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Haplotype diversity and molecular phylogeny of wild *Crambe* L. (Brassicaceae) taxa of Turkey

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Abstract: Revealing genetic diversity is both essential for plant systematics and also provides important information for agricultural sciences. *Crambe hispanica* var. *abyssinica* is an oil seed crop. Wild plants related to crops (Crop Wild Relatives) are important resources for the genetic improvement of cultivated species. In order to reveal the genetic diversity of wild *Crambe* taxa of Turkey, we used ITS and *trn*L-F markers to create the haplotype networks and phylogeny reconstruction. Thirty-nine accessions belonging to 8 *Crambe* taxa were used as the material of our study: *Crambe orientalis* var. *orientalis* with 18 accessions, *Crambe orientalis* var. *sulphurea* with 3 accessions, *Crambe orientalis* var. *dasycarpa* with 1 accession, *Crambe alutacea* with 1 accession, *Crambe grandiflora* with 1 accession, *Crambe tataria* var. *tataria* with 8 accessions, *Crambe tataria* var. *aspera* with 2 accessions, and *Crambe maritima* with 5 accessions. The phylogeny inference of ITS and *trn*L-F data revealed two major lineages: one consisted of *Crambe maritima* and *Crambe tataria* accessions, while the other consisted of *Crambe orientalis* subsp. *orientalis*, *Crambe orientalis* subsp. *sulphurea*, *Crambe orientalis* var. *dasycarpa*, *Crambe grandiflora*, and *Crambe alutacea.* In the haplotype networks based *trn*L-F region, all *Crambe maritima* accessions and most of the *Crambe. orientalis* accessions shared one haplotype. However, *Crambe maritima*, *Crambe orientalis*, and *Crambe tataria* shared no common haplotype in the networks based on ITS region. In both networks, *Crambe alutacea* shared one haplotype with some *Crambe orientalis*. *Crambe maritima* and *Crambe orientalis* shared the H1 haplotype. Other haplotypes differed from the most common haplotype (H1) by one or two base pairs. *Crambe orientalis* is the species with the highest haplotype diversity and IT6 haplotype has the highest seed oil content among CWR of *Crambe* in Turkey.

Key words: *Crambe*, crop wild relatives, haplotype network, oil seed crop, phylogeny

1. Introduction

The genus *Crambe* belongs to the family Brassicaceae. Brassicaceae has 338 genera and 3709 species in the world (Al-Shehbaz, 2006). Within this family, several species of crops (e.g., *Brassica* spp.), weeds (e.g., *Capsella*, *Lepidium*, *Sisymbrium*, and *Thlaspi*), and ornamentals (e.g., *Hesperis*, *Lobularia*, and *Matthiola*) (Couvreur, 2009) are particularly noteworthy. Brassicaceae members have characteristic cross-shaped corolla, tetradynamous stamens, and capsule fruits. The genus *Crambe* L., which has approximately 34 species in the world, is one of the largest genera of the tribe Brassiceae. It has a fairly wide distribution area from Macaronesia to the western Himalayas. It mainly includes hemicryptophytes, chamaephytes, and a small number of annuals. De Candolle (1821) divided the genus *Crambe* into three sections according to fruit morphology. These are *Dendrocrambe* DC., *Leptocrambe* DC., and *Sarcocrambe* DC. This genus classification is also in harmony with the geographical distributions of the species. The species of the section *Dendrocrambe* are endemic to the islands of Macaronesia, while *Leptocrambe* includes species that are distributed in the Mediterranean basin and East Africa. The section *Sarcocrambe* includes species that spread from East Europe to the Central Asian steppe areas and mountains. The type species of the genus was determined by Green (1925) to be *Crambe maritima* L. While this species previously belonged to the section *Sarcocrambe* of De Candolle's classification (De Candolle, 1821), it was later utilized by Prantl (1891) in forming the section *Crambe* L. and Schulz (1919) subsequently added new species to this section. With the latest contributions, 3 sections belonging to the genus *Crambe* are recognized today. These are *Leptocrambe* with 5 species and 5 subspecies (Prina, 2000), *Dendrocrambe* with 14 species (Prina and Martínez-Laborde, 2008), and *Crambe* (≡sect. *Sarcocrambe* DC.) with 16 species and 5 subspecies.

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In the first volume of *Flora of Turkey* (Davis, 1965), the genus *Crambe* had 4 taxa belonging to 2 species. In Volume 10 of that series (Davis et al., 1988), *Crambe maritima* was added as a new record for the Turkish flora. *Crambe hispanica* by Yıldıztugay et al. (2009), *Crambe orientalis* L. var. *sulphurea* Stapf ex O.E.Schulz from Şanlıurfa by Prina, and *Crambe grandiflora* DC. from Birecik were subsequently recorded. The record of *Crambe orientalis* L. var. *dasycarpa* O.E.Schulz was given from Mersin. Prina (2009) suggested some changes in the taxonomic revision of the section *Crambe*, according to which *Crambe orientalis* L. var. *sulphurea* Stapf ex O.E.Schulz was proposed as *Crambe orientalis* subsp. *sulphurea* (Stapf ex O.E.Schulz) Prina. *Crambe orientalis* L. var. *alutacea* (Hand.-Mazz.) Hedge & Hub.-Mor. was also reevaluated as *Crambe alutacea* Hand.-Mazz (Prina, 2009). With all of these new records and taxonomic changes, the total number of species in Turkey reached 6 species with 10 taxa.

In literature, there are molecular phylogeny and genetic diversity studies on *Crambe* taxa. Francisco-Ortega et al. (1999) constructed the phylogeny tree of the genus *Crambe* with the nuclear ITS marker and determined that the genus is monophyletic with three main lineages consisting respectively of the species from Macaronesia, the Mediterranean basin, and European Siberia-Asia. The taxa that grow in Turkey were grouped under three clades;*Crambe hispanica* to section *Leptocrambe* DC, *Crambe tataria, Crambe maritima*, and *Crambe aspera* to section *Crambe* L., and *Crambe orientalis* to section *Orientecrambe* I. Khalilov. *Crambe grandiflora* and *Crambe alutacea* were not included in that analysis. Briard et al. (2002) studied the morphology of natural *Crambe maritima* populations in France and used random amplified polymorphic DNA (RAPD) markers as well. In this research, the aim was to bring a systematic insight to enlarge the genetic basis of sea kale. Bond et al. (2005) used inter-simple sequence repeat (ISSR) markers while investigating the population structure of *Crambe maritima*. In their study, they tried to reveal the relationship between genetic diversity and population structure. To determine the genetic diversity of the *Crambe* taxa that naturally grow in Turkey, Tarıkahya-Hacıoğlu (2016) used ISSR markers and she revealed both the genetic relationships of populations and species.

Increasing industrial activities around the world have led to an increase in energy needs. For this reason, the use of oilseed plants and products has increased. Biodiesel, engine and machine lubricants, other lubricants, bioplastic, nylon, cosmetic, and dye industries are the leading consumers of oil seeds (Lalas et al., 2012). High seed oil content is a desired property for plant breeding and *Crambe* is a promising crop for this purpose. Cultivated *Crambe hispanica* var. *abyssinica* in China was found to have 34.48% oil content in whole seeds, of which 62.50% was erucic acid (Wang et al., 2000). Simon et al. (2019) also recorded varieties with 55% erucic acid content. Erucic acid (C22:1) is a fatty acid with industrial importance as it is used to produce erucamide, a key component in the plastic industry. Agronomy studies show that the inclusion of *Crambe* species into crop rotations can be beneficial because of their short life cycles, low fertility requirements, resistance to pests and diseases, and relative drought tolerance (Samarappuli et al., 2020). According to Samarappuli et al. (2020), the ability of the cultivated *Crambe abyssinica* Hochst, to survive in diverse environmental conditions, its unique oil composition, the high oil content, the suitability for the production of slip agents for plasticizers, the capacity to be easily included in common crop rotations, and the adaptability to equipment used for small grain cultivation have all renewed the interest in this emerging crop.

For agriculture, crop wild relatives (CWRs) can provide important resources for the genetic improvement of cultivated species (Miller and Khoury, 2018). Today, as the value of genetic resources is better understood, research on the genetic diversity and phylogenetic relations of CWRs has gained momentum. As Warwick et al. (2009) explained: "Wild relatives also possess a number of useful agronomic traits which could be incorporated into breeding programs, including cytoplasmic and nuclear male sterility; resistance to disease and insect and nematode pests; intermediate C3-C4 photosynthetic activity; and tolerance of cold, salt and drought conditions." The genetics of CWRs will have a key role in future breeding studies. According to investigations of CWR species of *Crambe*, the seed oil content of *Crambe orientalis* was 11% and that of *Crambe tataria* was 15% (Comlekcioglu et al., 2008). Comlekcioglu et al. (2008) researched the seed oil compositions of *Crambe orientalis* subsp. *orientalis* and *Crambe tataria* var. *tataria* from Kahramanmaraş, Turkey. They found erucic acid at rates of 39.29% in *Crambe orientalis* and 29.87% in *Crambe tataria*. The highest linolenic acid rate in *Crambe orientalis* oil was 21.21% and the highest rate of linoleic acid was 12.42%. The highest linolenic acid level in *Crambe tataria* oil was 15.01% and the highest level of linoleic acid was 9.00%. Subaşı (2020) investigated the seed fatty acid compositions and chemotaxonomy of wild *Crambe* taxa in Turkey. He determined 17 fatty acids and the oil contents ranged between 4.7% and 20.5% among the investigated accessions. Subaşı (2020) reported the seed oil content of *Crambe orientalis* to be 9.65%–12.16%, while that of *Crambe tataria* was 9.62%, that of *Crambe grandiflora* was 7.43%, and that of *Crambe maritima* was 13.34%. The main fatty acids of these species were palmitic, oleic, cis-11 eicosenoic, erucic, linoleic, and linolenic acid (Subaşı, 2020).

To determine the genetic diversity and the relations of the *Crambe* populations, we constructed a haplotype network. According to Schaal et al. (2003), "To determine a gene genealogy, pedigree data of the individuals is necessary. This is the only way to determine the true relationship between genes borne by individuals with the same haplotype. Pedigree information is generally unavailable for most population samples; however, within a sample of genes from several individuals, we can generally distinguish the relationship between genes differing by mutations. Genes at a locus that differ by mutations are known as alleles or haplotypes. Thus, the depiction of relationships between alleles or haplotypes within a species is known as a haplotype or allele tree or network. Networks, rather than the bifurcating trees used for between-species comparisons, are the most appropriate way to represent the relationships within a species (Schaal et al., 2003).

In the present study, we used ITS and *trn*L-F markers to create a haplotype network and phylogeny reconstruction. The results were then discussed with the previously given seed oil contents by Subaşı (2020). We believe that the information presented here on the genetic diversity of wild *Crambe* populations will be helpful both to plant taxonomists and seed oil plant breeders for further research.

2. Materials and methods

2.1. Sampling

Crambe accessions were collected in the 2012–2014 vegetation periods from Turkey. Thirty-nine accessions belonging to 8 taxa constituted the materials of this study: *Crambe orientalis* var. *orientalis* with 18 accessions, *Crambe orientalis* var. *sulphurea* with 3 accessions, *Crambe orientalis* var. *dasycarpa* with 1 accession, *Crambe alutacea* with 1 accession, *Crambe grandiflora* with 1 accession, *Crambe tataria* var. *tataria* with 8 accessions, *Crambe tataria* var. *aspera* with 2 accessions, and *Crambe maritima* with 5 accessions (Table 1). In addition, the *trn*L-F sequences of sister group species and ITS sequences of *Crambe hispanica* and *Crambe filiformis* taxa and 18 sister group species were taken from GenBank. The seed oil data was gathered from Subaşı (2020) for discussion with our results. Twenty-three accessions studied by Subaşı (2020) and us overlap (Table 1).

2.2. DNA isolation, amplification, and sequencing

Silica gel-dried fresh leaves were used for DNA isolation. Total genomic DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The ITS1 and ITS4 primers (White et al. 1990) were used to amplify the ITS1, ITS2, and 5.8 S rRNA (ITS) regions with the protocol given by Warwick et al. (2004). For amplification of the *trn*L-F region, the method of Ansell et al. (2007) was followed using primers C and F of Taberlet et al. (1991). DNA purification and sequencing were performed by BIOEKSEN (İstanbul, Turkey). The ITS and *trn*L-F datasets were unequal because of the amplification of degraded DNA or the extreme length of the spacer region of some accessions. A list of ITS and *trn*L-F amplified accessions is given in Supplementary File 1.

2.3. Haplotype network

Haplotype networks were constructed with 32 accessions for each region (Table 2). We generated a statistical parsimony haplotype network at the 95% connection limit using TCS Version 1.21 (Clement et al., 2000) for the ITS and *trn*L-F datasets separately to better understand the relationships among haplotypes. DNASP Version 5.10 (Librado and Rozas, 2009) was used to calculate haplotype (h) and nucleotide diversity (π). For ITS and *trn*L-F, a matrix of genetic distances between all pairs of accessions was estimated from the standard fixation index (F_{ST}) with DnaSP Version 6.12 (Librado and Rozas, 2009). Accessions that were relatively close to each other were grouped according to their geographic regions.

2.4. Phylogeny reconstruction and divergence time estimation

All sequences were edited with CodonCode Aligner (CodonCode Corp., Dedham, Massachusetts, U.S.A.), aligned by using MEGA v.6 (Tamura et al., 2013). The phylogenetic tree based on the ITS dataset was reconstructed by using MrBayes 3.2 (Ronquist et al., 2012). The best substitution model was selected by MEGA v.6 (Tamura et al., 2013) according to the Akaike information criterion (Aikake, 1974) and the GTR+G+I model was used for the ITS dataset. MrBayes 3.2 (Ronquist et al., 2012) was used for Bayesian analysis by using four simultaneous runs of Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) sampling for 10 million generations, and one tree sampled every 1000 generations. After stationarity was traced by Tracer v.1.5 (Rambaut & Drummond, 2007) and ensured, 25% of the trees were discarded as burn-in, and the remaining trees were summarized in a 50% majority-rule consensus tree. A dated maximum clade credibility tree, based on concatenated datasets (ITS+ *trn*L-F), was reconstructed for divergence time estimation using BEAST2 (Bouckaert & al., 2014). GTR+G+I model for ITS dataset and GTR+G model for *trn*L-F dataset were selected as substitution model using the Akaike information criterion (AIC) (Akaike, 1974), implemented in MEGA v.6 (Tamura et al., 2013) software. Two external calibration points, one at separation of Lineage III 20.57 Ma (95 % HPD = 18.93–22.17) and the second at the split of the core Brassicaceae at 21.26 (95 % HPD = 19.6–22.93) Ma, as introduced by Huang et al. (2020), were used for divergence time estimation. Accordingly, time estimations were done with the uncorrelated lognormal relaxed clock model (Drummond et al., 2006). Normal distribution was

Table 1. Accession codes, taxa, localities, and altitudes of the investigated accessions common accessions with Subaşı (2000) indicated with asterisk.

Accession code	Taxon	Locality	Altitude (m)
Cra1	*Crambe orientalis* var. *orientalis*	Ankara	1083
Cra2		Çankırı	715
Cra3		Kayseri	1204
Cra4		Kayseri	1539
Cra5*		Kahramanmaraş	943
Cra6*		Kahramanmaraş	1143
Cra7*		Malatya	918
Cra8		Adıyaman	1220
Cra9*		Adıyaman	1222
Cra10*		Van	1725
Cra11*		Elazığ	1118
Cra12		Gümüşhane	1107
Cra13*		Erzincan	1431
Cra14*		Erzurum	1897
Cra15		Bitlis	1824
Cra16		Erzurum	1558
Cra17*		Kars	1793
Cra18*		Kars	1677
Cra19*	*Crambe alutacea*	Malatya	924
Cra20	*Crambe orientalis* var. *sulphurea*	Kahramanmaraş	1118
Cra21*		Kahramanmaraş	1122
Cra22*		Kahramanmaraş	1456
Cra23	*Crambe orientalis* var. *dasycarpa*	Adana	1706
Cra24*	*Crambe grandiflora*	Şanlıurfa	777
Cra25	*Crambe tataria* var. *tataria*	Uşak	899
Cra26*		Ankara	1078
Cra27*		Ankara	950
Cra28*		Kırşehir	1096
Cra29*		Kayseri	1197
Cra30		Kayseri	1169
Cra31*		Kayseri	1162
Cra32		Malatya	1536
Cra33*	*Crambe tataria* var. *aspera*	Ankara	1059
Cra34*		Ankara	1127
Cra35*	*Crambe maritima*	Sinop	0
Cra36		Zonguldak	3
Cra37		Kastamonu	5
Cra38*		Kastamonu	4
Cra39		İstanbul	8

Table 2. List of accessions with shared *trn*L-F haplotypes and accessions with shared ITS type haplotypes with codes

Haplotype code (*trn*L-F)	**Accession code**	**Haplotype code (ITS)**	**Accession code**
H1	Cra3	IT1	Cra9
	Cra6	IT2	Cra35
	Cra11		Cra36
	Cra13		Cra37
	Cra18		Cra38
	Cra10		Cra39
	Cra20	IT3	Cra27
	Cra21		Cra30
	Cra35		Cra31
	Cra36		Cra33
	Cra37		Cra35
	Cra38	IT4	Cra26
	Cra39	IT5	Cra2
H2	Cra25		Cra24
	Cra26		Cra4
	Cra27		Cra14
	Cra28		Cra16
	Cra29		Cra10
	Cra30		Cra23
	Cra31	IT6	Cra11
	Cra32		Cra12
	Cra33		Cra13
H3	Cra7	IT7	Cra15
	Cra9		Cra17
	Cra19		Cra18
H4	Cra2	IT8	Cra21
	Cra23	IT9	Cra1
H5	Cra22		Cra5
H6	Cra14		Cra8
H7	Cra12		Cra19
H8	Cra17	IT10	Cra7
H9	Cra16		Cra22

used for secondary calibration points since it is regarded as the most appropriate prior distribution (Ho, 2007).

Four independent Markov Chain Monte Carlo (MCMC) runs were conducted with chain lengths of 40 million generations. Trees and associated parameter values were logged every 4000 states per independent MCMC run. Each run was checked using Tracer Version 1.7 (Rambaut et al., 2018) with the first 1000 sampled trees discarded as burn-in to verify that the independent MCMC runs had converged on the same distribution. After verification, the log files from each analysis were combined using LogCombiner Version 2.1.3 (Rambaut and Drummond, 2014). LogCombiner Version 2.1.3 was also used to combine the trees created in the three independent MCMC runs by removing the first 1000 sampled trees of each run as burn-in. The maximum clade credibility tree was obtained by using TreeAnnotator Version 2.6.2 (Bouckaert et al., 2019).

3. Results

3.1. Haplotype networks

The aligned ITS dataset included 32 sequences of 588 bp in length, of which 21 were variable and 17 were parsimony-informative. The *trn*L-F dataset included 32 sequences of 703 bp in length, of which 10 were variable and 3 were parsimony-informative.

The ITS region had higher genetic diversity than the *trn*L-F region. Summaries of haplotype diversity, nucleotide diversity, and F_{ST} are shown in Table 3 for both ITS and *trn*L-F regions. Genetic distances between species are given in Tables 4 and 5.

In both networks, *Crambe alutacea* shared one haplotype with some *Crambe orientalis* accessions (IT9 in Figure 1, H3 in Figure 2). Although there was no shared haplotype among *Crambe maritima*, *Crambe orientalis*, and *Crambe tataria* in the haplotype network based on the ITS region, all *Crambe maritima* accessions and most of the *Crambe orientalis* accessions shared one haplotype in the *trn*L-F analyses (H1 in Figure 2).

The haplotype network based on the *trn*L-F region had low frequency. The most widely distributed haplotype was H1 (Figure 2), which was shared by *Crambe maritima* and *Crambe orientalis*. Other haplotypes differed from the most common haplotype (H1) by 1 or 2 bp.

When the haplotype networks based on ITS and *trn*L-F were compared, 5 accessions shared the H1 and IT2 haplotypes and 4 accessions shared the H2 and IT3 haplotypes.

In the ITS dataset, both the haplotype and the nucleotide diversity of *Crambe orientalis* (0.852 and 0.004, respectively) were higher than those of *Crambe tataria*. Interestingly, in both datasets, *Crambe maritima* had no haplotype or nucleotide diversity.

ITS data revealed that only the F_{ST} values of the three accessions of *Crambe orientalis* were greater than 0 (Table 4), whereas the paired F_{ST} values of the other species were zero. Similarly, to the ITS data, the *trn*L-F data also revealed that only the F_{ST} values in the two accessions of *Crambe orientalis* were greater than zero (Table 5). However, the obtained F_{ST} values from the *trn*L-F data were lower than those obtained from ITS data (Table 5). Genetic distances between accessions of other species were low, but genetic distances between accessions of *Crambe orientalis* from southern, inner, and northeastern Anatolia were relatively high (Tables 4 and 5).

Table 3. Genetic characteristics of the Anatolian *Crambe* species included in this study (number of haplotypes (H), haplotype diversity (*h*), and nucleotide diversity (π)).

Species	ITS			*trn*L-F		
	H	*h*	π	H	*h*	π
Crambe orientalis	7	0.852	0.003	7	0.791	0.001
Crambe tataria	2	0.333	0.002	1	0	0
Crambe maritima	1	0	0	1	0	0
Crambe grandiflora	NE	NE	NE	NE	NE	NE

Table 4. Genetic distance (below diagonal) and paired F_{ST} values (above diagonal) between *Crambe orientalis* based on ITS region.

Populations	Inner Anatolia	Southern Anatolia	Northeast Anatolia
Inner Anatolia	---	0.122	0
Southern Anatolia	0.004	---	0.248
Northeast Anatolia	0.002	0.004	---

Table 5. Genetic distance (below diagonal) and paired F_{ST} values (above diagonal) between *Crambe orientalis* based on *trn*L-F region.

Populations	Inner Anatolia	Southern Anatolia	Northeast Anatolia
Inner Anatolia	---	0	0
Southern Anatolia	0.004	---	0.054
Northeast Anatolia	0.003	0.004	---

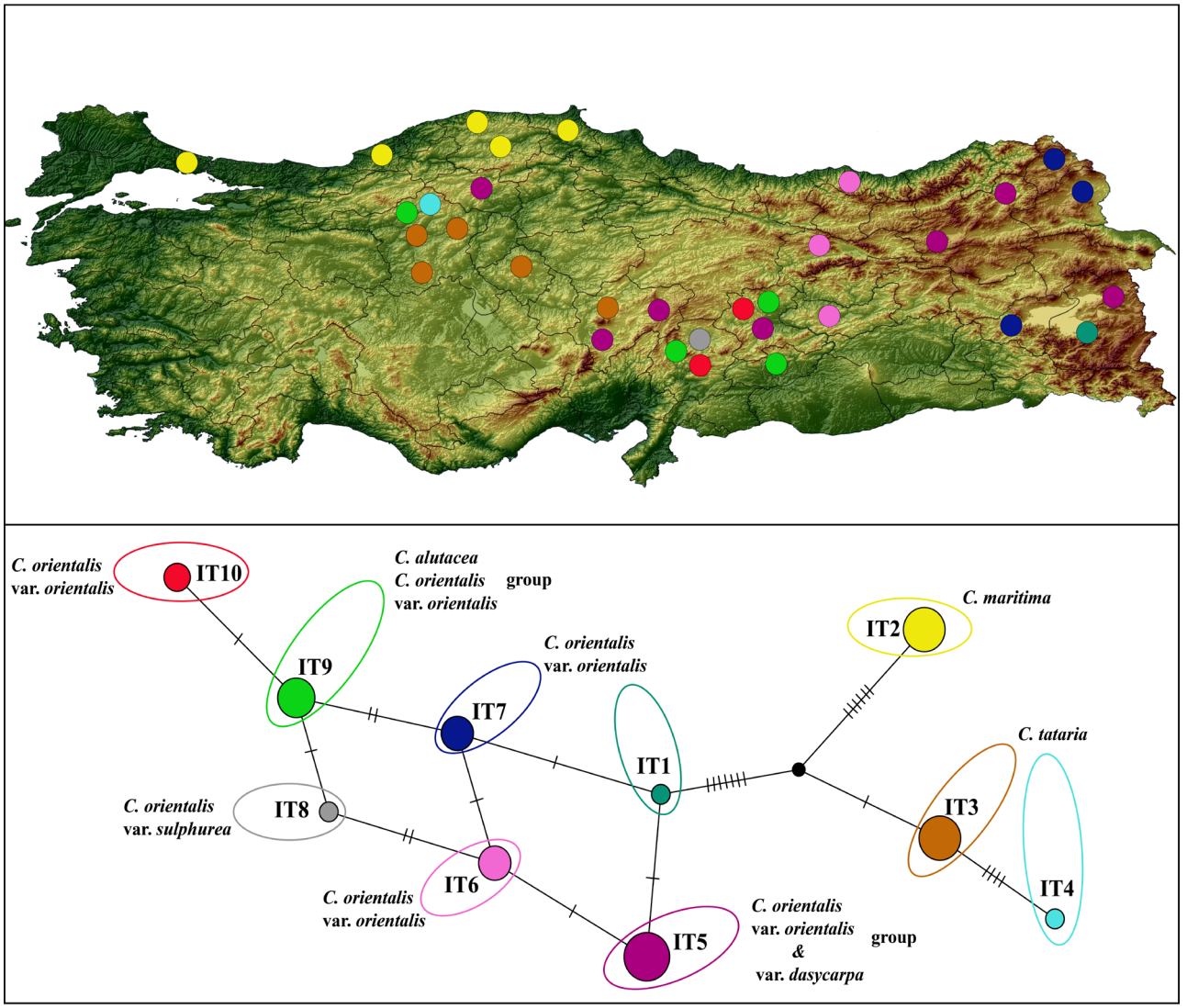


Figure 1. nrDNA ITS-type distribution and network analyses of the pairwise ITS-type differences. a) Locations of *Crambe* species. Each circle on the map represents a different ITS type. b) ITS-type network of the obtained ITS types. Each ITS type is presented with its own color and the size of the circle is proportional to that on the map.

3.2. Phylogeny and molecular dating

The phylogenetic trees presented in this research include mostly sampling accessions from Turkey and a few taxa from outside Turkey (Figures 3 and 4). The presented tree supported the monophyly of the genus *Crambe* and Turkish *Crambe* taxa (Posterior Probability-PP 100%). Within *Crambe*, two major lineages were formed: one consisted of *Crambe hispanica* and *C. filiformis* taxa from outside Turkey (PP 100%), second consisted *Crambe* taxa distributed in Turkey (PP 100%). Among the second clade, *Crambe maritima* and *Crambe tataria* accessions grouped together, and the remaining 5 taxa formed a separate group. All *Crambe maritima* accessions clustered together with a well-support (PP 100%). The phylogeny tree did not support the morphologically different varieties of *Crambe tataria* (var. *aspera* and var. *tataria*). *Crambe grandiflora*, and *Crambe alutacea* species were clustered with *Crambe. orientalis* accessions (subsp. *orientalis*, subsp. *sulphurea*, and var. *dasycarpa*). The phylogeny inference of ITS and *trn*L-F sequences revealed that the Turkish *Crambe* is monophyletic with 1 clade credibility and this is in accordance with the results of Francisco-Ortega et al. (1999). The concatenated dataset revealed that *Crambe* separated from its possible ancestor at ca. 1.76 Ma (95 % HPD = 0.50–4.26, Figure 3). Divergence time estimation analyses also revealed that the separation of Lineage III is

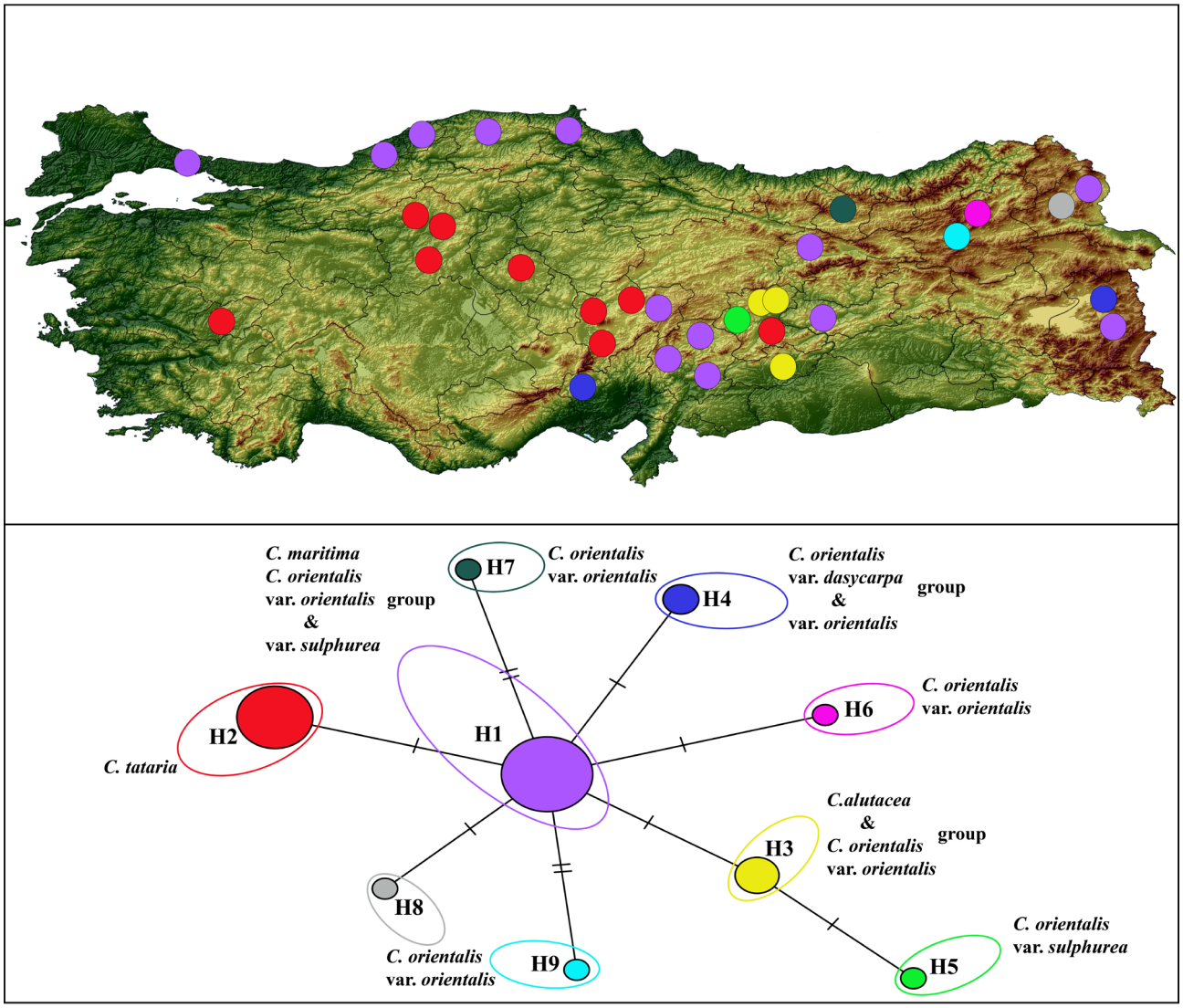


Figure 2. Plastid DNA haplotype network calculated with TCS Version 1.21. a) Locations of *Crambe species*. Each circle on the map represents a *trn*L-F haplotype. b) Haplotype network for the obtained *trn*L-F results. Each haplotype is presented with its own color and the size of the circle is proportional to that on the map.

19.07 Ma (95 % HPD = 17.29–22.41) and the split of the core Brassicaceae is at 19.66 (95% HPD = 17.29–22.41) Ma. These results are in accordance with the results by Huang et al. (2020) who proposed separation of Lineage III is 20.57 Ma (95 % HPD = 18.93–22.17) and the core Brassicaceae is at 21.26 (95% HPD = 19.6–22.93) Ma.

4. Discussion

Phylogeny reconstruction revealed that the genus *Crambe* is monophyletic as showed by Francisco-Ortega et al. (1999) and *Crambe* taxa that have distribution areas in Turkey are monophyletic as well. Although phylogenetic analyses of the Turkish taxa (Figures 3 and 4), revealed three major clades of species (*Crambe orientalis*, *Crambe tataria*, and *Crambe maritima*), haplotype analyses show that the genetic differences among the populations were low. The presence of geographically adjacent populations may result in gene flow among populations reducing the degree of genetic differentiation. On the other hand, the highest F_{ST} value (0.248) having been obtained between the Northeast and South Anatolian accessions of *Crambe orientalis* could be explained by geographic barriers in the form of high mountains.

The haplotype networks obtained from the *trn*L-F data showed *Crambe orientalis* and *Crambe maritima* clustering together. On the other hand, the phylogeny reconstructions

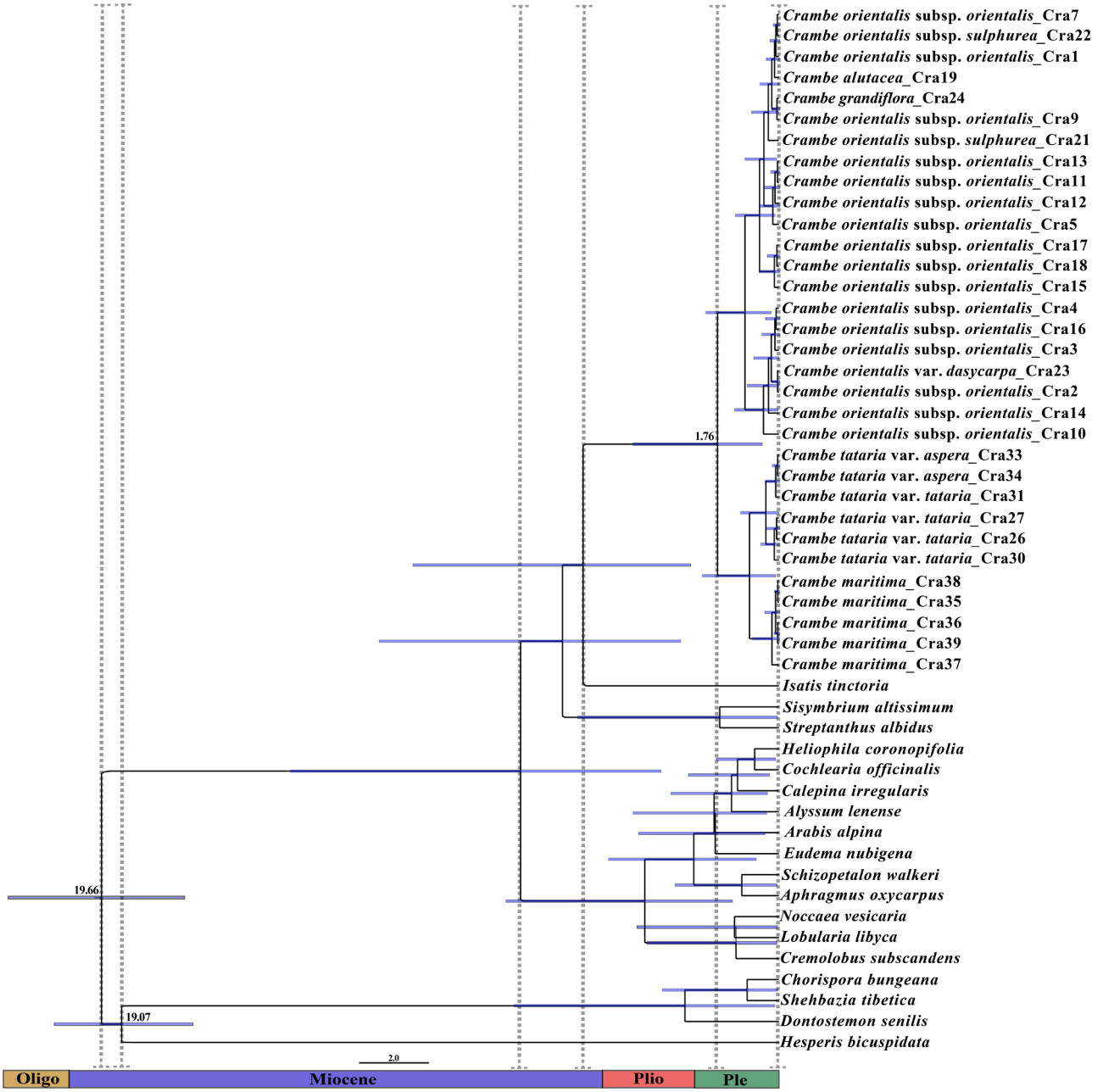


Figure 3. A dated maximum clade credibility tree based on concatenated ITS and *trn*L-F sequences with two secondary calibration points. Blue bars indicate the 95% highest posterior densities. The numbers at the nodes indicate the age (in million years) of core Brassicaceae, Lineage III and *Crambe* estimated in molecular clock studies. Divergence times of calibration points, Lineage III, Lineage I, and *Crambe* are aligned to timescale (Ple, Pleistocene; Plio, Pliocene, Oligo, Oligocene) with grey lines.

suggested that these specimens were significantly different from each other (Figures 3 and 4). Also, according to Francisco-Ortega et al. (1999), *Crambe. orientalis* belong to section *Orientecrambe* and *Crambe maritima* to section *Crambe*. Although some accessions of *Crambe orientalis* and *Crambe tataria* from Central and South Anatolia overlapped or had close ranges, introgression between these species was not reported in previous studies. Our findings have shown that these species are clearly different from each genetically (Figures 3 and 4).

In the networks established by both ITS and *trn*L-F, the 5 *Crambe maritima* accessions had no nucleotide diversity

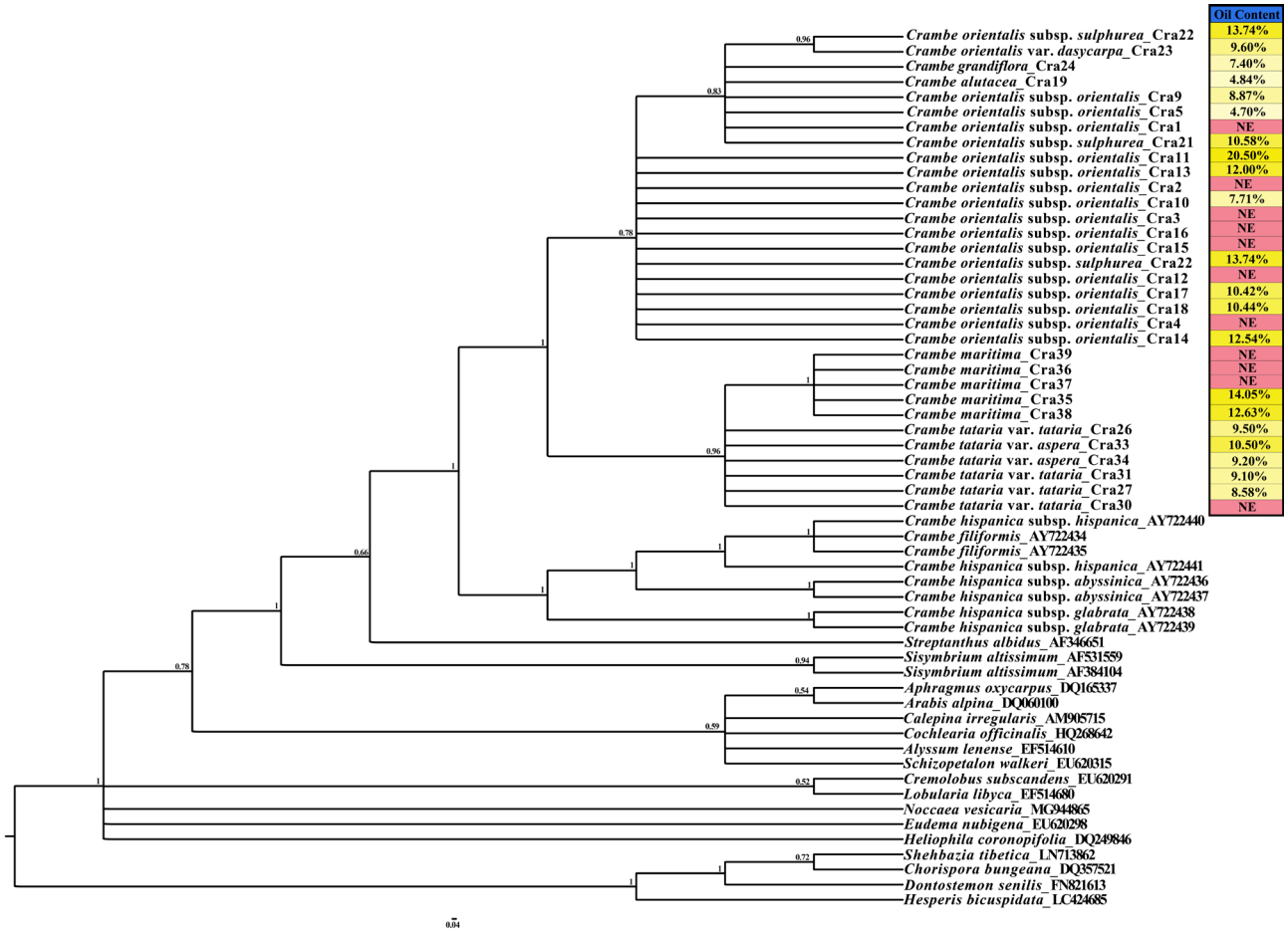


Figure 4. A Bayesian consensus tree based on ITS sequences. Posterior probability values (p > 0.5) are shown at the nodes. Seed oil percentages for each accession are labeled with colored bars. Missing data is abbreviated as "N/A".

(Table 3). As seen in Table 3, haplotype diversity based on *trn*L-F is lesser. It is known that the chloroplast genome is more conserved than the nuclear genome and it enables to reconstruct of plant phylogeny at higher taxonomic levels (Downie and Palmer 1992; Doyle et al., 1992). *Crambe maritima* is distributed along the north coastal side of Anatolia with no habitat differences; all populations grow in sandy habitats at approximately sea level. The *Crambe maritima* accessions shared a clade in the phylogeny tree, as well (Figures 3 and 4) and were sister to *Crambe tataria* group in accordance with previous phylogenetic analysis (Francisco-Ortega et al., 1999). *Crambe grandiflora* clustered with *Crambe orientalis* and *Crambe alutacea* in phylogeny trees (Figures 3 and 4). From this perspective, our current work supports that of Tarıkahya-Hacıoğlu (2016). In contrast to Prina (2009), the phylogeny trees reconstructed in our research support the close relationship of *Crambe grandiflora* and *Crambe orientalis* but not *Crambe tataria*. The infraspecific taxa of *Crambe orientalis* clustered together. The infraspecific taxa of *Crambe tataria* formed common clusters, as well. *Crambe alutacea* was grouped within the *Crambe orientalis* clade. CRA11, CRA12, and CRA13 were from close geographical areas among the *Crambe orientalis* accessions and these accessions were grouped in the same cluster in phylogeny trees (Figures 3 and 4). These three accessions also shared the IT6 haplotype. Among the *Crambe tataria* accessions, CRA27 and CRA33 were grouped together in the phylogeny inferences; both of them were from the Ankara region and shared the IT3 and H2 haplotypes.

Subaşı (2020) investigated the fatty acid compositions of the seeds of wild *Crambe* taxa in Turkey, concluding that cultivated *Crambe* (*Crambe abyssinica* Hochst.) had 30%–45% oil by seed weight while the oil contents of the wild *Crambe* species were lower (4.7%–20.5%). Since wild *Crambe* species fall within the gene pool of cultivated *Crambe abyssinica* R.E.Fr., information on both seed oil contents and rates is important for future breeding studies. In our research, we used some of the same accessions used by Subaşı (2020). According to the comparison of our

results with those of Subaşı (2020), the accessions with the highest oil contents were as follows: *Crambe orientalis* subsp. *orientalis* (CRA11-20.50%), *Crambe orientalis* subsp. *sulphurea* (CRA22-13.74%), *Crambe orientalis* var. *dasycarpa* (CRA23-9.60%), *Crambe grandiflora* (CRA24-7.40%), *Crambe tataria* var. *tataria* (CRA32-12.40%), *Crambe tataria* var. *aspera* (CRA34-10.50%), and *Crambe maritima* (CRA35-14.05%). Upon comparing the previous findings with our own, it was clear that the accessions with the highest oil contents shared the H1 haplotype (CRA3, CRA6, CRA11, and CRA35). H1 is a common haplotype shared by 13 accessions and distributed in North, Southeast, and East Anatolia. The mean oil levels of the haplotypes shared by more than 2 accessions were as follows: H1, 12.35%; H2, 9.73%; H3, 6.84%; IT2, 8.65%, IT3, 9.82%; IT5, 10.27%; 1T6, 16.25%; IT7, 10.43. Among the wild relative species of *Crambe*, 17 fatty acids were determined, with the 7 main fatty acids (>1%) being palmitic (C16:0), oleic (C18:1), linoleic (C18:2), cis-11 eicosenoic (20:1), linolenic (C18:3), erucic (C22:1), and nervonic (C24:1) acids (Subaşı, 2020). The level of palmitic acid was highest in CRA19 (5.78%) and lowest in CRA27 (0.21%); oleic acid was highest in CRA38 (32.49%), while it was lowest in CRA11 (15.02); linoleic acid was highest in CRA35 (17.34%) and lowest in CRA7 (3.94%); cis-11 eicosenoic acid was highest in CRA33 (21.20%) and lowest in CRA9 (10.76); linolenic acid was highest in CRA17 (9.57%) and lowest in CRA7 (2.18); erucic acid was highest in CRA9 (49.68%) and lowest in CRA38 (22.59); and nervonic acid was highest in CRA7 (2.864%) and lowest in CRA38 (0.796). According to the plastid DNA haplotype network, H1 included the accessions with the highest oleic (CRA38) and linoleic (CRA35) acid contents, while H3 included the accessions with the lowest linoleic (CRA7), cis-11 eicosenoic (CRA9), and linolenic (CRA7) acid contents. H1 included the accessions with the lowest oleic acid, erucic acid, and nervonic acid contents, namely CRA11, CRA38, and CRA38, respectively. According to the mean values of the accessions sharing H1, palmitic acid (2.64%), linoleic acid (12.19%), linolenic acid (6.78%), erucic acid (35.91%), and nervonic acid (1.36%) rates were highest. The mean rates of oleic acid (24.69%) and cis-11 eicosenoic acid (19.78%) were higher among the accessions sharing the H2 haplotype.

5. Conclusions

The genetic diversity of the CWR species of the seed oil crop *Crambe* from Turkey was investigated in this study using haplotype networks and phylogenetic analysis. In the phylogeny tree, 2 major *Crambe* lineages were formed: one consisted of *Crambe maritima* and *Crambe tataria* accessions, while the remaining 5 taxa formed the second one. *Crambe grandiflora* was seen to have a close relationship with *Crambe orientalis*, as demonstrated in our previous work (Tarıkahya-Hacıoğlu, 2016). *trn*L-F revealed 9 haplotypes and ITS yielded 10 haplotypes. The accessions with the highest oil contents shared H1, a common haplotype shared by 13 accessions and distributed in the north, southeast, and east of Anatolia. The accessions that shared H1 and IT6 had the highest mean oil rates. According to the results of our study, *Crambe orientalis* is the species with the highest haplotype diversity and the accessions sharing IT6 haplotype has the highest seed oil rates among CWR of *Crambe* in Turkey. H1 haplotype consists the species with the highest oleic and linoleic acid contents. We believe that the information presented here will be useful for future breeding studies.

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Supplementary File 1

Taxon	trnL-F	ITS
Crambe orientalis var. orientalis Cra1	-	+
Crambe orientalis var. orientalis Cra2	+	+
Crambe orientalis var. orientalis Cra3	+	-
Crambe orientalis var. orientalis Cra4	-	+
Crambe orientalis var. orientalis Cra5	-	+
Crambe orientalis var. orientalis Cra6	+	-
Crambe orientalis var. orientalis Cra7	+	+
Crambe orientalis var. orientalis Cra8	-	+
Crambe orientalis var. orientalis Cra9	+	+
Crambe orientalis var. orientalis Cra10	+	+
Crambe orientalis var. orientalis Cra11	+	+
Crambe orientalis var. orientalis Cra12	+	+
Crambe orientalis var. orientalis Cra13	+	+
Crambe orientalis var. orientalis Cra14	+	+
Crambe orientalis var. orientalis Cra15	-	+
Crambe orientalis var. orientalis Cra16	+	+
Crambe orientalis var. orientalis Cra17	+	+
Crambe orientalis var. orientalis Cra18	+	+
Crambe alutacea Cra19	+	+
Crambe orientalis var. sulphurea Cra20	+	-
Crambe orientalis var. sulphurea Cra21	+	+
Crambe orientalis var. sulphurea Cra22	+	+
Crambe orientalis var. dasycarpa Cra23	+	+
Crambe grandiflora Cra24	-	+
Crambe tataria var. tataria Cra25	+	-
Crambe tataria var. tataria Cra26	+	+
Crambe tataria var. tataria Cra27	+	+
Crambe tataria var. tataria Cra28	+	-
Crambe tataria var. tataria Cra29	+	-
Crambe tataria var. tataria Cra30	+	+
Crambe tataria var. tataria Cra31	+	+
Crambe tataria var. tataria Cra32	+	-
Crambe tataria var. aspera Cra33	+	+
Crambe tataria var. aspera Cra34	-	+
Crambe maritima Cra35	+	+
Crambe maritima Cra36	+	+
Crambe maritima Cra37	+	+
Crambe maritima Cra38	+	+
Crambe maritima Cra39	+	+
Crambe hispanica subsp. hispanica		**AY722440**

Crambe hispanica subsp. hispanica		**AY722441**
Crambe hispanica subsp. abyssinica		**AY722436**
Crambe hispanica subsp. abyssinica		**AY722437**
Crambe hispanica subsp. glabrata		**AY722438**
Crambe hispanica subsp. glabrata		**AY722439**
Isatis tinctoria	**KJ765852**	**AF384104**
Sisymbrium altissimum	**AY958545**	**AF531559**
Streptanthus glandulosus	**AY958575**	**AF346651**
Schizopetalon walkeri	**EU620378**	**EU620315**
Cremolobus subscandens	**EU620348**	**EU620291**
Eudema nubigena	**EU620355**	**EU620298**
Aphragmus oxycarpus	**DQ518350**	**DQ165337**
Heliophila coronopifolia	**DQ518369**	**DQ249846**
Cochlearia officinalis	**HQ268697**	**HQ268642**
Calepina irregularis	**AY751760**	**AM905715**
Arabis alpina	**JF705252**	**DQ060100**
Noccaea vesicaria	**MG925452**	**MG944865**
Alyssum lenense	**FN677633**	**EF514610**
Lobularia libyca	**DQ518372**	**EF514680**
Hesperis bicuspidata	**LC425244**	**LC424685**
Chorispora bungeana	**FN677730**	**DQ357521**
Dontostemon senilis	**AY559007**	**FN821613**
Shehbazia tibetica	**LN713862**	**FN677715**