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Evaluation of diversity and conservation status of *Matricaria chamomilla* (L.) and *Matricaria aurea* (Loefl.) Sch. Bip. in Lebanon

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Abstract

Matricaria chamomilla (L.), and *Matricaria aurea* (Loefl.) Sch. Bip., are two threatened wild species commonly used for medicinal purposes in Lebanon. An eco-geographic study was conducted, by assessing the distribution pattern of the two species in Lebanon and their related genetic diversity, to guide conservation efforts. The constructed distribution maps identified two richness areas namely Iaat and Hawch El Sayed Ali. Threats assessment to both species included urbanization, agriculture, unsustainable harvesting, overgrazing, and drought. Thirty-two sites were evaluated using eco-geographical surveys, and genetic analysis was done using Start Codon Targeted Polymorphism (SCoT) marker analysis. Neighbor Joining analysis clustered populations into four distinct groups with a gene flow of 0.4365 and genetic differentiation from 0.121 to 0.191. Results revealed a clear geographical isolation among populations with low gene flow between distinct populations. Principal Coordinate Analysis (PCoA) clustered 119 individuals within their populations and grouped them following the same pattern as the Neighbor Joining tree. Calculation of Nei's genetic diversity index revealed that genetic diversity in Mount Lebanon was lower than in the South. One area, Jezzine, appeared as the most genetically diverse and remained isolated. These findings indicate the need to develop a conservation strategy that would prevent the extinction of one of the most marketed medicinal and aromatic plants in Lebanon, with Jezzine area considered as a priority in conservation actions. Finally, chemical profiling should be conducted to valorise the Lebanese Matricaria species.

Keywords *Matricaria chamomilla* L; *Matricaria aurea* (Loefl.). Sch. Bip; Medicinal Plants; Genetic Diversity; In-Situ; Ex-Situ Conservation; Lebanon

Introduction

Matricaria L. is a genus in the family of Asteraceae (tribe Anthemideae), which is considered among the economically important plant families, including the edible cultivated lettuce (*Lactuca sativa* L.), sunflower (*Helianthus annuus* L.), artichokes (*Cynara cardunculus var. scolymusm* L.), as well as the edible wild tumbleweed (*Gundelia tournefortii* L.) and *Scorzonera mollis* M. Bieb. In addition to edible members of this family, other species, such as baccharises (*Baccharis* sp. L.), Wingstem Camphorweed (*Pluchea sagittalis* Less.), southern cone marigold (*Tagetes minuta* L.) and chamomile (*Matricaria* L.), are known to have medicinal properties.

The genus *Matricaria* comprises six to seven species native to Eurasia, North Africa and North America. In Lebanon, two wild species exist: *M. chamomilla* L., the wild matricary, and *M. aurea* (Loefl.) Sch. Bip., the golden chamomile [1]. They can be distinguished from each other mainly by the flowers ligules, which are present in *M. chamomilla* and absent in *M. aurea*. *M. aurea* grows to a height of 20 cm with a slender ascending stem. Its flower heads are golden yellow, dome shaped, mostly terminal or sometimes axillary [2]. On the other hand, *M. chamomilla* grows to a height of 10 to 80 cm. Its thin spindle-shaped roots penetrate the soil flatly. The stem is erect, mostly heavily ramified, bare, round, and filled with marrow. The leaves are long, alternate, and double to triple pinnatipartite, with narrow-linear prickly pointed sections being hardly 0.5 mm wide. The golden yellow tubular florets consist of five teeth 1.5 to 2.5 mm long, ending in a glandulous tube [3].

Chamomile is also an annual plant, considered to be one of the most common herbs used for medicinal purposes [4]. It has been known and used since the old times in ancient Egypt, Greece, and Rome [5]. Historically, the three Greek physicians Hippocrates, Galen and Asclepius referenced the plant in their writings [6]. In addition, the Arab herb physician Abul Abbas highlighted the spread of the use of this plant from the Middle East to Spain [7].

Matricaria chamomilla is native to the Old World, mainly Europe, North West Asia, and North Africa [5]. Yet, the plant is no longer restricted to the wild, since it is currently cultivated worldwide [8]. It is still used in both traditional and modern medicine, for diverse purposes such as anti-inflammation, anti-oxidation, wound healing, skin and digestive system problems among others [4].

On the other hand, *Matricaria aurea* is native to South Europe, North Africa, Middle East, South West to Central Asia [9]. The aerial extracts of the plant are known to possess significant antioxidant activity, which could be due to the phenolic compounds [8]. Phytochemical analysis of essential oil from *M. aurea* showed several chemicals compounds many of which are important antimicrobial and antioxidants [10].

Collectively, both species (locally known as "Babunaj") are used to treat colic pains, abdominal cramps, inflamed mucous membrane of mouth and throat, sore throat, and are also used as calmatives, for flavoring, and to induce sleep [9]. The flower heads are consumed in infusions as herbal tea.

Due to this medicinal importance, chamomile (*Matricaria chamomilla* and *M. aurea*) [11] have been subject to various studies aiming at investigating the nature of their chemical components and their therapeutic properties [5]. However, fewer studies focused on using molecular markers to study the plant's genetic diversity for conservation purposes. They were mainly focused in Germany and Iran, and revealed differences that were not detected at the morphological level [12-15].

In Lebanon, *Matricaria chamomilla* is distributed mainly in Mediterranean low altitude slopes, on cultivated and waste lands, while *M. aurea* is distributed in mountainous and continental areas, on road sides and waste lands, and it is characterized by very small and patchy populations with limited number of individuals and limited plant biomass [1,16]. The market demand for both species is met by wild harvesting from Lebanon and neighboring countries. Thus, the increase in demand of medicinal plants in general, and chamomile specifically, renders the species in danger of extinction if not sustainably addressed. Increased knowledge on the status, diversity and distribution of these species at different levels is a key prerequisite for priority setting of conservation needs and proper exploitation, especially that there are no comprehensive eco-geographic surveys for the two species and there is a complete gap regarding their genetic diversity.

The present study aims to -first- shed light on the distribution, habitat and threats of *Matricaria chamomilla* and *M. aurea* in Lebanon, and -second- to assess the genetic diversity of the populations of *M. chamomilla* in the identified distribution sites using SCoT markers. The overarching objective is to guide conservation efforts by assessing the distribution pattern of the two species in Lebanon and their related genetic diversity.

Material and Methods

Eco-geographic survey of *Matricaria chamomilla* and *M. aurea* in Lebanon

Distribution: Field visits were conducted from March to June 2014 in three regions (Mount Lebanon, Bekaa and South Lebanon). Sites' selection was based on related literature [1,16] and personal communications with perfumers, elderly residents, and municipalities' staff. The current distribution, occurrence, density, and status of the two species of Matricaria were assessed. The sites were described, and ecological and geographic parameters were recorded. Distribution and richness maps were generated for *M. chamomilla* and *M. aurea* using DIVA-GIS V.7.1.7 software.

The presence of the species as well as the impact of the habitat type, plant species composition, anthropological pressure on the occurrences were recorded. In addition, the observation of the phenological stages was considered and major threats were identified.

Prediction maps: DIVA-GIS was used to create prediction maps. Climatic data (temperature and precipitation) was used to predict the likelihood of occurrence and survival of the species. BIOCLIM modelling was used and the following six types of areas were mapped: areas with low likelihood to have the species (0-2.5 percentile), areas with medium likelihood (2.5-5 percentile), areas with high likelihood (5-10 percentile), areas with very high likelihood (10-20 percentile), and areas with excellent likelihood (above 20 percentile).

Threats: During the conduction of the survey, onsite observations were made for factors affecting the survival of the species and determining the most prominent threatening conditions. Presence of animal excretions and adjacent construction projects were used to make inferences.

Sampling technique for species richness: Out of the 32 visited sites in the three Lebanese regions, selected sites were targeted for plant sampling and identification. For *Matricaria chamomilla*, four sites in the three regions were selected for species identification and three plots, of 1m by 1m each, were randomly selected per site for sampling. For *M. aurea*, three sites were selected, and three plots were designated for the previously mentioned purposes.

Plant specimens of the two Matricaria species and associated species were collected and photographs were taken on site. Identification was conducted primarily in the field and confirmed later in the laboratory based on literature [1,16] by a botanist working at ICARDA (co-author on this publication).

Sampling technique for genetic diversity: Sites targeted for genetic diversity studies were selected based on different criteria (site accessibility, populations richness and plants availability, drought impact, etc.). The number of sampling sites was limited by the sporadic distribution pattern of the species, the concern to keep a minimum distance between sampled individuals, as well as the unexpected drought period in some regions.

Only sites with ample number of individuals and patches were targeted for sampling and further molecular analysis. Thus, due to the low number of individuals and patches of *Matricaria aurea*, and due to their presence in areas that are hard to reach due to the civil strife at the borders between Lebanon and Syria, the number of collected samples was not enough for genetic analysis. Consequently, only *M. chamomilla* was targeted for this purpose.

Governorate	Locality	Latitude	Longitude	Number of individuals collected
Mount Lebanon	Ain Remmaneh	33.8666	35.5183	19
	Tehwita	33.8711	35.5296	23
	Hadath	33.8702	35.528	11
	Mchrfeh	33.759827	35.658459	8
	Baalchmieh	33.824113	35.647226	14
	Fiadieh	33.847679	35.551929	11
	Jisr El Bacha	33.8647	35.5408	20
South Lebanon	Khelwat	33.418889	35.738611	20
	Jezzine	33.540837	35.586241	14

Table 1: Number of *Matricaria chamomilla* individuals collected for molecular analysis from different areas in Lebanon along with the respective GPS location of each area.

Young plants material was collected randomly to cover the population diversity in each site. The total number of sampled plants was 140 ranging between 8 and 23 plants per population of Matricaria chamomilla (Table 1). Out of the 140 samples, 119 were considered for analysis based on the quality of their genomic DNA. Collected samples were stored in filter paper with silica gel within plastic bags and kept in a dry place for further molecular processing.

DNA Extraction & quantification: Due to the high number of polysaccharides found in chamomile, DNA extraction from the young leaves of the plant was challenging. Different genomic DNA extraction protocols were tried [17-19] with multiple modifications. Total genomic DNA was eventually extracted from 30 mg young leaves using a plant DNA purification kit from Macherey-Nagel according to the manufacturer's protocol. The obtained DNA was stored at -20°C until further use. Genomic DNA purity and concentration were assessed using Nanodrop 2000UV-Vis Spectrophotometer (Thermo Scientific).

Polymerase Chain Reaction (PCR) amplification: Out of the 12 SCoT primers screened, 10 primers producing reproducible and polymorphic bands were selected for the analysis of the genetic variability and relationships among *Matricaria chamomilla* populations (Table 2). All primers were 18-mers with an annealing temperature of 50°C.

SCoT Primer	Sequence (5′-3′)
1	CAACAATGGCTACCACCA
2	CAACAATGGCTACCACCC
6	CAACAATGGCTACCACGC
11	AAGCAATGGCTACCACCA
13	ACGACATGGCGACCATCG
22	AACCATGGCTACCACCAC
32	CCATGGCTACCACCGCAC
34	ACCATGGCTACCACCGCA
35	CATGGCTACCACCGGCCC
36	GCAACAATGGCTACCACC

Table 2: Sequences of SCoT primers used for *Matricaria chamomilla* DNA screening.

PCR reactions were carried out in a total volume of 20 µL consisting of 5 ng template DNA, 1x Taq buffer with KCl, 2 mM $MgCl_2$, 200 µM dNTPs, 30 µmol of primer, 0.2 units of Taq DNA Polymerase, 0.5 µg BSA. Amplification was performed in LifePro Thermal Cycler programmed for an initial denaturation step at 94°C for 3 mins, followed by 45 cycles of 1 min at 94°C, 1 min at 50°C and 2 mins at 72°C, with a final extension at 72°C for 5 mins. After amplification, PCR products were resolved by loading 20 µL of the reaction product on 2% (w/v) agarose gel in 1x TBE buffer stained with ethidium bromide (0.5 µg/ml) at 75-80 V for 2-3 hours. DNA fragments were then visualized under UV light and photographed using a Gel Doc System (Quantity One, BioRad).

Data analysis- species richness: For each plot, the associated plant diversity was assessed by keeping record of the number and information of each of the identified species.

Genetic analysis: Since SCoT marker is a dominant marker, each band was assumed to represent a single bi-allelic locus [20,21]. All detectable bands were scored for their presence (1) or absence (0) and compiled into a data binary matrix. Bands were assumed to be independent, and those of identical size were assumed to have identical sequences.

The resulting present/absent data matrix was analyzed using POPGENE 32 Version 1.31 [22] by calculating the following genetic diversity parameters: Number of polymorphic band (NPB), percentage of polymorphic band (PPB), number of observed (Na) and effective alleles (Ne), Shannon information index of diversity (I), Nei's gene diversity (H), and genetic distance (D).

Polymorphic information content (PIC) values were calculated for each locus according to the following formula: PIC=2*fi*(1-fi); where fi is frequency of present band at ith locus and (1-fi) is frequency of absent band. The average of PIC values for all loci of each primer was calculated as the PIC of the corresponding primer.

Analysis of molecular variance (AMOVA) was performed in GenAlEx v.6. 1 [23] to partition the total SCoT variation into within populations and among populations.

Principal coordinate analysis (PCoA) was also conducted using GenAlEx v.6.1 to figure out the genetic relationships between the populations and the distribution at the individual level in a two-dimensional graph.

Pairwise genetic distance was determined by calculating Nei's genetic distance with 1000 bootstrapped replicates and was converted to a dendrogram displaying populations relationships using R software [24]. A consensus tree showing the relationships between the populations was generated as well by the NJ method of cluster analysis using PHYLIP 3.57C package [25].

Results

Eco-geographic survey of *Matricaria chamomilla* and *M. aurea* in Lebanon

Survey time-phenological stages: Variations in terms of flowering, maturity and density were directly related to the climatic conditions, particularly to rainfall. *Matricaria aurea* flowering period is supposed to be in March according to literature. However, the plants in some sites were post-maturity when visited in March.

Survey area and distribution pattern: *Matricaria chamomilla* was mainly found in densely inhabited slopes and in continental areas of variable altitude from sea level to 1000 m.a.s.l., whereas *M. aurea* was restricted to relatively higher altitude, from 600 to 1100 m.a.s.l, in the semi-arid continental part of the country.

Distribution and richness maps were generated for the two targeted species (Figure 1). Out of the 32 surveyed sites, 22 sites were occupied by *Matricaria chamomilla* exclusively, and 8 sites were occupied by *M. aurea* exclusively, leaving only two sites with overlapping distribution of both species present in small patches. Both sites, identified as richness areas at the national scale, are situated in the Northern Bekaa (North East Lebanon), namely in Iaat (Central Bekaa) and Hawch El Sayed Ali (near the Syrian borders).

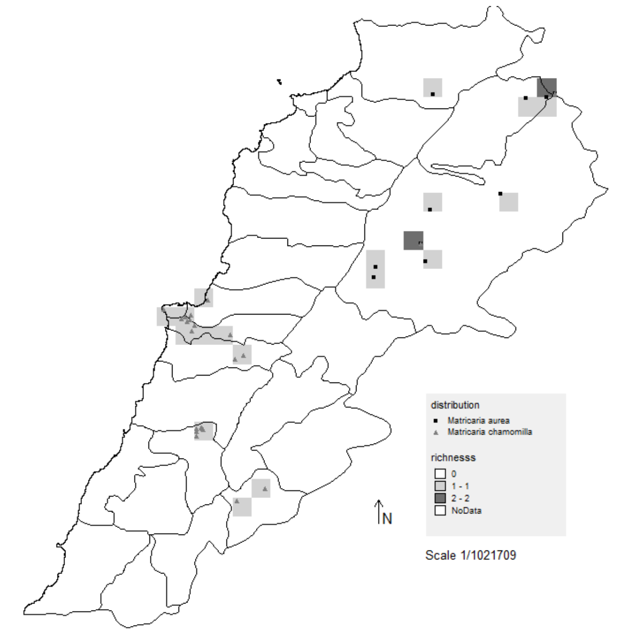

Figure 1: Map showing distribution and richness of *Matricaria chamomilla* and *M. aurea* in the surveyed sites. Black squares show areas of occurrence of *M. aurea*. Triangles show areas of occurrence of *M. chamomilla*. Dark gray squares show areas where both species occur. Light gray squares show areas where only one of the two species occur.

Natural habitats in the different sites varied from disturbed grasslands and roadsides, to abandoned fields and pine woodlands. However, none of the richness areas' sites fell within the boundaries of protected areas. Moreover, no records were reported on the presence of the target species in protected areas, and since our surveys were guided by literature, protected areas were not surveyed, and the presence or absence of these species was not confirmed. Nevertheless, our knowledge of the management practices of protected areas in Lebanon strongly suggests that no targeted management plan for these species is implemented in such areas.

Matricaria species were found in almost every site previously reported in literature [1,16]. Thus, our findings came as a confirmation to the literature, with few exceptions such as Dbayeh site, on the coastal region, which was completely urbanized so neither of the two species was found. On the other hand, additional sites were identified in areas that were not listed in the literature, such as Iaat in Bekaa, Baalchmieh, and Mcherfeh in Mount Lebanon.

Prediction maps: A pre-requisite for the conservation strategies of reintroduction by cultivation and direct sowing is determining areas that are suitable for the growth requirement of the plant, which could be done through the identification of locations "within the known historical range of the species but outside its current distribution area" [26] in case of availability. In our case, these locations included the areas in which the presence of *Matricaria chamomilla* was previously recorded by Post [27], yet was not found during our conducted survey. An alternative and more systematic way would be to construct prediction maps to identify areas in Lebanon that have similar environmental conditions to the areas of the current natural distribution of chamomile.

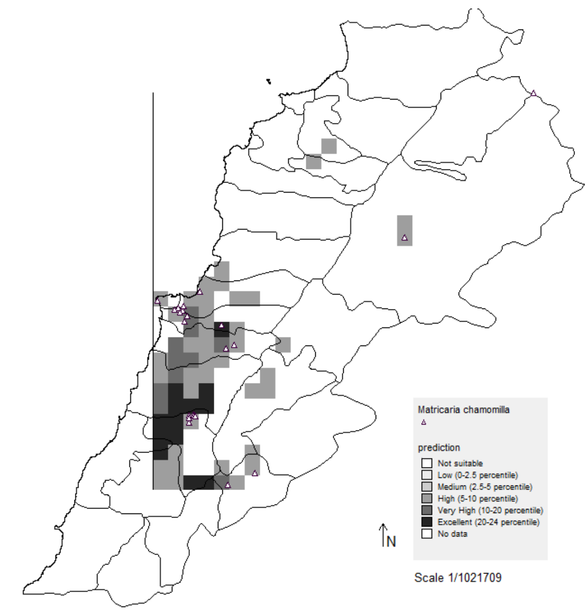

Figure 2: Predictive map for *Matricaria chamomilla*. The black squares show areas that are excellent sites suitable to house the species. The dark gray squares are very high suitable sites to house the species. The light gray squares are highly suitable sites to house the species. Triangles are the distribution sites.

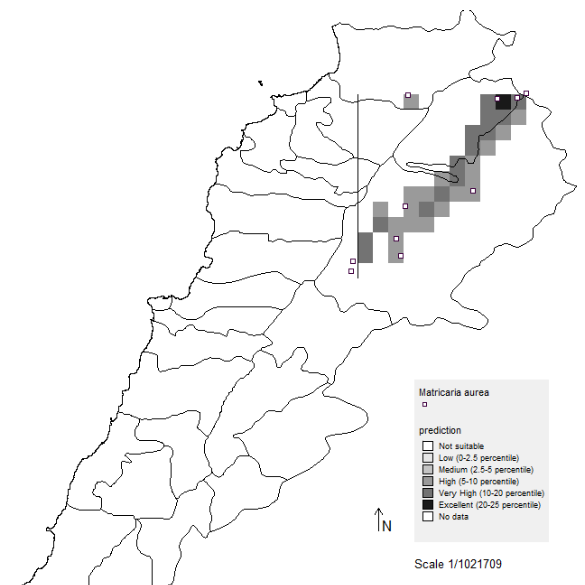

Figure 3: Predictive map for *Matricaria aurea*. The black square show areas that are excellent sites suitable to house the species. The dark gray squares are very high suitable sites to house the species. The light gray squares are highly suitable sites to house the species. Small white squares are the distribution sites.

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The reconstructed map (Figure 2) shows that the areas highlighted in black (El Chmeice, Mazraat El Chouf, Baakline, Gharife, Harf, Bisri, Kfar Melki, El Rihane) and in dark grey (Ain Saade, Bchamoun, Ammiq, Debieh) are excellent and very high suitable sites that are most likely to house Matricaria chamomilla. On the other hand, the predictive map for M. aurea (Figure 3) shows that Nasriyeh is an excellent site that is most likely to host the species, and areas in orange (Chwaghir, Hermel, Wadi Faara, Hlabta, Nabha, Knaissat, Yammoune, Mazraet Beit Mcheik) are highly suitable sites, however, ground truth survey needs to be conducted to verify their presence/absence. It is worth noting that prediction maps are constructed using only two environmental factors: rainfall and elevation. In case of absence, more factors such as temperature, soil type, habitat type, land use, and associated species can be used for refinement.

Threats: Despite the ability of Matricaria to survive in various environments, our observations identified the following seven factors that affected both species: lack of awareness, construction (urbanization), agriculture (land use and ploughing), unsustainable harvesting, overgrazing, drought, and fire (Table 3).

	M. chamomilla		M. aurea	
Threatening factor	Mount Lebanon	South Lebanon	Bekaa	North West Lebanon
Agriculture		X	X	
Drought				X
Overgrazing				X
Urbanization	X	X	X	
Lack of awareness	X	X	X	
Harvesting		X	X	
Fire	X			

Table 3: List of different threatening factors that affect Matricaria plant species per Governorate. X indicates the presence of the threatening factor in the respective governorate.

Matricaria chamomilla is suffering from the fragmentation of its distribution due to human pressure, mainly by urbanization, which limits the ability of the plant to grow freely in different areas of the site (Figure 4a and 4b). Lands that were previously occupied by the plant are ploughed either in preparation for construction, or for agriculture purposes (Figure 4c and 4d).

On the other hand, *Matricaria aurea* seems to be "endemically" restricted to dry regions where the ecosystem is particularly vulnerable to climate change, putting such a species at risk (Figure 4e and 4f).

Figure 4: Different threats affecting *M. chamomilla* and *M. aurea* in Lebanon. (a) Construction site in Tehwita, Mount Lebanon Governorate. (b) Urbanization site in Hasbaya, Mount Lebanon Governorate. Both sites were previously occupied by the plant. (c,d) Agriculture and land use site in Jisr El Bacha, Mount Lebanon Governorate. During monitoring visits of the site, the land was tilled with a plow and sub-populations were destroyed in full blooming stage. (e,f) Dry site in Nasriye, North West Lebanon Governorate. Plants are subject to drought.

Plant species composition: Many different plant species are associated with Matricaria in Lebanon. Most of them are common in disturbed areas. The identification of the associated species with *M. chamomilla* in the four target sites (3 plots per site) in the regions of Mount Lebanon and South Lebanon, showed a total of 23 different genera belonging to 11 families. Nine of these genera were seen commonly in both regions, the most abundant were namely *Hordeum* sp. (Poaceae), *Malva* sp. (Malvaceae), *Sisymbrium* sp. (Brassicaceae), *Cirsium* sp. (Asteraceae), *Erigeron* sp (Asteraceae), *Capsella* (Brassicaceae), *Geranium* sp. (Geraniaceae), *Polygonum* sp. (Polygonaceae), and *Taraxacum* sp. (Asteraceae). Some of the genera were reported only in sites of Mount Lebanon, namely: *Crepis* sp. (Asteraceae), *Chrysanthemum* sp. (Asteraceae), *Oxa lis* sp (Oxalidaceae), *Daucus* sp (Apiaceae), *Urtica* sp (Urticaceae), *Medicago* sp. (Fabaceae), *Dactylis* sp. (Poaceae) and *Trifolium* sp. (Fabaceae) (Table 4), whereas South Lebanon sites were unique in the association of *Phalaris* sp. (Poaceae), *Avena* sp. (Poaceae), *Raphanus* sp. (Brassicaceae), and *Calendula* sp. (Asteraceae) (Table 4).

	Mount Lebanon							South Lebanon				
	Jisr El Bacha				Tehwita			Hadath			Bteddine El Lucch	
Species	Plot-1	Plot-2	Plot-3	Plot-4	Plot-1	Plot-2	Plot-3	Plot-1	Plot-2	Plot-3	Plot-1	Plot-2
Sisymbrium sp.	X										X	
Oxalis sp.	X		X									

Hordeum sp.								X	X	X	X	X
Cirsium sp.		X					X				X	
Phalaris sp.											X	
Erigeron sp.				X							X	
Capsella sp.								X				X
Raphanus sp.												X
Malva sp.	X	X	X	X					X			X
Geranium sp.				X			X				X	
Polygonum sp.	X				X							X
Taraxacum sp.		X				X						X
Crepis sp.	X				X	X		X		X		
Daucus carota	X											
Urtica sp.	X											
Avena sp.												X
Medicago sp.			X				X					
Dactylis sp.		X				X						
Chrysanthemum sp.		X	X	X	X			X		X		
Trifolium sp.						X			X			
Calendula sp.											X	X

Table 4: Associated plant species with M. chamomilla in the identified plots.

North West Lebanon								
	Nasriye			**Sahel Nasriye**			**Addous**	
Species	Plot-1	Plot-2	Plot-3	Plot-1	Plot-2	Plot-3	Plot-1	Plot-2
Hordeum sp.	X			X		X	X	
Cirsium sp.	X	X			X			X
Erigeron sp.				X				
Raphanus sp.							X	
Malva sp.	X	X	X		X	X		X
Geranium sp		X						
Polygonum sp.			X					
Taraxacum sp.		X		X	X			
Crepis sp.							X	
Medicago sp.							X	X
Dactylis sp.			X					
Capsella bursa pastoris							X	X

Chrysanthemum sp.		X					X	

Table 5: Associated plant species with *M. aurea* in the identified plots.

For *Matricaria aurea* (Table 5), a total of 13 genera were recorded in all the nine plots of the three targeted sites belonging to seven families all of which were identified to genus level. The most common were *Hordeum* sp. (Poaceae), *Malva* sp. (Malvaceae), *Cirsium* sp. (Asteraceae), *Taraxacum* sp. (Astraceae), *Medicago* sp. (Fabaceae) and *Capsella bursa-pastoris* (Brassicaceae).

SCoT Polymorphism: The 10 SCoT primers used in the study generated a total of 262 bands, ranging from 19 (SCoT6) to 28 (SCoT11, SCoT13, SCoT22, SCoT34, SCoT35) bands per primer, with an average of 26.2 bands. The percentage of polymorphic bands (100%) was uniform among all primers and populations. The total number of bands and the level of polymorphism generated for each primer over the nine populations are presented in Table 6.

SCoT Primer	TB	PB	PPB (%)	PIC
SCoT 1	26	26	100	0.23
SCoT 2	27	27	100	0.26
SCoT 6	19	19	100	0.32
SCoT 11	28	28	100	0.26
SCoT 13	28	28	100	0.31
SCoT 22	28	28	100	0.29
SCoT 32	26	26	100	0.37
SCoT 34	28	28	100	0.33
SCoT 35	28	28	100	0.33
SCoT 36	24	24	100	0.36
Mean	26.2	26.2	100	0.31
Total	262	262		
TB: Total Bands, PB: Polymorphic Bands, PPB: Percentage of Polymorphic Bands, PIC: Polymorphic				

Table 6: Polymorphism detected using SCoT markers.

Polymorphic information content (PIC) varied from 0.231 (SCoT1) to 0.37 (SCoT32) with an average of 0.31. The primers used were clearly able to generate polymorphic profiles as shown in Figure 5.

Genetic diversity analysis among populations: Genetic diversity among populations of *Matricaria chamomilla* was estimated through the analysis of multiple parameters (Table 7). The number of polymorphic bands (NPB) was highest for Fiadieh (139) followed by Jezzine (135), while the lowest was obtained for Ain Remmaneh (94) and Mchrfeh (93). The percentage of polymorphic bands (PPB) followed the same previous pattern.

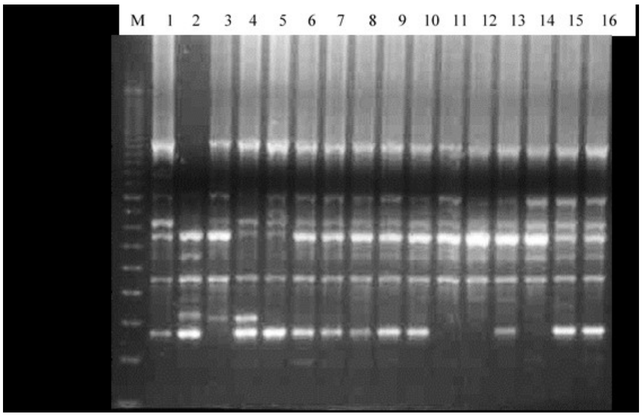

Figure 5: SCoT profile of PCR-amplified products from Khelwat population using primer #6. M: 100 bp PCR molecular ladder. 1-16: *Matricaria chamomilla* individuals.

The observed number of alleles values (N_a) ranged from 0.840 for Tehwita population to 1.179 for Jezzine population, with an average of 0.979, whereas the effective number of alleles values (N_e) ranged from 1.204 in Tehwita to 1.265 and 1.328 in Khelwat and Jezzine respectively, with an average of 1.240. Shanon's information index of diversity (I), ranged from 0.183 in Tehwita to 0.237 in Khelwat and 0.286 in Jezzine. The calculation of Nei's genetic diversity index (h) revealed that the genetic diversity in Mount Lebanon was lower than in the South. The gene flow among populations (N_m) was 0.4365. The genetic distance between pairs of populations of *M. chamomilla* was generally low ranging from 0.161 between Ain Remmaneh and Khelwat to 0.326 between Tehwita and Fiadieh (Table 8). The overall genetic distance between pairs of populations appeared moderate, as most of them did not exceed 0.3.

Localities	Population	Sample size	NPB	PPB (%)	N_a	N_e	I	h
South Lebanon	Jezzine	18	135	51.53%	1.073 ± 0.06	1.242 ± 0.02	0.233 ± 0.016	0.15 ± 0.011
	Khelwat	18	102	38.93%	0.866 ± 0.059	1.209 ± 0.02	0.19 ± 0.016	0.125 ± 0.011
	Ain Remmaneh	14	94	35.88%	0.84 ± 0.057	1.204 ± 0.02	0.183 ± 0.016	0.121 ± 0.011
	Hadath	11	110	41.98%	0.973 ± 0.058	1.231 ± 0.02	0.215 ± 0.016	0.141 ± 0.011
	Mchrfeh	7	93	35.50%	0.893 ± 0.0556	1.234 ± 0.022	0.2 ± 0.017	0.135 ± 0.012

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Mount Lebanon	Baalchmieh	12	121	46.18%	1.034 ± 0.058	1.265 ± 0.022	0.237 ± 0.017	0.157 ± 0.012
	Fiadieh	11	139	53.05%	1.179 ± 0.057	1.328 ± 0.023	0.286 ± 0.018	0.191 ± 0.012
	Jisr El Bacha	16	116	44.27%	0.947 ± 0.06	1.209 ± 0.019	0.2 ± 0.016	0.129 ± 0.011
	Tehwita	14	120	45.80%	1.008 ± 0.059	1.242 ± 0.021	0.223 ± 0.017	0.146 ± 0.011
	Mean	13.4	114.4	43.68%	0.979 ± 0.019	1.24 ± 0.007	0.219 ± 0.006	0.144 ± 0.004

Table 7: Genetic variability within nine geographic regions of M. chamomilla detected by SCoT markers.

	JEZ	KLW	AR	HDT	MCH	BAL	FIA	JB	THW
JEZ	0								
KLW	0.19	0							
AR	0.184	0.161	0						
HDT	0.239	0.259	0.228	0					
MCH	0.279	0.278	0.284	0.324	0				
BAL	0.27	0.255	0.256	0.302	0.045	0			
FIA	0.304	0.297	0.27	0.281	0.236	0.215	0		
JB	0.222	0.242	0.239	0.26	0.306	0.264	0.305	0	
THW	0.217	0.199	0.227	0.268	0.281	0.237	0.326	0.162	0
JEZ: Jezzine; KLW: Khelwat; AR: Ain Remmaneh; HDT: Hadath; MCH: Mchrfeh; BAL: Baalchmieh; FIA: Fiadieh; JB: Jisr El Bacha; THW: Tehwita									

Table 8: Nei's genetic distance.

Genetic diversity analysis within populations: AMOVA analysis showed that 49% of variation was present within populations, 50% among populations and 1% among localities, indicating that the total genetic variation was equally divided between inter and intra population variations.

Cluster analysis: Genetic relationship among populations of M. chamomilla was carried out using two different approaches: The Neighbor Joining (NJ) clustering and the Principal Component Analysis (PCoA). A dendrogram was constructed with R software using data based on weighted Neighbor Joining (NJ) cluster analysis (Figure 6). Principal coordinate analysis followed the same pattern of cluster analysis provided by the dendrogram, showing an overall view of genetic diversity at both population and individuals levels (Figure 7). The outcome of this analysis is consistent with the genetic diversity analysis. All the individuals of the same populations remained clustered together, except the population of Jezzine in the South where individuals are more scattered, indicating a genetic diversity within the population that appears isolated as shown in the Neighbor Joining tree.

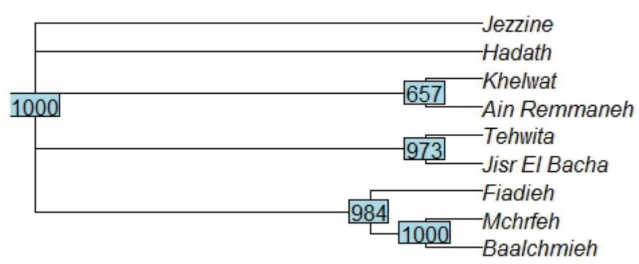

Figure 6: Dendrogram based on weighted NJ cluster analysis of 9 *Matricaria chamomilla* populations. Bootstrap values are indicated at each node.

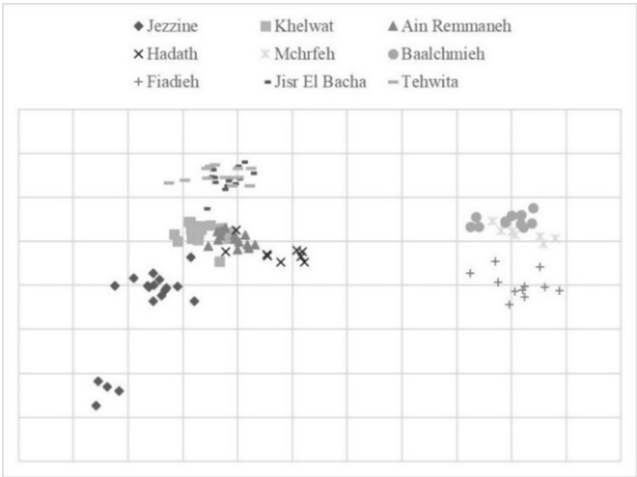

Figure 7: Principal coordinate analysis showing the distribution of 119 individuals of *Matricaria chamomilla* from 9 populations from Lebanon according to genetic distance.

Discussion

Genetic diversity and conservation

Measures of genetic diversity are very important to manage and conserve natural populations wisely. "Greatly diverse or differentiated populations could be targeted for conservation, while genetically penurious populations might be targeted for management plans to restore diversity" [28].

In Lebanon, the evaluation of the genetic variability in *Matricaria chamomilla* represents the first attempt to acquire knowledge about the structure of populations of this species and consequently suggest corresponding conservation strategies. Our study has shown that there is a clear geographical isolation among populations since most of populations from close geographical locations were clustered together indicating low gene flow between distinct populations. Moreover, the population of Jezzine showed the highest genetic diversity, remained isolated, and its individuals were the most scattered in the principal coordinate analysis. These findings indicate that Jezzine population is the most genetically diverse and should be considered a priority in conservation strategies (in-situ and ex-situ).

In the case of Matricaria in Lebanon, urbanization and agriculture are causing habitat fragmentation, leading to small populations. This poses an additional challenge since it will lead to lower genetic diversity and higher risk of inbreeding. Moreover, their surveillance will be more critical, given they are particularly sensitive to changes. Additionally, visits to the same sites were conducted after 2 years of the first visit to check the status of the plant, and it has been noted that the size of the populations is decreasing, further highlighting the need for urgent conservation action. The status of in-situ conservation of the two-target species remains to be verified. There is a need for a more thorough survey for protected areas to confirm the presence/absence of these species. In case of their presence, targeted management plans need to be developed so that these important medicinal species are not overlooked.

Recommended conservation approaches

The two approaches of conservation (ex-situ and in-situ) can be applied to chamomile, which allows the creation of a sustainable source of medicinal plants, thus satisfying the market's needs and reducing pressure on natural resources. Ex-situ conservation involves the establishment and maintenance of viable collections through cultivation in home gardens and botanical gardens, direct sowing in marginal lands, non-arable land and in corridors, as well as on-farm fields in location outside the zone of their natural occurrence.

Further steps related to cultivation and harvesting consist of having better knowledge on the proper time of harvesting, since the yield and nature of the plant's active compounds varies between different seasons [29]. In the case of chamomile, most active compounds are found in its flower, thus a proper time to harvest would be in its flowering season between March and June. Monitoring harvesting locations and the percentage of harvested plants are also of high importance and can be done by establishing a collection cycle under rotation to prevent repeated exploitation of the same sites [30]. The recommendation in this respect is to conduct a chemical profiling study on these two species to confirm the phenological stage at which the active compounds are at the highest level.

In addition to cultivation, maintaining a stock of the plant's genetic material through seed banks is of high importance as a precaution and backup against extinction [31]. Only few collections are available in the countries of the species' natural origin. The representation of populations in collections, and their use in ex situ conservation, is not enough to safely ensure the survival of the species considering the high threat level. Furthermore, conserved individuals are not properly documented, due to the lack of information on the origin of the plant material, their taxonomic status, and/or the cultivation history, therefore making them inappropriate for scientific studies and conservation programs. Thus, the selected population of Jezzine, which is the most diverse, can be targeted for a collection mission and the collected seeds can be conserved at the national seed bank at the Lebanese Agricultural Research Institute (LARI) [32].

In situ conservation, on the other hand, is more challenging, but at the same time more useful on the long-term than ex situ conservation. It is worth noting that, in Lebanon, no initiative has been taken so far to declare any protected areas directly oriented to the presence of wild relatives of agricultural crops [26]. However, there is a high chance that the target species (*Matricaria chamomilla* and *M. aurea*) do occur in protected areas, despite the absence of any targeted management plans to ensure their conservation. Hence, a suggestion would be to conduct more comprehensive surveys for the current designated protected areas to detect the occurrence of these species and customize management practices accordingly.

In case both target species do not occur in the protected areas or in viable populations, then micro-reserves can be established in selected areas (Figures 2 and 3). After ground truthing, new distribution maps can be constructed, which might in turn result in different prediction maps. These areas can also be considered as suitable areas for reintroduction either by cultivation or by direct sowing.

As a conclusion, a great proportion of medicinal plants is facing serious threats of extinction worldwide, detailed information is lacking, and little effort is being put to conserve them [33]. Given its high medicinal value, setting a detailed conservation and management plan for *M. chamomilla* in Lebanon can serve as a model and should be the leading step in a movement calling for the conservation of all endangered plants in Lebanon, especially endemic ones. The first phase is recommended to be the preparation of a full eco-geographical survey on the status of the plant in Lebanon, followed by ex situ conservation and cultivation of *M. chamomilla* in nurseries or special areas as a temporary measure to prevent it from extinction. Finally, it is important to use the results of our study and similar studies of the genetic level of the plant as a starting point, while working on setting a full action plan for its conservation.

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