dependent on several life-history traits, population history and also type of speciation process (Hamrick & Godt 1990; Booy & al. 2000; Dodd & Helenurm 2002). Values of genetic diversity of *D. bolosii* are somewhat lower than mean values found for other perennial larkspurs (compiled in López-Pujol & al. 2003), which suggests that the explanation for the lack of variability should be looked for in its historical traits. Currently it is considered a derivative species from a widespread progenitor, *D. fissum*, and thus should have suffered a phenomenon of dysploidy (from 2n =16 to 2n = 18; Blanché 1991), a loss of genetic variation and a drop in the number of alleles, according to the recently arrived at progenitor-derivative species model (Gottlieb 1973; Crawford 1983).
Analysis of fixation indices in *D. bolosii* revealed that populations are very close to Hardy-Weinberg equilibrium, even showing a slight excess of heterozygotes. These data are somewhat surprising though this species is primarily outcrossing, because autogamy can achieve 20% and limited rhizomatous growth is present. This can be due to selection against homozygotes due to heterozygote advantage; the two populations may be composed of highly heterozygous individuals that are probably long established since formation of new seedlings is relatively rare within populations. Similar phenomena have been described for *Gentiana pneumonanthe* (Raijmann & al. 1994) and *Arnica montana* (Kahmen & Poschlod 2000; Luijten & al. 2000).
The high value of F_{ST} (0.310) reveal that the two populations of *D. bolosii* are genetically very divergent, as is to be expected for populations separated by a great distance (more than 60 km). Given the most likely absence of gene flow between populations, genetic drift has probably been acting within populations, first leading to changes in allele frequencies

Table 4. Genetic variation for 15 loci in 2 populations of *D. bolosii*, and 5 loci in 8 populations of *T. loscosii*. ***N:*** sample size; ***P:*** percentage of polymorphic loci; ***A:*** mean number of alleles per locus; ***Ho:*** observed heterozygosity; ***He:*** expected panmictic heterozygosity.

Species	***N***	***P***	***A***	***Ho***	***He***
D. bolosii	203	20	1.23	0.067	0.065
T. loscosii	257	85	3.0	0.472	0.442

and finally to the loss of rare alleles. This, in addition to the likelihood of introduction of new alleles by mutation, could explain the substantial genetic divergence found between the two populations.

Thymus loscosii

Thymus loscosii Willk. (*Lamiaceae*) is an endemic plant restricted to the Ebro river basin in the northeastern part of the Iberian Peninsula. It is distributed over Catalonia, Aragon, La Rioja, Castilla-León, Navarra, and Euskadi. It has hundreds of thousands of individuals occupying 52 UTM (10×10 km squares), and an extent of occurrence of 28,000 km^2. Previous to this study it had been reported from 19 10×10 km UTM in Catalonia.
T. loscosii is a gynodioecious perennial plant, 9-15 cm tall, with abundant stoloniferous branches. Inflorescences are erect, formed by whorls containing about 6-10 zygomorphic, whitish, small flowers. It is a tetraploid species ($2n = 54$, Morales 1986). Some hybrids with other species have been described: *Thymus* ×*rubioi* Font Quer = *T. loscosii* × *T. vulgaris* subsp. *vulgaris*, *T.* ×*riojanus* Uribe-Echebarria = *T. loscosii* × *T. mastichina*; *T. x aragonensis* G. Mateo, M. B. Crespo & Mercadal = *T. loscosii* × *T. zygis* subsp. *zygis*. But field search on the *locus classicus* did not reveal hybrid evidence after pollen viability tests and chromosome counting, but only some morphological variation interpretable as intraspecific variation. Outside the studied territory (Catalonia), the hybrid effectively exists.
Conservation status: besides the category assigned to it in the BOE, it has been locally reported as "rare" in Aragon, where a conservation strategy for the species has been developed (Sainz-Ollero & al. 1996), "vulnerable" in Navarra (BON 1997) and "of special interest" in Euskadi (IKT 2001). Recently, due to the increasing number of new populations of *T. loscosii* found, it has been excluded from the Red List of Spanish Vascular Flora (Aizpuru & al. 2000), which indicates that it is considered to be at a low level of threat in Spain as a whole. However, the natural erosion of the substrate and external factors, such as grazing or tourism, are threats that may increase the *T. loscosii* vulnerability (Sainz-Ollero & al. 1996).

Demography
In Catalonia *T. loscosii* has been reported from 19 UTM 10×10 km squares in which in 11 of the squares, after intensive research, it has not been found again. The 8 UTM with confirmed presence of *T. loscosii* contain in total 33 populations. This is probably due to a previous confusion with other taxa such as *T. fontqueri* (Jalas) Molero & Rovira or because the species was represented by a very few isolated individuals that were not found. An estimation of the number of individuals in the 8 UTM in 2002 is illustrated in table 3.

Reproductive biology of T. loscosii
Occurrence of gynodioecy in this species, i.e. the presence of hermaphrodite and female individuals in the same populations, ranged from 0 to 18 % of female plants (at least in Catalonia populations). These values are low in comparison with what has been reported for other *Thymus* species (Morales 1986; Manicacci & al. 1998; Valdés & al. 2001; Thompson & al. 2002). Morphologically it is relatively easy to differentiate the gender,

given that hermaphrodite flowers are larger (4.82 ± 0.44 mm of length, n = 284) than female ones (3.68 ± 0.37, n = 41, ANOVA $F_{1,323} = 244.6$ $P = 0.000$). Females (male steriles) may sometimes have stamens, but these are rudimentary and do not produce pollen (Rovira 1979). Hermaphrodites are protandrous, a mechanism that serves to avoid self-fertilization and to promote allogamy. The blooming period is in June and lasts from 3 to 4 weeks.

The most frequent visitors to *T. loscosii* were *Apis mellifera* (about 58 % of total visited flowers in 2002) and several species of *Bombylius* (35 %), although others such as halictid bees, syrphidae or diurnal butterflies also visited, contributing to pollination. Pollinator services in terms of deposition of pollen loads on the stigmas are low (the mean number of pollen grains of all species was approximately 20 and around 12 were conspecific). Heterospecific pollen belongs mainly to *Asperula cynanchica* and *Salvia lavandulifolia*. Reproductive success in natural populations was relatively low (about 15-25 % of seed set), but similar to other Iberian *Thymus* species (Manicacci & al. 1998; Valdés & al. 2001). An insect exclusion test revealed a marked dependence on insect pollination, although the species may be able to set seeds by self-pollination (and thus is self-compatible), but at very low rates.

The study of the effects of gynodioecy on pollination and reproduction showed that hermaphrodite flowers in general received more visits than females, possibly as a response to visual attraction due to the different flower size. They also received more conspecific pollen loads. Nevertheless, seed set from open pollinated flowers was similar between genders. Even female seeds were slightly heavier and began to germinate earlier. Female bagged flowers were able to produce seeds without pollen intervention and thus by apomictic mechanisms, which can assure reproduction in a pollinator scarcity situation.

Seeds exhibited moderate germination rates (0-50 %), higher than values reported previously (Morales 1986; Sainz-Ollero & al. 1996) but lower than values reported by Albert & al. (2002). Germination conditions are important and should be considered in a management plan.

In addition to sexual reproduction, the species presents vegetative propagation by stolons, profusely observed both in the field and under greenhouse conditions.

Genetic diversity studies on T. loscosii

Allozyme data support the hypothesis suggested by Morales (1986) that *T. loscosii* is an autotetraploid. No evidence of fixed heterozygosity was found in *T. loscosii*, and all loci except two showed both homozygotes and heterozygotes (balanced and unbalanced). These two loci displayed only heterozygous phenotypes, but these were both balanced and unbalanced. Tetraploidy allows for the presence of three or four alleles at a single locus since loci are duplicated (Soltis & Rieseberg 1986; Soltis & Soltis 1989; Mahy & al. 2000). In *T. loscosii*, 37.2% of all plants possessed three or four alleles for at least one of the examined loci, a value close to that obtained for other autotetraploid taxa (Soltis & Soltis 1989; Mahy & al. 2000).

Genetic diversity was high in the eight studied populations of *T. loscosii* (Table 4), as is expected for autotetraploids as a consequence of tetrasomic inheritance (Soltis and Soltis 1989), and its high values of genetic variation are within the range of other autotetraploids (compiled in López-Pujol & al. 2004). As observed in the field, *T. loscosii* can undergo vegetative propagation through stolons, which can maintain genetic variation, once produced, within populations.

Negative values for the fixation index were found at most loci and for most populations of *T. loscosii*, indicating an excess of heterozygotes compared to Hardy-Weinberg expectations, which could be explained by: (i) selection against homozygotes, (ii) random stochastic events, (iii) barriers to inbreeding, and (iv) sampling error. The excess of heterozygotes detected in *T. loscosii* might be explained by some barriers to inbreeding, e.g. occurrence of gynodioecy, together with a marked protandry and low rates of selfing. Alternatively, vegetative propagation may have contributed to the maintenance of the heterozygote excess.

The very high genetic identities found between populations (only around 3% of allozyme variation in *T. loscosii* is due to differences between populations) may be explained by recent and rapid fragmentation from a wide continuous area resulting in genetically similar populations. Chorologic research in recent years has revealed the existence of many additional populations, which may indicate that *T. loscosii* formerly had a continuous distribution along the Ebro river basin, an area that has been extensively replanted with forest during the last decades (Blanco & al. 1997) and is currently experiencing a fragmentation process caused by change of land use.

Conclusions

The results of these 3-year studies, together with the reevaluation of category using 2001 IUCN criteria (IUCN 2001), which are more restrictive than those of 1994, lead to different conclusions for the two species studied (Table 5). *D. bolosii* should be considered an endangered species, with a notably high threat level (EN) in the context of Catalan, Iberian and/or Mediterranean s.l. flora, whereas in *T. loscosii*, the lack of severe threats, the sufficient number of individuals to counteract environmental stochasticity, and a proper biologic and reproductive performance suggest the need to lower the threat category for this species (despite its rarification in some territories), considering it as 'of special interest' according to Spanish law and as 'least concern' (LC) according to IUCN nomenclature. Its inclusion in the CNEA was probably due to insufficient knowledge available before the species was listed. The present results show one of the side effects of including species in conservation lists: greater attention drawn to these taxa promote research priorities resulting in improved knowledge of a given species, hence leading to the need of updating those lists periodically. However, some populations of *T. loscosii* are locally endangered (Ebro Depression) or may contain valuable genetic combinations. Thus we suggest the establishment of a low level management plan including 3(5)-year reevaluation and conservation of some selected populations as an alternative to the high-level recovery plan established by law (BOE 1990). Conservation strategy should be focused on Conservation Units (CU) with higher fragility -higher diversity- or improved coordination with other conservation activities.

According to specifications set by law, the conservation status of *D. bolosii* suggests the need of a recovery plan, which should be similar to the existing plan for the closest species *D. fissum* subsp. *sordidum* (DOCM 2002), including the actions described below. Prior to the management of this species, legal actions must be considered, such as promoting the inclusion of *D. bolosii* in Annex 2 of the EU Directive "Habitats". Conservation *in situ* is

Table 5. Adaptation to current protection measures: revaluation using IUCN (2001) criteria, proposed Spanish Catalogue (CNEA) categories and proposed conservation measures.

Species	IUCN evaluation (2001)	Proposed CNEA Category	Proposed measures
D. bolosii	EN B1ab(iii,iv,v)+2ab(iii,iv,v)C1	Endangered (*en peligro de extinción*)	Recovery Plan
T. loscosii	LR lc	Special interest (*de interés especial*)	Management Plan

thought to be the best measure of protection, because it maintains the interactions between the plant and its ecosystem without detaining the evolutionary processes (Barrett & Kohn 1991). The establishment of Natural Reserves for Flora to protect the two existing populations probably constitutes one of the most feasible *in situ* measures, and is a figure already contemplated in the Catalan law but still not developed for plant species although some close territories proved the usefulness of those small reserves (Microreserves, as in the Valencian Country, cfr. Laguna 2001). Additionally, intensive research of possible new populations in the vicinity of the known localities should be performed. Given the low number of individuals of this species and the extinction of former populations, another worthy *in situ* measure is the establishment of a reintroduction plan, i.e. the re-establishment of a new population in the extinct locality (historical range). According to this study, 600 seeds per year (during ten years) should be collected in the larger population (i.e. Rubió de Baix, Lleida) in order to obtain 120 self-sustainable individuals, after taking into account germination and survival rates. On the other hand, several *ex-situ* conservation measures should be included. The most frequent is seed preservation in a germplasm bank and cultivation in botanical gardens. The number of seeds that should be collected is around 350 in total, in accordance with the formula proposed by the Center for Plant Conservation of St. Louis (CPC 1991). Nevertheless, we also recommend the establishment of a 3-year monitoring plan. Research on Conservation Biology mainly focused on demography is suggested, though this is not strictly speaking a conservation measure, but should provide scientific guidelines for adequate conservation management. Another aspect which must be enhanced is education, e.g. funding an education plan aimed at wildlife agents and the general public.

For *T. loscosii* a management plan (not a 'conservation plan' in Spanish legal terms) is proposed. This option agrees with the scientific data suggesting the lowering of the protection category but allows protecting some ESU (Evolutionary Significant Units) or CU (Conservation Units) in accordance with the suggestions from other regions (Aragon, Navarra and Euskadi).

In relation to legal actions, the possibility should be considered of adding some interesting populations of *T. loscosii* growing in protected natural areas as "Serra del Montsant", "Muntanyes de Prades" and "Ancosa-Montagut", to Annex 3 of the PEIN (Pla d'Espais d'Interès Natural de Catalunya), which includes a list of protected species in each area.

Table 6. Populations of *T. loscosii* to be conserved and corresponding arguments.

Population and UTM (1×1 km)	**Argument**
Vallclara (Conca de Barberà) 31TCF3283	The sum of these 3 populations includes 100 % of all detected alleles for *T. loscosii*
Albarca (Priorat) 31TCF2474	
La Granadella (Garrigues) 31TCF0380	
La Palma d'Ebre (Baix Ebre) 31TCF0172	Small population size
Ulldemolins (Priorat) 31TCF2075, 31TCF2076, 31TCF2174, 31TCF2175, 31TCF2176, 31TCF2178	Set of populations and subpopulations on the Montsant PEIN range which include the greatest part of total individuals in Catalonia
Montagut (Alt Camp) 31TCF6885	Particular morphological phenotype

One of the 'low level' *in situ* conservation measures proposed for this species is the preservation of the populations' health listed in the Table 6, selected using criteria of representativity and maximum coverage of genetic diversity. On the other hand, long term monitoring of conservation status should include two levels of control. The first level is in the short run: 2- year general surveys by visual inspection on the 6 selected localities to evaluate the population size, conservation conditions, habitat trends and possible incidences that may affect the species. The second level is long term: 10-year assessment by means of a new census and analysis of trends during this period. It should include a reevaluation of the applied measures and reconsideration of the management and monitoring plan. In order to guarantee the *ex situ* conservation of the genetic diversity of *T. loscosii*, seeds from four populations (Albarca, La Granadella, Vallclara and Montagut), in amounts of 350 seeds per population, should be collected to be preserved in a germplasm bank and cultivated in botanical gardens. The minimum seed amount is calculated using the formula proposed by the Center of Plant Conservation (CPC 1991). Last but not least we should mention the need to promote a shared conservation program with the other territories or administrative areas where there is a presence of *T. loscosii*.

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