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Distribution of Tunisian beet wild relatives (*Beta* sp.) according to morphological characteristics and eco-geographical origin

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ABSTRACT

Beta vulgaris subsp. *maritima* (L.) Arcang. and *Beta macrocarpa* Guss. are crop wild relative taxa belonging to the primary gene pool. They constitute a crucial gene reserve for enhancing cultivated *Beta* species (*B. vulgaris* subsp. *vulgaris* L.). Climate change poses a significant threat to genetic reservoir in Tunisia. We evaluated the morphological diversity of ten populations of *B. vulgaris* subsp. *maritima* and five populations of *B. macrocarpa* growing in different Tunisian bioclimatic and ecological areas using a set of 9 quantitative and 14 qualitative traits to promote the preservation and exploration of this germplasm. Variance component analysis of the quantitative data showed an important spectrum of variability, both within and between populations. The principal component analysis (PCA) allocated this wild *Beta* collection into three groups. G1 included the populations of *B. macrocarpa* that were characterized by the largest glomerules and heaviest seeds, while G2 included all *B. vulgaris* subsp. *maritima* populations except one, i.e., N1015 that clustered into G3, which was characterized by the highest values of leaf characters. Similarly, qualitative traits exhibited a high diversity level (H'index $\geq$0.6) for almost all characters. The PCA divided these 15 populations into three groups as well: G′1 concerned the island *B. vulgaris* subsp. *maritima* populations, characterized by prostrate growth habit and red inflorescences; G′2 included all *B. macrocarpa* populations characterized by erect-procumbent growth habit and very synchronous flowering pattern; and G′3 was formed by the mainland *B. vulgaris* subsp. *maritima* populations, characterized by erect growth habit and hairy, curly leaves. The observed eco-geographic distribution patterns suggest that these wild relatives are highly adaptable to diverse and even extreme conditions (salinity, heat, and drought), highlighting their potential as resilient gene sources for beet breeding under the challenges of accelerating climate change.

Abbreviations: INRAT, National Institute of Agronomic Research of Tunisia; NGBT, National Gene Bank of Tunisia.

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1. Introduction

Crop Wild Relatives (CWRs) are wild plant species closely related to domesticated species of socio-economic value, including those used for food, fodder, forage, condiments, medicine, ornamental, forestry, and industrial purposes [1]. They present greater genetic diversity than their cultivated relatives [2], constitute an important component of both natural environment and agro-ecosystems, and play a crucial role in the functioning and sustainability of ecosystem services [3,4]. CWRs can also be Wild Harvested Plants (WHPs), which are undomesticated species typically harvested from the wild by local populations [5]. They are valuable supplements in diet and medicine because they often are primary sources of healthy, functional foods [5,6].

Because they are not domesticated, CWRs are expected to have high levels of genetic diversity, specifically adaptive genes to the edaphic and climatic constraints underlying the process of local adaptation [7]. They constitute potential sources of important traits, such as resistance to pests and diseases, yield improvement, and/or stability [8], and could ensure food security via the creation of new varieties that are more tolerant to environmental stress and have higher productivity and/or greater nutritional value [1,9].

CWRs account for a substantial 83 % (2445 taxa) of the total identified plant taxa (2912) in Tunisia, encompassing 137 families and 643 genera, thereby amounting to 2243 distinct species. This rich biodiversity cements Tunisia's status as a critical hub of CWR diversity within the Mediterranean Basin [5]. Focusing on the *Beta* genus, two taxa in particular, *Beta vulgaris* subsp. *maritima* (L.) Arcang. and *Beta macrocarpa* Guss., which are the only wild beet species growing in Tunisia, demonstrate a closely linked genetic structure [10] and belong to the primary gene pool (GP1) of cultivated beets [8]. *Beta vulgaris* subsp. *maritima* is considered the ancestral species of all cultivated beets and their closest relatives [11]. It is distributed across the coasts of the Mediterranean Sea and European North Atlantic Ocean [12], and occurs in natural habitats such as cliff coasts, sand beaches, salt marshes, and ruderal sites [13]. Because of its high genetic variability [13–15], *B. vulgaris* subsp. *maritima* has adapted to survive under extreme conditions, such as high salinity and water scarcity [13]. Furthermore, it serves as gene donor for cultivated beet, imparting tolerance against various pests and diseases [11,16].

Beta macrocarpa, described as an annual self-compatible species with predominant autogamy [17], is typically found among halophyte clumps in Tunisia in saline soils bordering Sebkhas (salty marginal areas) [18,19]. This species demonstrates remarkable salt tolerance (up to 200 mM NaCl) because of its morphological, structural, and functional adaptations [20]. Owing to its relatively high protein content (18 %), this species can be used as a fodder additive in marginal arid ecosystems [21]. It has been used to improve productivity [22] and drought tolerance of cultivated relatives [7].

However, the genetic integrity of the Tunisian wild beet germplasm is imperiled by climate change and human activities, leading to

Table 1
Taxon name, geographic and bioclimatic characteristics of Tunisian beet wild relative populations studied here.

Inventory number	Taxon name	Geographic characteristics			Bioclimatic characteristics		
		Governorate, Location	Longitude (E)	Latitude (N)	Annual precipitation (mm)	Minimum temperature of the coldest month (°C)	Maximum temperature of the hottest month (°C)
NGBTUN1012	*B. vulgaris* subsp. *maritima*	Medenine, Djerba island	10.939546	33.869704	217	8	32.8
NGBTUN1013	*B. vulgaris* subsp. *maritima*	Medenine, Djerba island	10.89444	33.874444	213	8.1	32.6
NGBTUN1014	*B. vulgaris* subsp. *maritima*	Nabeul, Sidi Khalifa	10.439822	36.243787	429	6.1	32.8
NGBTUN1015	*B. vulgaris* subsp. *maritima*	Tunis, Botanical garden of INRAT	10.184508	36.84403	466	6	32.2
NGBTUN1016	*B. vulgaris* subsp. *maritima*	Tunis, Sijoumi sebkha	10.122226	36.756951	446	6.2	32.5
NGBTUN1017	*B. vulgaris* subsp. *maritima*	Bizerte, Menzel Jémil	9.861633	37.231746	610	7.4	31.2
NGBTUN1018	*B. vulgaris* subsp. *maritima*	Bizerte, Ghar El-Melh	10.182032	37.167015	530	6.4	31.5
NGBTUN1019	*B. vulgaris* subsp. *maritima*	Nabeul, Soliman sebkha	10.469690	36.721856	427	5.9	32.8
NGBTUN1020	*B. vulgaris* subsp. *maritima*	Ariana, Raoued sebkha	10.235304	36.951067	440	6.7	32
NGBTUN1021	*B. vulgaris* subsp. *maritima*	Sfax, Kerkennah island	11.148465	34.702102	235	7.9	30.5
NGBTUN1022	*B. macrocarpa*	Sousse, Kondar (Kelbia) sebkha	10.224292	35.866166	441	5.8	32.9
NGBTUN1023	*B. macrocarpa*	Sousse, Sidi El-Heni sebkha	10.427189	35.466017	293	6	33.8
NGBTUN1024	*B. macrocarpa*	Tunis, Sijoumi sebkha	10.122226	36.756951	446	6.2	32.5
NGBTUN1025	*B. macrocarpa*	Zaghouane, El-Fahs sebkha	9.790092	36.413458	457	4.6	34.4
NGBTUN1026	*B. macrocarpa*	Nabeul, Soliman sebkha	10.469690	36.721856	427	5.9	32.8

genetic erosion [5,7] and loss of advantageous traits [23,24]. Conservation of such germplasm is imperative for agriculture's sustainability and food security's continuance [25]. Diligent efforts are needed to survey, sample, and characterize these genetic resources to manage both *in-situ* and *ex-situ* conservation, assess their diversity, and exploit them better [25]. Different tools, such as morphological, biochemical, and molecular characterization, are used to evaluate the diversity within and between populations [26]. The morphological characterization constitutes the first step for the taxonomic identification of species, helping in the classification and the selection of germplasm with desirable traits [27,28].

The present study was conducted to (i) assess the pattern of phenotypic diversity beet wild relatives (*B. vulgaris* subsp. *maritima* and *B. macrocarpa*) growing in their natural habitats in Tunisia using a set of 9 quantitative and 14 qualitative descriptors, (ii) establish the relationships between phenotypic diversity and eco-geographic characteristics, and (iii) evaluate the potential adaptation of specific germplasm to abiotic stresses of interest for crop breeding. It is anticipated that these data will be of great interest for conserving and further utilizing of these wild species in breeding programs for beet. The assessment of phenotypic diversity in relation to eco-geographic distribution may guide the preservation of representative, non-redundant germplasm with potential tolerance to abiotic stress, especially drought and salinity.

2. Materials and methods

2.1. Plant material and study area

The study material consisted of 15 wild beet relative populations (10 populations of *B. vulgaris* subsp. *maritima* and 5 populations of *B. macrocarpa*) collected from 12 localities extending from northeast to southeast Tunisia and belonging to four different eco-geographical zones with different bioclimatic characteristics (Table 1).

The northeast Tell zone, which included populations N1014, N1015, N1016, N1017, N1018, N1019, N1020, N1024, and N1026, is characterized by an average rainfall of between 400 and 600 mm and temperatures varying from 6.4 °C (in the coldest month) to 32 °C (in the hottest month). In this zone, the soils are highly varied and complex and can be vertisols, rendzines, lithosols, or deep calci-magnesic soils. The dorsal zone (that included population N1025) is marked by rainfall of about 450 mm/year and strong thermal amplitude at around 30 °C (from 4.6 to 34.4 °C). The soils in this region are similar to those in the northeast Tell zone. The low steppe

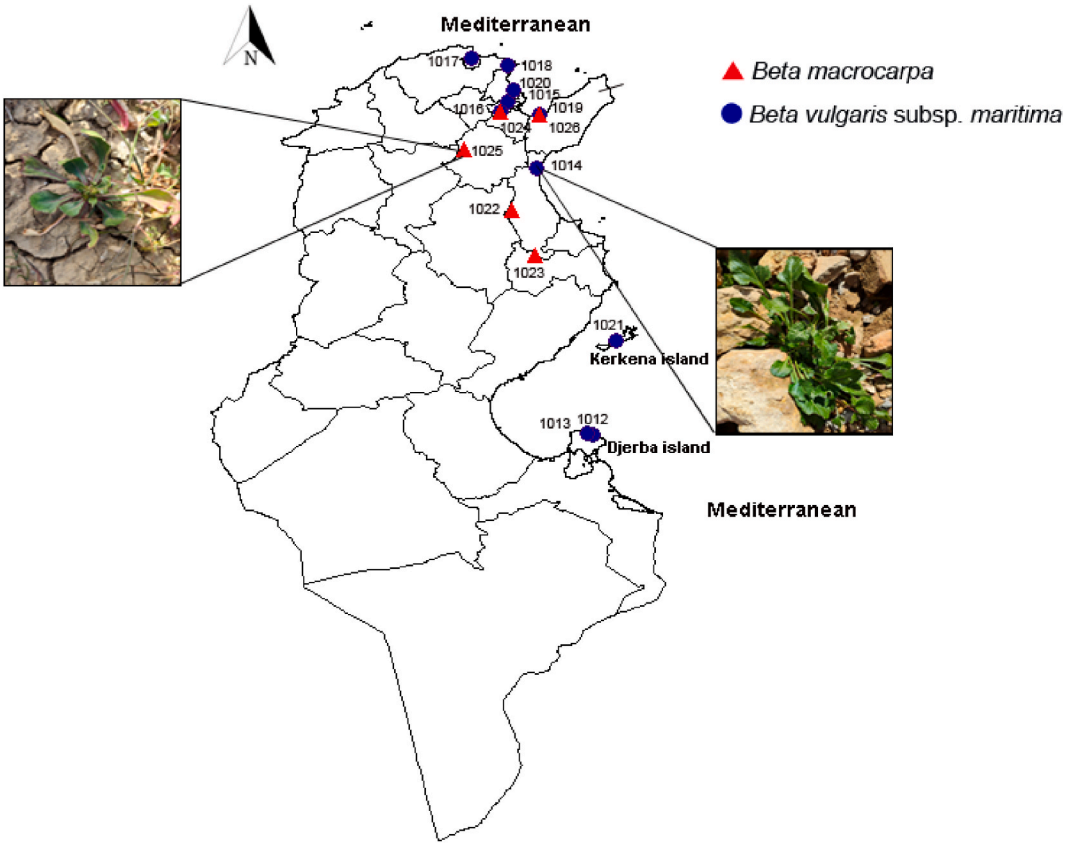

Fig. 1. Geographic distribution of the Tunisian beet wild relatives populations examined in this study. Inventory number as assigned by NGBT are shown in the figure.

zone, which included populations N1022 and N1023, is characterized by an average rainfall ranging from 300 to 440 mm, mean temperature between 5.8 and 33.8 °C, and sandy soils. The southeast islands zone (Djerba and Kerkennah islands), containing populations N1012, N1013, and N1021, are marked by the lowest annual precipitation (213–235 mm), thermal amplitude of about 24 °C, and sandy soils (Table 1).

The collection sites were identified according to Pottier-Alapetite [29]. Primary taxonomic identification was performed in the field according to plant growth habits, leaf morphology, and flowering morphology [29].

Passport data and inventory numbers were assigned to each population according to the National Gene Bank of Tunisia database; full details are available from the Germplasm Resources Information Network (http://www.tn-grin.nat.tn/gringlobal/search). Geographic Positioning System coordinates were imported into the DIVA-GIS program version 7.5 (https://diva-gis.org/) and served as input data for mapping the populations (Fig. 1).

2.2. Bioclimatic data

For each sampling location, the main climate parameters, such as annual rainfall, average maximum temperature of the hottest month, and average minimum temperature of the coldest month were collected from the WordClim database (https://www.worldclim.org/) at a spatial resolution of 2.5 arc-minutes (approximately 5 km^2 at the equator) and used to calculate the pluviothermic Emberger coefficient Q2 according to the following formula:

$$Q2 = 2000P/M^2 - m^2$$

where P is the annual rainfall (mm), M is the average of the maximum temperature of the hottest month and m is the average of the minimum temperature of the coldest month.

2.3. Morphological characterization

Populations were examined for 9 quantitative and 14 qualitative characters (traits) related to plant architecture (growth habit), leaves, stems, bracts, inflorescences, and seeds (Table 2). Observations were made on 20 randomly selected individuals per population. The traits were chosen following the descriptor lists for *Beta* (*Beta* spp.) of the International Plant Genetic Resources Institute (IPGRI) [27]. Additional characters, such as inflorescence height (IH), bract shape (BS), and glomerule diameter (GD), were included according to previous studies [30–32]. All morphological characters, except 1000 seeds weight (SW), were measured at the sampling site. Quantitative traits were measured with a ruler or digital caliper, whereas qualitative characters were based on scoring and coding according to IPGRI [27] descriptors. The SW trait was measured in the laboratory using a precision balance, and seeds from all

Table 2
Morphological descriptors, descriptor states and their codes for numerical analysis on wild beet relative populations in Tunisia.

Trait/descriptor	Acronym	Type	Classes/unit
Plant architecture			
Growth habit	GH	QL	1 = Erect, 2 = Erect and procumbent, 3 = Procumbent, 4 = Erect and prostrate, 5 = Prostrate
Stem			
Stem color	SC	QL	2 = Yellow/light green, 3 = Green
Stem pigmentation	SP	QL	0 = Absent, 1 = Stripped,
Stem hairiness	SH	QL	1 = Globrous, 2 = Hairy
Leaf			
Leaf color	LCol	QL	1 = Yellow/light green, 2 = Green
Leaf pigmenation	LP	QL	0 = Absent, 1 = Spotted, 2 = Red vein, 3 = Entire red, 4 = Red border
Petiole color	PC	QL	2 = Yellow/light green, 3 = Green, 4 = Pink, 5 = Red
Leaf curliness	LCur	QL	3 = Smooth, 5 = Medium, 7 = Curled
Leaf hairiness	LH	QL	1 = Glabrous, 3 = Very sparse, 5 = Hairy, 7 = Very hairy
Leaf shape	LS	QL	1 = Spatulate, 2 = Ovate
Leaf blade length	LBL	QN	cm
Leaf blade width	LBW	QN	cm
Petiole length	PL	QN	cm
Petiole width	PW	QN	cm
Cuticule thickness	CT	QN	mm
Bract			
Bract shape	BS	QL	1 = Rhomboic, 2 = Lanceolate
Bract thickness	BT	QN	mm
Inflorescence			
Inflorescence color	IC	QL	1 = Green, 2 = Green and red, 3 = Red
Multigermicity	MG	QL	3 = Mono and bigerm, 5 = Muligerm (2–4), 7 = Multigerm (>5)
Flowering-pattern between plants	FP	QL	1 = Very asynchrounous, 9 = Very synchronous
Inflorescence height	IH	QN	cm
Glomerule diameter	GD	QN	mm
Seeds			
1000 seeds weight	SW	QN	g

populations were conserved in the NGBT and INRAT seed banks.

2.4. Data analysis

Statistical analyses were conducted using packages in R software version 4.4.0, which is available from the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org.

For quantitative traits, we applied a Variance Component Analysis (VCA) using the Ime4 package [33] to study the variation within and between populations for each parameter. Furthermore, Pearson correlation analysis was performed using the Corre package [34], providing insight into linear relationships to explore the relationships among the assessed quantitative parameters.

The frequency distributions of the qualitative traits were calculated using the Proc Freq procedure in SAS software version 9.1. To quantify the diversity present in each qualitative characteristic, we computed the estimates of variability using the Shannon–Weaver Diversity Index. The Shannon–Weaver Index, denoted as H', is a measure of diversity that considers the frequency and richness of categories within a dataset, offering a standardized approach to evaluating variability [35] and is calculated using the following formula:

$$H' = -\frac{\sum_{i=1}^{n} pi.\log_2(pi)}{\log_2(n)}$$

where pi is the frequency proportion of each descriptor state and n is the number of states for each descriptor.

The diversity index was coded as high ($H' \geq 0.60$), intermediate ($0.40 \leq H' < 0.60$) or low ($0.10 \leq H' < 0.40$) as described by Eticha et al. [36]. Means determined for each quantitative parameter and frequencies determined for each state of qualitative parameters were calculated for each population and subjected to Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) using the FactoMineR package [37] to determine the relationships among the different parameters and group populations into homogenous classes according to their morphological characters.

3. Results

3.1. Morphological diversity

3.1.1. Quantitative traits

VCA performed on the quantitative data displayed an important spectrum of variability, both within and between populations. Glomerule diameter (GD), seed weight (SW), inflorescence height (IH), and bract thickness (BT) had high percentages of variability, which were attributed to differences among populations. In contrast, traits such as cuticle thickness (CT), petiole width (PW), and petiole length (PL) exhibited lower between-population variability (Table 3).

Beta vulgaris subsp. *maritima* had the highest mean values for leaf blade length (LBL), leaf blade width (LBW), petiole length (PL) and inflorescence height (IH), whereas *B. macrocarpa* displayed the highest values for glomerule diameter (GD) and 1000 seeds weight (SW). Among *B. vulgaris* subsp. *maritima* populations, N1015 stood out from all the other populations. It had the highest values for four traits related to leaves (i.e., LBL, LBW, PL, and PW). For *B. macrocarpa* populations, N1023 and N1024 were distinguished by their highest mean values for glomerule diameter (GD) and 1000 seeds weight (SW) (Supplementary Table 1).

Pearson correlation coefficients (r) were calculated to determine the linear relationships between all quantitative parameters. The correlation matrix showed that the four features were correlated at $p < 0.05$ significance level (Fig. 2). Leaf blade width (LBW) showed the highest correlation coefficients with leaf blade length (LBL; $r = 0.97$) and petiole length (PL; $r = 0.95$). LBL also positively and significantly correlated with PL ($r = 0.91$). A significant positive correlation ($r = 0.95$) was observed between glomerule diameter (GD) and 1000 seeds weight (SW).

The PCA (Supplementary Fig. 1), showed that 67.7 % of variance was scored using the first two principal components. The first principal component (PC1) explained 40.4 % of the total variability and was highly associated with leaf-related traits such as LBL,

Table 3
Variation among and between Tunisian beet wild relative populations for nine quantitative characters.

Trait [a]	Variance Component (between-Populations)	Variance Component (within-Populations)	Total Variance	% Variance within Populations	% Variance between populations
LBL	8.463	8.114	16.577	48.95 %	51.05 %
LBW	3.114	3.449	6.563	52.55 %	47.45 %
PL	10.45	16.06	26.51	60.58 %	39.42 %
PW	0.5066	0.7966	1.303	61.13 %	38.87 %
CT	0.0076	0.0158	0.023	64.40 %	32.60 %
GD	3.3913	0.8249	4.216	19.57 %	80.43 %
IH	53.43	47.21	100.64	46.91 %	53.09 %
BT	0.0501	0.0497	0.099	49.80 %	50.20 %
SW	111.98	36.36	148.34	24.51 %	75.49 %

[a] For trait abbreviation, see Table 2.

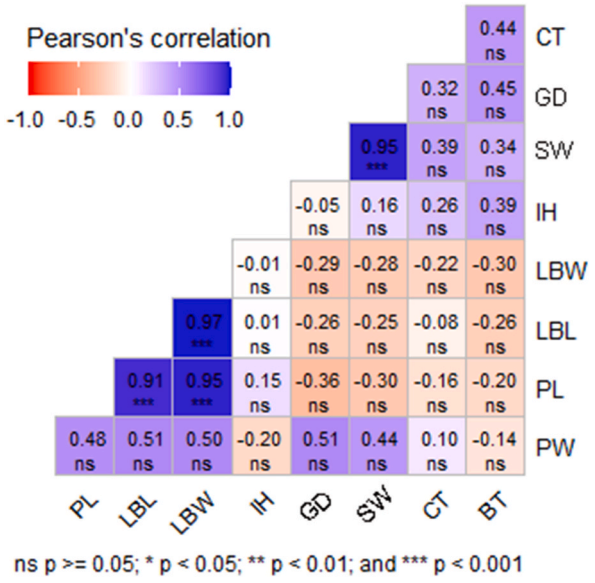

Fig. 2. Pearson correlation between quantitative traits of the Tunisian beet wild relative populations.

LBW, and PL. The second principal component (PC2), which accounted for 27.3 % of the total variation, was mainly correlated with GD and SW.

The scatter plot of the PCA in the plan defined by the first two components separated the 15 populations into three groups based on their quantitative traits (Fig. 3). The first group (G1) was formed of *B. macrocarpa* populations. The second group (G2) was represented by all *B. vulgaris* subsp. *maritima* populations except for N1015, which diverged from all other populations and formed the third group (G3).

3.1.2. Qualitative traits

The frequency distribution of the 14 qualitative characteristics (discontinuous variables) is shown in Fig. 4. Growth habits and leaf pigmentation had the highest levels of polymorphism. Most populations were characterized by erect-procumbent (39.16 %) or erect (23.78 %) growth habits, while the leaves were light green (76.22 %), devoid of pigmentation (80.42 %), smooth (55,95 %), and glabrous (49.65 %). The stems were mainly light green (82.52 %), glabrous (72.03 %), stripped (48.36 %), or non-pigmented (56.64

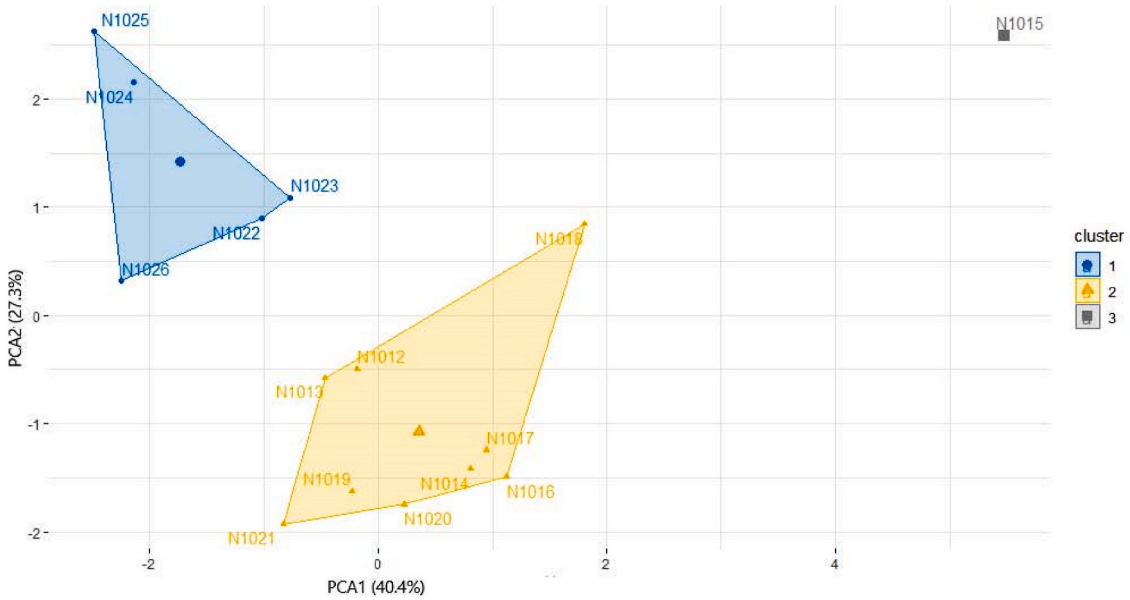

Fig. 3. Scatter plot grouping of the Tunisian beet wild relative populations on the first two principal components analysis using quantitative traits.

Fig. 4. Distribution of 14 qualitative traits among Tunisian beet wild relative populations. For each trait, the % of individuals falling into each of the different classes considered for descriptors is indicated.

%). The flowering pattern between plants was mostly asynchronous (68.53 %), with green (54.55 %) or green and red (39.86 %) inflorescences and two types of multigermicity, i.e., mono-bigerm (42.66 %) and multigerm (55.55 %).

Analysis of the frequency distribution of qualitative traits between species revealed more variability in *B. vulgaris* subsp. *maritima* than in *B. macrocarpa* for stem hairiness and leaf-related traits (color, curliness, hairiness, and shape). These traits were polymorphic in *B. vulgaris* subsp. *maritima* and monomorphic in *B. macrocarpa*. Growth habit, stem pigmentation, and inflorescence color were polymorphic in both species, whereas the flowering patterns between plants were monomorphic (Supplementary Fig. 2). The comparison between populations showed that the island *B. vulgaris* subsp. *maritima* populations (N1012, N1013, N1021) had the highest levels of polymorphism in stem color (SC), stem pigmentation (SP), and inflorescence color (IC) traits. Growth habit (GH) was the most polymorphic in the N1016, N1019 (*B. vulgaris* subsp. *maritima*), N1024, and N1026 (*B. macrocarpa*) populations, exhibiting all trait classes (erect, erect-procumbent, procumbent, erect-prostrate, and prostrate) (Supplementary Fig. 3). The estimate of variation using the Shannon–Weaver diversity index (H′) showed a high level of diversity (H' $\geq$ 0.6) for all studied characters, except for leaf pigmentation (LP), which exhibited an intermediate H' (0.42). The most polymorphic trait was leaf shape (LS; H' = 0.99), followed by stem pigmentation (SP; H' = 0.97), bract shape (BS; H' = 0.97), and growth habit (GH; H' = 0.91). High variation indicates an equitable distribution of the different states, whereas low variation indicates the dominance of a one-character state over the others, as shown by the frequency distribution. The mean diversity index for all qualitative traits recorded across all populations was 0.80, indicating significant phenotypic diversity within the collection. The number of classes observed for some traits, such as petiole color, stem color, stem pigmentation, and multigermicity, was less than that mentioned in IPGRI descriptors list [27]. In contrast, LP character exhibited a new class (red border) not reported in the descriptors (Table 4).

By comparing the two species, we noted that the diversity index was significantly higher in *B. vulgaris* subsp. *maritima* species for some traits related to leaf and stem, such as leaf color (H' = 0.93), leaf hairiness (H' = 0.9), leaf curliness (H' = 0.83) and stem hairiness (H' = 0.97). In *B. macrocarpa*, these characters are monomorphic (H' = 0). The highest index values (H' = 1) within *B. vulgaris* subsp. *maritima* populations were obtained for leaf color in the N1017 population, petiole color in the N1014 and N1018 populations, and stem color, stem pigmentation and inflorescence color in the island populations (Supplementary Table 2).

The PCA performed on qualitative traits showed that the first two principal components explained 46.4 % of the total variation. The first principal component (PC1) recorded 28,2 % of the total variance and was positively correlated with leaf-related traits, such as curliness (smooth; LCur3 or medium; LCur5), hairiness (glabrous; LS1) and shape (spatulate; LS1 or ovate; LS2). The second principal component (PC2) accounted for 18,2 % of the total variance and was mainly correlated with leaf color (light green (Lcol1) or green (Lcol2)) and stem color (light green (SC2) or green (SC3)) (Supplementary Fig. 4).

The scatter plot of PCA in the plan defined by the first two components separated the wild *Beta* collection into three groups based on their qualitative characters. The first group (G′1) included the three island *B. vulgaris* subsp. *maritima* populations N1012, N1013, and N1021, which were distinguished from the other populations by their prostrate growth habit, deep green leaves, and red-colored inflorescences. The second group (G′2) was formed by the mainland *B. vulgaris* subsp. *maritima* populations (N1014–N1020), and was mainly defined by erect growth habit and curled and hairy leaves. The third group (G′3) contained all *B. macrocarpa* populations (N1022-N1026), and was characterized by erect and procumbent growth habits, green inflorescences, and a synchronous flower pattern between plants (Fig. 5).

3.2. Clustering of Beta populations based on combined qualitative and quantitative characters

A hierarchical cluster dendrogram combining qualitative and quantitative characters was constructed using AHC to evaluate the general pattern of variability within Tunisian wild *Beta* populations and establish the genetic relationship between them. The dendrogram separated the wild *Beta* populations into several clusters, with distances ranging from 0 to 10. At the average distance of 5, the dendrogram identified three main clusters (i.e., Cl1, Cl2 and Cl3), which included the same groups (G′1-G′3) as identified using the qualitative data. However, population N1015, which consistently showed the highest values for leaves characters, was separated from all other mainland *B. vulgaris* subsp. *maritima*. Populations N1022 and N1023, which had similar quantitative characters were grouped

Table 4
Characteristics of fourteen qualitative traits used for morphological characterization of Tunisian beet wild relatives populations.

Trait	Number of total classes	Number of observed classes	Diversity index (H′)
Growth habit	5	5	0.91
Leaf color	2	2	0.79
Leaf pigmentation	4	5	0.42
Petiole color	5	4	0.79
Leaf curliness	3	3	0.79
Leaf hairiness	4	4	0.84
Leaf shape	2	2	0.99
Stem color	3	2	0.67
Stem pigmentation	3	2	0.97
Stem hairiness	2	2	0.85
Inflorescence Color	3	3	0.78
Bract shape	2	2	0.97
Flower-pattern between plants	2	2	0.89
Multigermicity	4	3	0.72

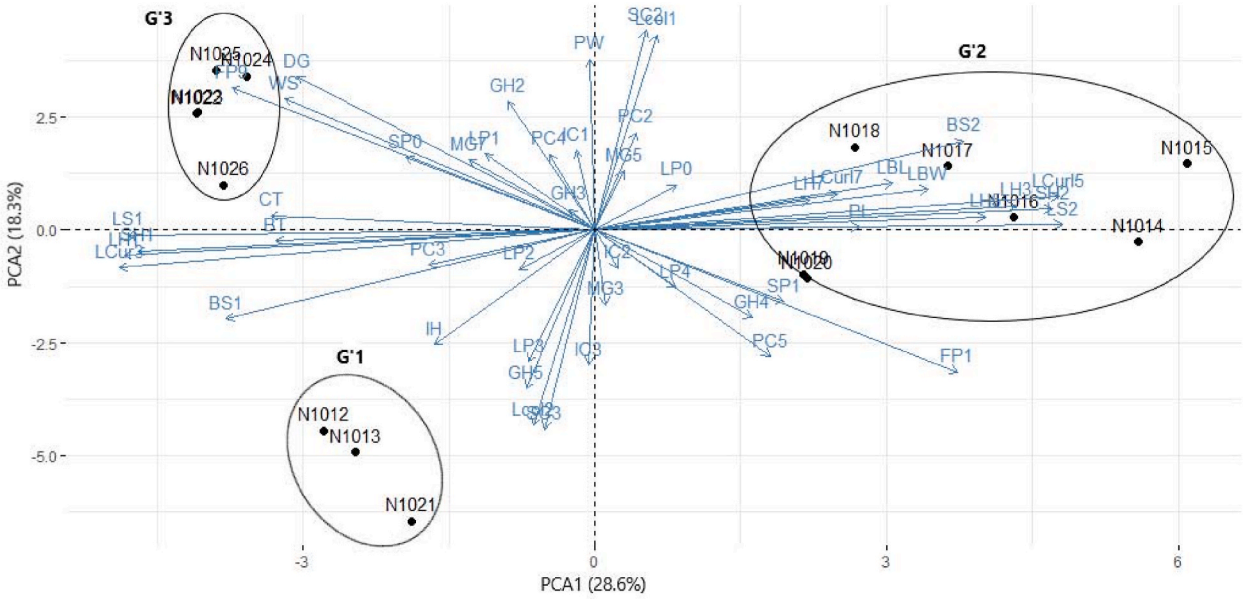

Fig. 5. Scatter plot grouping of the Tunisian beet wild relative populations on the first two principal components analysis using qualitative traits.

and separated from the other *B. macrocarpa* populations (Fig. 6).

3.3. Geographical and climatic distribution

The geographic distribution pattern of beet wild populations is shown in Fig. 1. A large spatial pattern was observed in *B. vulgaris* subsp. *maritima* species; thus, three populations were island and seven populations were mainland. Among the seven populations, four (N1014, N1017, N1018, and N1020) originated from coastal regions, two (N1016 and N1019) from Sebkhas, and one (N1015) from the botanical garden of INRAT. All *Beta macrocarpa* populations (N1022, N1023, N1024, N1025, and N1026) were exclusively found at the edges of the Sebkhas. This was also observed in sympatry with *B. vulgaris* subsp. *maritima* in the Soliman and Sijoumi Sebkhas.

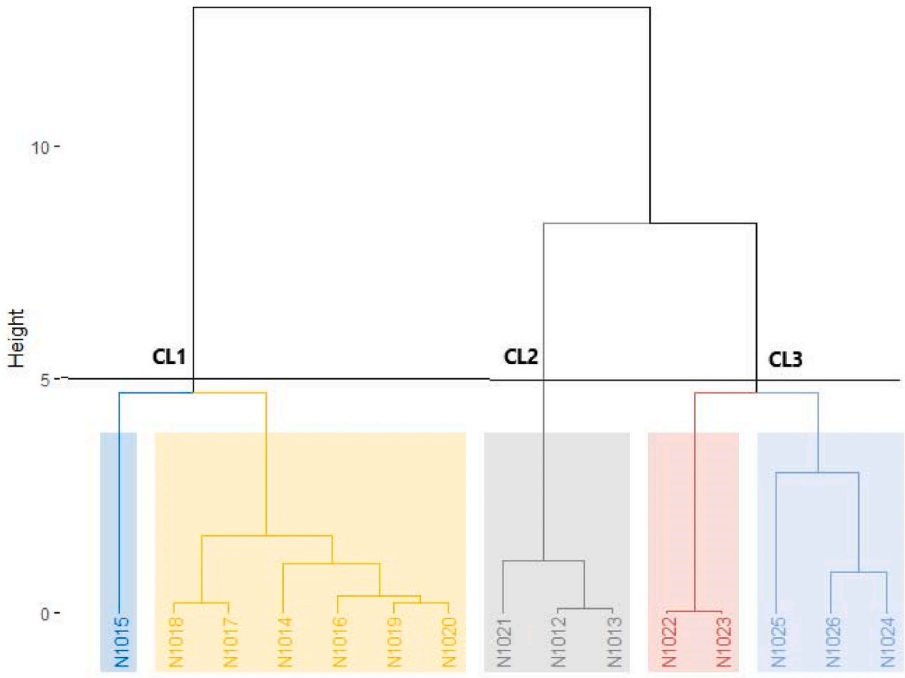

Fig. 6. Dendrogram obtained from Hierarchical cluster analysis of Tunisian wild beet populations using qualitative and quantitative data.

According to Emberger's climogram (Fig. 7), a large variability in growing conditions for the 15 wild beet populations was observed for both species; thus, populations of *B. vulgaris* subsp. *maritima* belonged to three bioclimatic zones: arid/warm winter areas for the three island populations (N1012, 1013, and N1021), sub-humid/temperate winter areas (for the mainland population N1017), and semi-arid/temperate winter areas for the remaining mainland populations (N1014, N1015, N1016, N1018, N1019, and N1020). Populations of *B. macrocarpa* belonged to either the arid/temperate winter areas (N1023) or the semi-arid/temperate winter areas (N1022, N1024, N1025, and N1026).

4. Discussion

Despite their significant potential roles in enhancing crop varieties, mitigating climate change, preserving ecosystems, and bolstering food security, Crop Wild Relatives (CWRs), including wild beet species, remain underexplored in Tunisia. To the best of our knowledge, apart from the pioneering work by Pottier-Alapetite [29], which was dedicated to identifying *Beta* species within Tunisia, there appears to be a lack of research addressing the eco-geographic distribution and morphological diversity of Tunisian *Beta* wild relatives based on internationally recognized standards.

Our investigations concerned 15 populations of *Beta* wild relatives, *B. vulgaris* subsp. *maritima* and *B. macrocarpa,* growing in different regions of the country, extending from the northeast to the southeast.

Eco-geographic characterization showed that these populations inhabit different ecological (island and mainland, including coastal and salty areas) and climatic (sub-humid, semi-arid, and arid) areas. This large distribution reflects (i) the high adaptability of the Tunisian wild *Beta* germplasm to different ecological and climatic environments and (ii) its capacity to tolerate severe drought and high salinity under hostile conditions in arid climates [13,20].

Morphological analysis, incorporating quantitative and qualitative parameters, revealed substantial variability within and between populations. In particular, VCA performed on quantitative data revealed that some traits, such glomerule diameter (GD), seed weight (SW), inflorescence height (IH) and bract thickness (BT), had high percentages of variability attributed to differences among populations. This suggested that genetic differentiation is driven by adaptation to local environmental conditions; however, genetic analyses must be performed to confirm this hypothesis. In contrast, traits such as cuticle thickness (CT), petiole width (PW) and petiole length (PL) exhibited lower between-population variability, suggesting a more stable expression across different environments, which could indicate stabilizing selection pressures. Such morphological diversity observed in the wider Mediterranean region is likely a result of adaptation to dynamic and varied Mediterranean habitats [31,38], signaling the evolutionary resilience of these species and their potential value in agricultural advancements. The capacity of a given genotype to express different phenotypes in response to shifts in environmental conditions, known as phenotypic plasticity, is a key mechanism by which plants cope with changing climates [39,40]. This can lead to new phenotypes with adaptive capacities [41].

Among *B. vulgaris* subsp. *maritima* populations, the N1015 population growing in the botanical garden of INRAT, distinguished by having the largest leaves, diverged from all the others and formed a separate group. This population stood out from all others by having significantly higher values for the four traits related to leaves. Human practices such as irrigation are favorable for the development of vegetative organs, including leaves. This population was considered an outlier because it was not subjected to the same stressful conditions as the other populations. In contrast, the remaining populations, belonging to the G1 and G2 groups and characterized by smaller leaves, grew on unfavorable island, coastal, and salt marsh areas. Similar observations have been reported for wild sea beets growing under abiotic stress in the Madeira's archipelago [32]. These observations indicate that *Beta* species have adaptive phenotypic plasticity to harsh environmental conditions based on modifying growth patterns [42]. Reducing leaf size allows *Beta* plants to resist to water loss by evapotranspiration. Globally, this is a result of genotype × environment interactions or gene interference [43]. As reported by You et al. [44], Tunisian coastal areas are important habitats for salt- and drought-tolerant plants. The occurrence of *Beta* species next to the sea or at the edge of Sebkhas in semi-arid or arid climates suggested the presence of drought and salt stress tolerance genes within our germplasm. Similarly, the presence of *D. carota* subsp. *gummifer* growing in close proximity to the Mediterranean Sea suggested that this taxon is a potential candidate for salt stress tolerance [45]. Additionally, the eco-geographic characterization of carrot wild relatives in Tunisia identified *D. syrtcicus* Murb. and *D. carota* subsp. *capillifolius* (Gilli) Arbizu as endemic taxa with potential adaptation to high temperatures and low precipitation; both heat and drought stress tolerance have been reported for *D. carota*

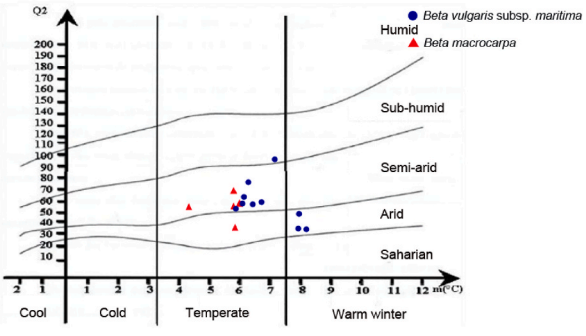

Fig. 7. Climogram showing the distribution of the Tunisian beet wild relative populations according to the Emberger's coefficient.

subsp. *capillifolius* in field trials. Finally, the high level of polymorphism observed within the studied wild *Beta* populations was similarly observed in Tunisian collections of *Daucus* L. [46,47], *Thymus capitatus* (L.) Hoffm. et Link. [48], and *Quercus coccifera* L [49].

Qualitative traits also exhibited considerable diversity among populations, as shown by the mean Shannon–Weaver diversity index of 0.8.

The stem pigmentation (SP) trait recorded the highest diversity index (H' = 0.99) because of the equitable distribution between class 1 (absence of pigmentation; 46.15 %) and class 2 (stripped stem; 53.85 %). This pigmentation is due to the presence of betalain pigments synthesized in *Beta* plant organs, including petals, fruits, leaves, stems, and roots [50,51]. Their high accumulation could explain plant responses to abiotic stresses, such as drought and salinity, to scavenge free radicals [52]. This information corroborates our findings on the island *Beta vulgaris* subsp. *maritima* populations, which develop red-colored inflorescences as a form of adaptation to severely arid conditions.

The growth habit (GH) was also characterized by a high diversity index (H' = 0.91) with an equitable distribution between all morphological states (erect, erect-procumbent, procumbent, erect, and prostrate). Similar results have been reported in wild beet populations in Sicily. Occasionally, different types were observed in only one population [31]. The erect (23.78 %) and erect-procumbent (39.16 %) growth habits were the dominant morphotypes in the Tunisian population. A similar tendency was described in Italian *B. vulgaris* subsp. *maritima* populations [31], whereas Masutani et al. [53] reported that the prostrate type was predominant. In our study, prostrate GH was mainly found in the island *B. vulgaris* subsp. *maritima* species, suggesting an adaptive behavior to wind exposure, as hypothesized by Ascarini et al. [32].

According to our results, *B. vulgaris* subsp. *maritima* exhibits greater phenotypic diversity than *B. macracarpa* especially in terms of stem hairiness, leaf color, curliness, hairiness, and shape. High variability within *B. vulgaris* subsp. *maritima* was previously reported by Ribeiro et al. [13].

Based on these qualitative traits, the clustering of Tunisian wild *Beta* populations into three groups, i.e., G′1 (island *B. vulgaris* subsp. *maritima*), G′2 (mainland *B. vulgaris* subsp. *maritima*) and G′3 (*B. macrocarpa*), was evidenced by the PCA analysis. This clustering reflected the influence of the ecological origin in the case of *B. vulgaris* subsp. *maritima* populations (island *vs* mainland) and the genetic factor (*B. vulgaris* subsp. *maritima vs B. macrocarpa*). The distribution pattern of *B. vulgaris* subsp. *maritima* populations in the two distinct groups could be explained by their differential adaptations to their respective ecosystems. The same distribution was obtained by using a combination of quantitative and qualitative traits.

5. Conclusions

The main achievement of this study was to assess the phenotypic diversity and eco-geographic distribution of Tunisian beet wild relatives (*Beta* spp.). Knowledge of the morphological variability within collections is critical for taxonomic identification and trait use in crop breeding [47]. This can be helpful in establishing conservation priorities and better managing conserved germplasm. Therefore, it is necessary to work with representative, non-redundant specimens with wide genetic diversity [54]. According to our results, the high level of diversity observed within our germplasm highlights the need for an urgent conservation program to avoid the risk of losing genetic resources, especially for endangered species such as *B. macrocarpa* [8]. Eco-geographic characterization has provided considerable information on the resilience of local germplasms to stressful environmental conditions, such as elevated salinity, drought, and extreme temperatures. This implied that this germplasm is a potential candidate for testing abiotic stress tolerance and can be used as a gene donor for beet breeding programs. The contribution of such traits from wild relatives has not advanced their use in currently grown beet cultivars. Identification of germplasm sources for these important traits is valuable for coping with the anticipated abiotic challenges in future agricultural production. Further studies are required to acquire additional populations to enrich this collection. This research will progress by integrating morphological and molecular data, reinforcing the observed diversity, and enhancing the management of *Beta* germplasm.

CRediT authorship contribution statement

Kaouther Ben Mahmoud: Writing – original draft, Resources, Investigation, Data curation, Conceptualization. **Najla Mezghani:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Youssef Ouakrim:** Software, Formal analysis. **Neila Mezghani:** Writing – review & editing, Software, Formal analysis. **Noura Jemai:** Resources. **Ahmed Jemmali:** Supervision, Project administration.

Ethics statement

All participants in this study gave their informed consent to the publication of this manuscript. This study contains no bioethically questionable data and therefore did not require ethics committee review or approval'.

Data availability statement

Data supporting the findings of this study are provided in the publication and Supplementary Materials.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41773.

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