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Habitat characterization and decline of Critically Endangered *Onobrychis conferta* subsp. *conferta*

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ABSTRACT

Rare endemic plant species are very susceptible to anthropogenic threats and natural changes, thereby facing a high risk of extinction. The Mediterranean Basin supports a rich flora with high percentages of endemic species. An iconic case of Mediterranean narrow endemic plant taxon is represented by *Onobrychis conferta* subsp. *conferta* (Desf.) Desv. (Fabaceae). Management of this species requires better information on its habitat requirements and population status. Field surveys and historical records were used to characterize the distribution of *O. conferta* in Tunisia. Habitats were characterized using topography, soils, climate, and land cover variables. Expeditions on ten previously reported sites revealed the presence of *O. conferta* in only one of the previous recorded localities and, for the first time, in two new localities. We recorded a sharp decreasing trend for the total number of plants per population in all locations over 6-year period. Our findings showed that the current status of *O. conferta* in Tunisia is Critically Endangered (CR). *Onobrychis conferta* faces an extremely high risk of extinction in the wild. Therefore, prompt comprehensive conservation actions are needed to ensure its survival. We propose a conservation and recovery recommendations including *in situ* and *ex situ* conservation actions. *In situ* actions are aimed at minimizing the effects of the identified threats and to create new populations of *O. conferta* in its characterized habitat. *Ex situ* actions include an environmental education campaign and seed preservation.

1. Introduction

The Earth is currently undergoing its sixth mass extinction event, primarily caused by human activities, which have escalated the extinction rate to 100–1000 times the natural pace (Pyron and Pennell, 2022; Fenu et al., 2023). The diversity of plant species worldwide faces a significant threat, with approximately 40 % of identified plant species at risk of extinction, according to recent estimates (Kew, 2020). Over the last two decades, there has been a notable rise in the number of vascular plant species classified as threatened (IUCN, 2023). The Mediterranean Basin is renowned for its high plant diversity and is acknowledged as the second largest among the 34 Global Biodiversity Hotspots (Mittermeier et al., 2004). It is characterized by high plant diversity that is mainly concentrated in restricted areas such as islands, peninsulas, and high mountains (Cañadas et al., 2014; Fenu et al., 2020). These areas are characterized by a concentration of endemic species, notably range-restricted taxa that are of conservation importance because of their often reduced, isolated and threatened populations (Orsenigo et al., 2018; Fenu et al., 2020). In fact, the Mediterranean Basin supports a rich flora with c. 25,000 native species, coupled to high percentages of endemic species (50–59 %) (Greuter, 1991; Médail & Quézel, 1997). Narrow endemism is the cornerstone of Mediterranean plant diversity, with c. 60 % of all native taxa occurring only in this region and c. 37 % being narrow endemic species (Thompson, 2020), with the latter comprising c. 11,000 taxa (Médail & Baumel, 2018).

On many occasions, narrow endemic species grow in specialized habitats with specific requirements (Levin, 2019). Thus, the decline of suitable habitats is a major threat for these endemic species (Miranto et al., 2012; Allen et al., 2014). These endemic species are exposed to a severe biotic and abiotic factor that have led to a high degree of habitat fragmentation as a result of soil overexploitation, the conversion of much of the pristine vegetation into agricultural landscapes, and climate change since the Mediterranean Basin is a global warming hotspot (Fenu et al., 2020, 2023; Benítez-Benítez et al., 2022; El-Khalafy et al., 2024).

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In addition, climate change is considered one of the most important drivers of the current loss of biodiversity (Varela et al., 2022; Benítez-Benítez et al., 2022). Biodiversity loss is a problem that needs to be urgently addressed, particularly with the uncertainties of climate change (Bobo-Pinilla et al., 2020).

An iconic case of Mediterranean narrow endemic plant taxon is represented by the pastoral legume species from semi-arid Tunisian dorsal mountains *Onobrychis conferta* subsp. *conferta* (Desf.) Desv. (Fabaceae). This plant species is present only in a few disjunct localities in Tunisia and Algeria, always in small populations. Very little is known about the ecology, distribution and population size of *O. conferta* subsp. *conferta*, and even less about its conservation status. Information on *O. conferta* subsp. *conferta* is not available from herbarium materials, literature, and database of the Tunisian National Gene Bank. *Onobrychis conferta* subsp. *conferta* is a rare perennial species thriving as isolated individuals in arid and semiarid calcareous skeletal soils on the western Tunisian ridge and the Saharan Atlas above 1000 m above sea level (Abdelguerfi & Laouar, 2002; Le Houérou, 1995; Le Houérou, 1987). Pottier-Alapetite (1979) reported the presence of *O. conferta* subsp. *conferta* only in ten locations in Tunisia. Since 1979, there have been no additional records of this taxon, and it is not known to be present in any *ex situ* conservation collections. *Onobrychis conferta* subsp. *conferta* has been characterized as highly palatable, consumed by cattle, sheep, goats, camels, horses and donkeys (Le Houérou, 1980). Schoenenberger (1972) reported that permanent pastures can be created using local pastoral species, such as *O. conferta* subsp. *conferta* giving high yields. Thus, *O. conferta* subsp. *conferta* emerges as an intriguing biotool for rehabilitating degraded areas (Le Houérou, 1995; Le Houérou, 1987). So far, no research has been carried out on the conservation of *O. conferta* subsp. *conferta*. Therefore, the aim of this study was to inventory and characterize all populations of *O. conferta* subsp. *conferta* in Tunisia, to determine its current conservation status and to develop conservation recommendations for this endemic taxon. We hypothesized that the distribution of *O. conferta* subsp. *conferta* in Tunisia would be limited to very few locations, where the populations of this endemic taxon might be suffering from human-mediated environmental impacts, such as overgrazing, and experiencing a sharp decline.

2. Material and methods

2.1. The study species

Onobrychis conferta (Desf.) Desv. is distinguished from other *Onobrychis* species by its fruits of 8–10 mm pubescent pods with a radiating crest with short triangular prickles (Pottier-Alapetite, 1979). Pilosity variations have led to the distinction of two subspecies: subsp. *hispanica* (Širj.) Guittonneau & Kerguélen with leaflets and calyx teeth not very hairy native to the Pyrenees and east Iberian Peninsula, and subsp. *conferta* (Desf.) Desv. with silky-silvery leaflets and calyx teeth (Tison & de Foucault, 2014) native to arid and semiarid *Pinus halepensis* Mill. forests in North Africa (Pottier-Alapetite, 1979). *Onobrychis conferta* can have various ecological and socio-economic functions such as supplying forage to grazing animals, nitrogen fixation, providing nectar for bees, hosting wildlife and controlling erosion (Rios et al., 1991).

2.2. Population survey and plant material collection

Onobrychis conferta subsp. *conferta* plants were identified following Guittonneau & Kerguélen (1991). Based on data published by Pottier-Alapetite (1979), field prospecting missions were carried out to verify the data on all previously ten known locations of *O. conferta* subsp. *conferta* in north-western Tunisia during May, June and July 2016. Prospected sites and distribution map of *Onobrychis conferta* subsp. *conferta* in Tunisia was performed using software ArcGis 10.6.1 (Esri®, California, USA). Those localities where *O. conferta* subsp. *conferta* was recorded were re-surveyed annually until 2021. The geographic locations of the collection sites were recorded using a GPS (Garmin 72H receiver, Olathe, Kansas, USA). Herbarium specimens of the sampled *O. conferta* subsp. *conferta* populations were preserved at the Herbarium of Higher School of Agriculture of Moghrane.

Data obtained from each population of *O. conferta* subsp. *conferta* included: latitude, longitude, altitude above sea level, bioclimatic zone according to Emberger's (1976), surface (m^2), maximum and minimum annual air temperature (°C), number of rainy days per year, mean annual precipitation (mm), slope orientation and inclination (%), soil texture (percentages of pebbles, sand, silt and clay), soil pH, electrical conductivity (EC), organic matter (%), total nitrogen (N) (ppm), total phosphorus (P) (ppm), and exchangeable potassium (K) (ppm). Climatic information was obtained from the Ministry of Agriculture, Hydraulic Resources and Fisheries of Tunisia based on the normal data from a 25-year weather period. Soil samples (n = 3 samples per population) were collected from 0 to 30 cm depth as close to *O. conferta* subsp. *conferta* stands as possible, dried to constant dry weight and sieved through a 2-mm sieve. Soil texture was determined according to the diagram of USDA Soil texture triangle (Pradeep et al., 2021). Soil substrate was characterised according to Toluei et al. (2012). Because of the presence of extensive rock surfaces, a visual estimation of rock percentage covering the sampling areas was carried out. Soil pH was recorded in 1 M KCl (soil:extractant mixture 1.0:2.5 v:v) using a pH-meter (Sartorius PB-20, Germany). Soil electrical conductivity (EC) was measured by using distilled water extract (soil:distilled water, 1:5 v:v) using a conductivity meter (Hanna EC 215, Lingolsheim, France). Soil organic matter content (%) was recorded following Tjurin method (Rinkis et al., 1987). Total nitrogen (N) content was determined using the Kjeldahl method (Hesse, 1971). Total phosphorus (P) was determined spectrophotometrically according to Tandon (1993) using a spectrophotometer (Jenway 6300, Cole-Parmer, United States). Exchangeable potassium (K) content in soil was extracted using neutral normal ammonium acetate solution using flame photometry (Jenway PFP7, Cole-Parmer, United States) (Thomas, 1982). Total limestone was determined according to the Bernard calcimeter method (Lamas et al., 2005). The active calcium carbonate equivalent or active lime was determined using the NH_4 oxalate method as described by Drouineau (1942).

The three populations were found at south-facing slopes, occupying a very restricted area between 10–22 m^2 at elevations ranging from 532 to 930 m a.s.l. in upper semiarid bioclimatic zones with temperate or cool winter. Minimum and maximum annual air temperatures ranged from 3.7 to 5.0 °C and 35.1 and 36.5 °C, respectively. Mean annual precipitation was between 384.6 and 454.8 mm distributed in 58–61 rainy days. The soils were classified as fine clay loam or loam with relatively low contents of organic matter (0.7–2.7 %), high total limestone percentage ranging (50–58 %), and alkaline pH ranging from 7.2 to 8.7 (Table 1).

Each population of *O. conferta* subsp. *conferta* was characterized by counting the number of individual plants. The population structure was evaluated by grouping individual plants into three different size classes: seedlings (diameter < 30 cm), mature plants without reproductive structures (Without RS; diameter > 30 cm) and mature reproducing plants (With RS) that had already flowered. The vegetation in every *O. conferta* subsp. *conferta* population was recorded applying the sampling method of Braun–Blanquet (Westhoff & Van der Maarel, 1978) in 5 plots per population, sized 25 m^2, from 2016 to 2020. The cover-abundance scale was based on 7 levels: r (<5% range of cover; one individual), + (<5% range of cover; few individuals), 1 (<5% range of cover; numerous individuals), 2 (5.1–25 % range of cover), 3 (25.1–50 % range of cover), 4 (50.1–75 % range of cover) and 5 (75.1–100 % range of cover). The constancy was determined as the number of times each taxon occurred over the five plots. Plant taxa identification was made at species level using floras for Tunisia (Cuénod, 1954, Pottier-Alapetite, 1979; 1981), Algeria (Quézel & Santa, 1962–1963) and North Africa (Maire, 1952–1987).

Table 1
Ecogeographical variables for the three recorded populations of *Onobrychis conferta* subsp. *conferta* in Tunisia.

	Population OC1	Population OC2	Population OC3
Province, location	Kef, Dyr El Kef	Siliana, Fedj El Hdoum	Siliana, Aïn Dyssa
Latitude	36°12′35.55″N	36°20′28.70″N	35°57′47.89″N
Longitude	8°44′32.90″E	9° 7′50.53″E	9°15′22.16″E
Elevation (m above sea level)	930	532	868
Bioclimatic zone[a]	USATW	USATW	USACW
Population surface (m^2)	22	10	14
Maximum air temperature (°C)	35.1	36.5	36.0
Minimum air temperature (°C)	3.7	5.0	4.9
Rainy days per year (#)	59.52	61.84	58.5
Annual precipitation (mm)	428.7	454.8	384.6
Slope orientation	Southeast	Southwest	Southwest
Slope inclination (%)	10	30	25
Pebbles (%)	30	75	5
Silt (%)	30.4 ± 3.7	40.9 ± 2.3	35.8 ± 8.0
Clay (%)	39.8 ± 3.0	20.4 ± 5.7	37.8 ± 9.4
Sand (%)	29.8 ± 3.6	38.7 ± 3.1	26.4 ± 2.2
Soil pH	7.8 ± 0.7	8.7 ± 0.3	7.2 ± 0.9
Soil organic matter (%)	1.3 ± 0.6	0.7 ± 0.3	2.7 ± 1.0
Soil N (ppm)	120.2 ± 18.0	160.4 ± 10.7	208.4 ± 11.60
Soil total P (ppm)	36.9 ± 7.2	49.8 ± 11.8	90.3 ± 9.1
Soil available K (ppm)	89.6 ± 4.5	42.3 ± 7.1	70.8 ± 11.8
Electrical conductivity (dS m^{-1})	0.4 ± 0.07	0.5 ± 0.2	0.7 ± 0.2
Total limestone (%)	57.9 ± 6.8	52.6 ± 9.3	50.0 ± 6.0
Active limestone (%)	28.6 ± 4.2	29.3 ± 4.3	30.7 ± 2.6

[a] Bioclimatic zone are defined according to Emberger's (1976) coefficient: USATW, upper semiarid variant at temperate winter; USACW, upper semiarid at cool winter. a.s.l.: above sea level.

2.3. Population status and conservation

In an attempt to assess the global extinction risk by studying the Tunisian and Algerian *O. conferta* populations, we consulted the Algerian flora and the botanists, no data or recent records were available in relation to the distribution area of the species in Algeria. Therefore, the preliminary conservation status of *O. conferta* subsp. *conferta* populations was assessed using the IUCN Red List criteria at regional level (IUCN, 2012a). For criterion B, geographic range of the species in the form extent of occurrence (EOO) and area of occupancy (AOO) was estimated using Geospatial Conservation Assessment Tool (http://geocat.kew.org/; Bachman et al., 2011). The estimate of the AOO, according to the IUCN guidelines, has been calculated using fixed grid of 2 × 2 km (IUCN, 2019). In the distribution area of *O. conferta* subsp. *conferta*, threats and stresses, to which its populations were subjected, were identified following the classification of IUCN (2012b, 2012c). For each threat and stress, the extent, occurrence, frequency, severity, certainty of causality and level of priority for conservation were assessed as reported by García-Beltrán et al. (2022). The extent and occurrence of the threat was considered according to its existence in space (point, if it affected ≤ 25 % of the population area, or broad, if it affected > 25 %) and time (past, present, imminent or not imminent). The frequency of the threat was assessed as its periodicity (once, seasonal, continuous or recurrent). Severity was considered high if it affected at least 75 % of individuals, medium if it affected 11–74 % of individuals, and low if it affected less than 10 %. Certainty of causality was treated as high if there was evidence linking threat and stress, medium if there was a correlation between threat and stress, and low if the relationship was assumed or logical. The priority of each threat for conservation was considered at two levels: high (if it was key to the recovery of the species) and low (if it influenced the recovery of the species) (García-Beltrán et al., 2022). For each threat and stress, the causal relationships between them were identified. Based on the results obtained, the conservation status of *O. conferta* subsp. *conferta* was reassessed according to IUCN categories and criteria (2012a, 2019). Finally, recovery recommendations for the species were proposed on the basis of the identified threats.

2.4. Data analysis

A Principal Component Analysis (PCA) was performed to reduce the number of environmental variables recorded in the *O. conferta* populations, analysing the correlation matrix using PAST software v. 4.03 (Hammer et al., 2001). Data of population decline over years were fitted to the best non-linear regression model using Sigma Plot 2012 v.12.0 (Systat Software Inc., San Jose, California, USA). Non-linear regression with 4 parameters sigmoid model has been used to calculate R^2.

3. Results

3.1. Population characterization

Onobrychis conferta subsp. *conferta* was only found in 3 of the 10 surveyed locations (Fig. 1., S1). The surveyed sites were *O. conferta* was absent have a converted habitat and were affected by natural disturbance and anthropogenic activities. These sites were characterized by a high domestic and wild herbivores pressure, as well as by the many human activities (i.e. trampling and off-road activities). The Principal Component Analysis (PCA) extracted two components with eigenvalues higher than one. The first component (PCA1) explained 53.7 % of the total variance and was positively correlated with the altitude, bioclimatic zone, substrate, clay percentage, total N, organic matter and active limestone, and negatively correlated with the number of rainy days, annual precipitations, pebble percentage, texture, sand percentage and pH. The second component (PCA2) explained 46.3 % of the total variance and was positively correlated with minimum and maximum air temperatures, slope inclination and orientation, silt percentage, total N, available K, electrical conductivity and active limestone percentage. PCA2 was negatively correlated with altitude, total P and total limestone (Table 2).

Twenty-seven species were recorded in the three recorded *Onobrychis* populations, including two endemic species to Tunisia (*Medicago tunetana* Murb. and *Onobrychis kabylica* (Bornm.) Širj.) (Table 3). The number of observed plant species was 11 for Population OC3, 8 species for populations OC1 and 9 species for OC2. Trees were represented by two species (*Pinus halepensis* Mill. and *Olea europea* L.), shrubs by one species (*Nerium oleander* L.), and herbs by 25 species. *Hedysarum coronarium* L. was the only species present in all study locations. Populations OC1 and OC2 shared 4.3 % of species, while OC1-OC3 and OC2-OC3 shared 12.5 % of species. Population OC1 was distinguished by the presence of *Sanguisorba minor* Scop., Population OC2 was characterized by the occurrence of *Olea europaea* L., and Population OC3 included some waterlogging tolerant species such as *Trifolium fragiferum* L., *Melilotus segetalis* (Brot.) Ser. and *Nerium oleander* L. (Table 3). Overall, half of the species have constancy equal to 20 % while 35.7 % of them have constancy of 40 %. Only 10.7 % of the species have a constancy higher than 60 %. All the species where OC3 occurs have a constancy of 20 %.

The total number of *Onobrychis* plants showed a sharp decrease for all populations over the 6-year monitoring period, varying from 196 individuals in 2016 to only 74 individuals in 2021. The three recorded populations were characterized by a low number of mature reproducing plants which, on average, varied between 69 and 182 individuals, depending on the year. The number of *Onobrychis* plants decreased 56 % in Population OC3 (R^2 = 0.9187), 61 % in Population OC1 (R^2 = 0.9187), and 65 % in Population OC2 (R^2 = 0.8468) from 2016 to 2021.

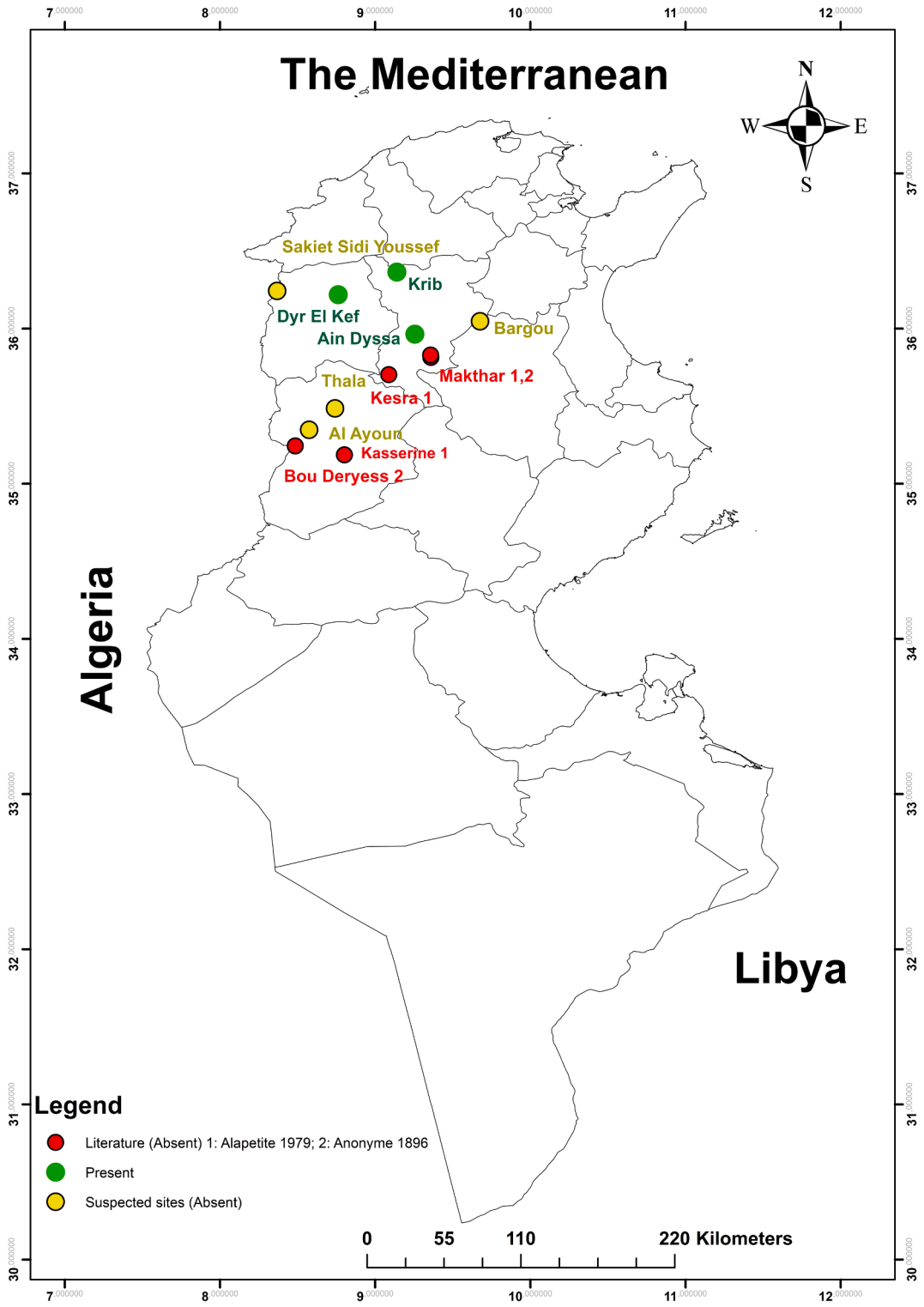

Fig. 1. Prospected sites and distribution map of *Onobrychis conferta* subsp. *conferta* in Tunisia.

In 2021, population OC3 presented only 18 mature individuals with only 3 individuals producing seeds. Only one seedling was recorded in populations OC2, and none in populations OC1 and OC3 (Fig. 2).

3.2. Population status and conservation

By considering only the Tunisian populations and using the IUCN Red List criteria at regional level (IUCN 2012a), we have classified *O. conferta* subsp. *conferta* as Critically Endangered (CR) B1ab(ii, iii, v); B2ab(ii, iii, v); C1 + 2a(i). The threats and their associated stresses affecting the conservation status of *O. conferta* subsp. *conferta* are presented in Table S1.

Applying the Criterion B, the Geospatial Conservation Assessment Tool estimates that the Extent of Occurrence (EOO) and Area of Occupancy (AOO) of *O. conferta* subsp. *conferta* were 2200 Km^2 and 12 km^2, respectively, therefore less than the EN category threshold of < 500 Km^2

Table 2
Factor loadings (PC) of the individual variables obtained by a Principal Component Analysis (PCA) on traits of the three collected populations of *Onobrychis conferta*.

	PC 1	PC 2
Soil organic matter	**0.975**	0.223
Bioclimatic zone	**0.867**	0.498
Substrate	**0.867**	0.498
Clay percentage	**0.814**	−0.581
Altitude	**0.783**	**−0.622**
Total N	**0.728**	**0.686**
Active limestone	**0.656**	**0.755**
Total P	0.597	**−0.802**
Electrical conductivity	0.571	**0.821**
Exchangeable potassium	0.545	**0.839**
Slope orientation	0.002	**1.000**
Minimum air temperature	−0.067	**0.998**
Slope inclination	−0.238	**0.971**
Total limestone	−0.325	**−0.946**
Maximum air temperature	−0.351	**0.937**
Silt percentage	−0.484	**0.875**
Texture	**−0.865**	0.502
Sand percentage	**−0.968**	0.252
Rainy days	**−0.975**	0.221
Annual precipitation	**−0.990**	−0.144
Soil pH	**−0.998**	0.070
Pebble percentage	**−1.000**	0.002

for the EOO and 5000 Km^2 for the AOO; furthermore, the species is known at only one location (*sensu* IUCN) and a decline of the number of mature individuals, area of occupancy as well as area, extent and quality of habitat are quantified (Fig. 2). Under criterion B, the species is assessed as Critically Endangered (CR) B1ab(ii, iii, v) + 2ab(ii, iii, v).

Appling the Criterion C, the populations of *O. conferta* subsp. *conferta* comprises 69 mature individuals which is less than the CR category threshold of < 250. Furthermore, an observed and continued decline of 62 % of total number of plants was recorded between 2016 and 2021 which is higher than the CR category threshold of 25 % (Fig. 2). The number of mature individuals in each subpopulation (*sensu* IUCN, 2019) is less than 50. Under criterion C, the species is assessed as Critically Endangered (CR) C1 + 2a(i).

Given the limited size of the population, the Criterion D has also been considered. Since it is based exclusively on the number of mature individuals, the species matches with the EN category as the species comprises 69 mature individuals (⟨250).

As the species should be listed under the highest category of threat, we propose (CR) B1ab(ii, iii, v); B2ab(ii, iii, v); C1 + 2a(i) as the conservation status for *O. conferta*. Overgrazing, by livestock such as grazing by cattle, goats, and sheep, has led to the fragmentation of the habitat, trampling and herbivory of seedlings and juveniles and the death of *Onobrychis* plants. The construction of roads has caused fires, the dumping of waste and deforestation. Fires, usually associated with human activities, have affected mainly seedlings and juveniles *Onobrychis* plants, although they can cause the death of mature plants (Table S1).

Recovery suggestions are proposed to improve the conservation status of *O. conferta* subsp. *conferta*. The suggestions include various actions structured into four components and pursues three main objectives: (1) to reduce habitat loss, degradation and fragmentation, so as to slow the loss of mature individuals and restore natural recruitment; (2) to encourage the establishment of protected areas in the species historical range; and (3) to create new populations in that protected area (Table 4).

4. Discussion

According to our hypothesis, our study reveals that *O. conferta* subsp. *conferta* is classified as Critically Endangered in Tunisia, where it is found in only one location (*sensu* IUCN), with a declining number of plants over time and very low recruitment, primarily due to overgrazing, wildfires, and habitat conversion. This endemic taxon may be one of the most endangered plants in northern Africa.

Recruitment levels can be influenced by seed production, availability of favourable microsites, dispersal, seed germination, and seedling establishment (García-Beltrán et al., 2022). In the case of *O. conferta* in Tunisia, trampling and herbivory on seedlings and mature reproducing plants by livestock, including cattle, goats, and sheep, have limited recruitment, which was null in one of the three populations (Population OC3). Nevertheless, the presence of *Pinus halepensis,* thorny woody species such as *Erinacea anthyllis* Link, and abundant rocks and pebbles

Table 3
Cover-abundance in five sampling plots, frequency, biogeographical status and conservation status for plant species growing in the three recorded population of *Onobrychis conferta* subsp. *conferta* in Tunisia. Cover-abundance scale:. (absent), r (<5% range of cover; one individual), + (<5% range of cover; few individuals), 1 (<5% range of cover; numerous individuals), 2 (5.1–25 % range of cover), 3 (25.1– 50 % range of cover). Constancy: I = 20 %, II = 40 %, III = 60 %, IV = 80 % and V = 100 %. Biogeographical status: END = Endemic to Tunisia; NAT = Native.

	1	2	3	4	5	Constancy	Biogeographical status
Number of species	26	5	5	8	7		
Population OC1	2	.	.	.	.	I	END
Hedysarum coronarium L.	3	1	.	+	+	IV	NAT
Melilotus segetalis (Brot.) Ser.	2	.	.	.	1	II	NAT
Aegilops sp.	1	.	.	.	1	II	NAT
Pinus halepensis Mill.	+	.	.	+	.	II	NAT
Lotus corniculatus L.	+	.	.	+	.	II	NAT
Hippocrepis minor Munby	.	.	3	.	+	II	END
Sanguisorba minor Scop.	.	+	.	.	.	I	NAT
Population OC2	2	.	.	.	+	II	END
Hedysarum coronarium L.	1	+	r	+	+	V	NAT
Oxalis corniculata L.	+	+	.	1	2	IV	NAT
Erinacea anthyllis Link	r	r	.	r	.	III	NAT
Hedysarum carnosum Desf.	+	.	1	+	.	II	NAT
Plantago lanceolata L.	2	.	1	.	.	II	NAT
Ampelodesmos mauritanicus (Poir.) T.Durand & Schinz	+	.	+	.	.	II	NAT
Olea europaea L.	+	.	.	+	.	II	NAT
Onobrychis kabylica (Bornm.) Širj.	r	.	.	.	.	I	END
Population OC3	+	.	.	.	.	I	END
Hedysarum coronarium L.	2	.	.	.	.	I	NAT
Hedysarum spinosissimum L.	1	.	.	.	.	I	NAT
Medicago tunetana Murb.	+	.	.	.	.	I	END
Pinus halepensis L.	+	.	.	.	.	I	NAT
Trifolium fragiferum L.	2	.	.	.	.	I	NAT
Plantago lanceolata L.	1	.	.	.	.	I	NAT
Plantago serraria L.	1	.	.	.	.	I	NAT
Melilotus segetalis (Brot.) Ser.	2	.	.	.	.	I	NAT
Sanguisorba minor Scop.	+	.	.	.	.	I	NAT
Nerium oleander L.	+	.	.	.	.	I	NAT

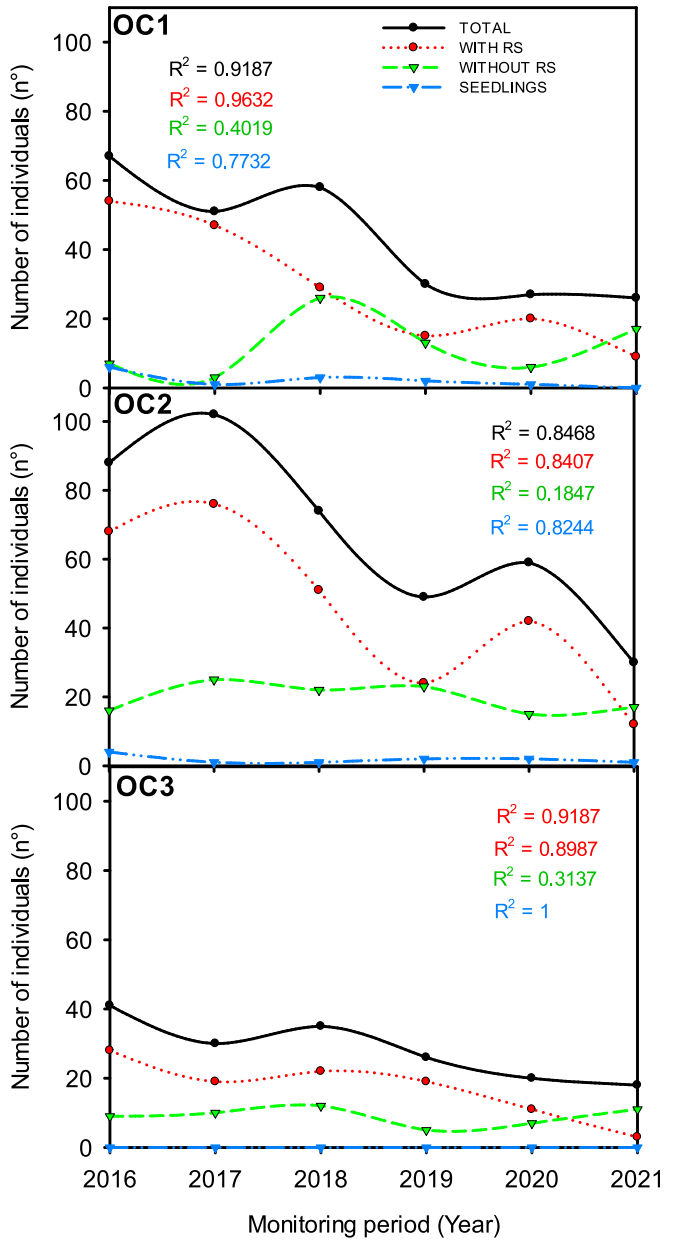

Fig. 2. Number of seedlings (diameter < 30 cm), mature plants without reproductive structures (Without RS; diameter > 30 cm) and mature reproducing plants (With RS) of the three populations of *Onobrychis conferta* subsp. *conferta* in Tunisia from 2016 to 2021.

on the soil surface created microsites suitable for germination and establishment of *O. conferta* due to lowered radiation and evapotranspiration levels, improved nutrient levels and protection against trampling and herbivory (Pernús & Sánchez, 2021). In this sense, Population OC3, where *Onobrychis* plants grew in the proximity to or under shrub canopy, showed higher levels of soil organic matter than those of the other two populations. In addition to grazing, *O. conferta* subsp. *conferta* was found growing on semiarid bioclimatic zones with dry grassland communities that reduced the survival of *Onobrychis* plants, as is the case for other endangered species in the Mediterranean Basin (Pasta et al., 2022). Furthermore, the habitat of *O. conferta* subsp. *conferta* has being converted to human settlements and large scale cultivated farmlands with *Triticum aestivum* L. and *Olea europaea* L. This process of habitat conversion is responsible for endangering the conservation of many plant species around the Mediterranean Basin (Dewees et al., 2022; Mendoza-Fernández et al., 2022).

On view of our results, the limited and decreasing presence and abundance of *O. conferta* subsp. *conferta* classified this species under Critically Endangered category. This classification should be the start-up of immediate and comprehensive conservation actions crucial to the survival of this species. *Onobrychis conferta* subsp. *conferta* is facing a very high risk of extinction in the wild in Tunisia. None of its three populations are currently within protected areas. The preservation of the three locations of *O. conferta* subsp. *conferta* was also supported by the presence of other Critically Endangered endemic species, such as *Medicago tunetana* and *Onobrychis kabylica*. Although *M. tunetana* was recently documented from seven new localities, it was not reported to be present in Ain Dyssa (OC2) (Ferchichi et al., 2021). In addition, *O. kabylica*, mentioned as quite rare by Quézel & Santa (1962), was not encountered during extensive targeted surveys within its occurrence range in northern Algeria (Abdelguerfi & Laouar, 2002).

Based on the identified threats and the stakeholders present in the study locations, we have proposed a conservation recommendation for *O. conferta* subsp. *conferta* in Tunisia (Table 4). These recommendations integrate both *in situ* and *ex situ* conservation actions highlighting the incorporation of management activities (i.e. erection of fences). *In situ* actions are aimed at minimizing the effects of the identified threats and to establish new populations of *O. conferta* in its characteristic habitat: south-facing locations in semiarid climates with alkaline soils, featuring a high active limestone percentage and ranging from 532 to 930 m a.s.l.

Ex situ actions include an outreach environmental education campaign highlighting the ecological and socio-economic values of the species. During this study, we collected seeds of *O. conferta*, which are currently preserved in the greenhouse of Higher School of Agriculture of Moghrane. These *ex situ* collections will serve as effective media for further research and for public education and awareness on plant biodiversity and conservation. These collections can support *in situ* conservation programs by providing plants needed to create new populations, to reintroduce extirpated or declining populations as well as conservation translocation for halting and reversing species decline (Heywood, 2017; Abeli et al., 2020; Fenu et al., 2023). Furthermore, there is a pressing need for research on conservation strategies specific to *O. conferta* subsp. *conferta*. Understanding the genetic structure of the population is critical for formulating accurate conservation policies that can support and sustain the conservation status of *O. conferta* subsp. *conferta*. Research on propagation and cultivation will also be instrumental in ensuring the growth of the *in situ* population of *O. conferta* subsp. *conferta*. Finally, studies on spatial distribution modelling will facilitate the reintroduction process and contribute to landscape conservation policies for *O. conferta* subsp. *conferta*. Research on propagation and cultivation is essential for improving both the *in situ* population and *ex situ* collections of *O. conferta* subsp. *conferta*. It is imperative to address several risk factors that could impede the progress of these conservation recommendations. The main factor would be the lack of financial and logistic support. Proposed actions must be maintained for at least five years to achieve the objectives outlined in our recommendations. Another important risk factor is the absence of an approved and officially managed protected area within the historical range of the species. Additionally, the lack of local leaders to support recovery actions needs to be considered. Moreover, the limited existing information on the optimal times for collecting seeds of native species in the area and their requirements for germination and establishment reduce the capacity to restore the habitat of *O. conferta* subsp. *conferta*.

CRediT authorship contribution statement

Anis Sakhraoui: Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Hela Belhaj Ltaeif:** Writing – original draft, Investigation, Conceptualization. **Jesús M. Castillo:** . **Slim Rouz:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization.

Table 4
Proposed conservation and recovery recommendations for *Onobrychis conferta* subsp. *conferta* in Tunisia.

Components	Recommended actions	Goals
Species reintroduction	1. Reintroduce *O. conferta* in the 7/10 sites where the species is known to have existed but is now extinct. 2. Enrichment planting: This involves introducing *O. conferta* seedlings into existing wild populations which have reduced to very few numbers. 3. Reintroduce/translocate *O. conferta* in a site outside of the known historical range of the species, as that is the only place safe from the threats that brought the species to extinction.	Creation of new, self-sustaining of *O. conferta* populations in/ within their historic range and characteristic habitat.
Genetic enrichment	4. Genetic enrichment by identifying suitable genetic banks which function as *in situ* 'sinks' into which gene pools from various 'source' sites are introduced and maintained that will facilitate the maintenance of a 'global' allelic set of *O. conferta*.	Represent the widest possible spectrum of genetic variability of *O. conferta*.
Rehabilitation	5. Rehabilitate the ecosystem has been converted (deforestation and vegetation clearing etc.). 6. Establishment of nurseries for *O. conferta* and accompanying flora such as the thorny woody species *Erinacea anthyllis* Link serving as microsite for *O. conferta* germination and establishment. 7. Fencing around populations of *O. conferta* to avoid grazing and anthropic disturbances.	Recovery and continuity to survive.
Monitoring	8. Collect information on the extent of reduction in the population size and identify specific threats, either intrinsic (auto-incompatibility, low viability, poor regeneration, etc.) or extrinsic (habitat loss, overgrazing, etc.) on a periodical basis will ensure that for any unforeseen threats, corrective measures can be taken up.	Establishment of a viable population that maintains the genetic variability of *O. conferta* and produces successful offspring.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anis Sakhraoui reports financial support was provided by Laboratory of Agricultural Production Systems and Sustainable Development (LR03AGR02) of the Higher School of Agriculture of Moghrane, University of Carthage.

Data availability

Data will be made available on request.

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Authors' contributions

AS, HBL and SR conceived and designed the study. AS, HBL and SR collected samples. AS, HBL, and SR conducted the laboratory analyses. AS and SR conducted the statistical analyses. AS, HBL, JMC and SR prepared the original draft of the manuscript. SR and JMC validated, reviewed, and edited the manuscript. SR and JMC visualized and supervised the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2024.126603.

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