MOLECULAR GENETIC ANALYSES IN *ORIGANUM* (LAMIACEAE) TAXA IN TÜRKİYE

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ABSTRACT

MOLECULAR GENETIC ANALYSES IN *ORIGANUM* (LAMIACEAE) TAXA IN TÜRKİYE

Medicinal and aromatic plants (MAPs) belonging to the genus, *Origanum* L. (Lamiaceae), are called "oregano". They are economically important and beneficial for trade, medicine, food, cosmetics, and ornamental purposes with their bioactive compound diversity and richness. Although Türkiye is the gene center for generation, speciation, and diversification of oregano throughout the world, their uncontrolled consumption and other factors threaten their status. According to Ietswaart (1980), there are ten morphological sections in the genus. Of these, 25 taxa (including 13 endemics) and 13 hybrids from eight sections grow naturally in Türkiye. The cross-pollinating and gynodioecious nature of oreganos makes their taxonomic classification difficult. In this dissertation, molecular markers (EST-SSRs and SRAPs) were used to assess the complex evolutionary relationships in a herbarium and the Turkish national AARI Gene Bank collection. Cross hybridization due to high gene flow was found to be the main source of genetic diversity within both collections. In both collections, the highest gene flow was observed between two sections, *ANA* and *BRE*, with diecious flowers which supports their frequent hybridization when compared to gynodioecious oreganos in nature. The Aegean and Mediterranean regions had the highest gene flow among all regions, while five province pairs had the highest gene flow among all provinces. In conclusion, molecular markers were shown to be a useful tool for examinations of genetic diversity and evolution in oregano.

ÖZET

TÜRKİYE'DE *ORIGANUM* (LAMIACEAE) TAKSONLARINDA MOLEKÜLER GENETİK ANALİZLER

Origanum (Lamiaceae) cinsine ait tıbbi ve aromatik bitkiler, kekik olarak adlandırılırlar. Biyoaktif bileşen çeşitlilikleri ve zenginlikleri ile ticaret, tıp, gıda, kozmetik ve dekoratif amaçlar için ekonomik olarak önemli ve faydalıdırlar. Türkiye dünyada kekiğin üretimi, türleşmesi ve çeşitlenmesi için gen merkezi olsa da, kontrolsüz tüketimi ve diğer faktörler türünü tehlikeye sokmaktadır. Ietswaart'a göre (1980), bu cinse ait 10 morfolojik seksiyon vardır. Bu 10 seksiyon içerisinden sekizine ait 25 takson (13'ü endemik olmak üzere) ve 13 hibrit Türkiye'de doğal olarak yetişmektedir. Çapraz döllenme ve doğası gereği hem dişi hem erkek üreme organı taşıyan çiçekli bireylerin varlığı kekiklerin taksonomik sınıflandırmalarını zorlaştırmaktadır. Bu tezde moleküler belirteçler (EST-SSR ve SRAP markörleri) bir herbaryum ve bir Ege Tarımsal Araştırma Enstitüsü (ETAE) Gen Bankası ulusal Türk kekiği koleksiyonlarındaki kompleks evrimsel ilişkileri belirlemek için kullanıldı. Yüksek miktarda gen akışına bağlı olarak çapraz hibritleşmeler bu koleksiyonlarda genetik çeşitliliğin ana kaynağı olarak belirlendi. Her iki koleksiyonda da kendileşen türlere nazaran en yüksek gen akışı her ikisi de dış döllenen *ANA* ve *BRE* seksiyonları arasında tespit edildi. Ege ve Akdeniz bölgeleri tüm bölgeler arasında en fazla gen akışına sahipken, birbiriyle eşleşen beşer il diğer illere göre en fazla gen akışına sahip olarak belirlendi. Sonuç olarak, moleküler belirteçler kekikte genetik çeşitliliği ve kekiğin evrimini araştırmada yararlı olarak gösterilmiştir.

This dissertation is dedicated to my one and only family; my eternally beloved mother, father, and sister.

PREFACE

Wild native oregano species are one of the most economically and biochemically valuable perennial culinary herbs and are under threat due to the high endemism frequency within the genus *Origanum*. Oregano species grow on rocky, calcareous, and limestone soils at certain heights above the sea level and under certain climatic conditions and ecosystems.

Türkiye has geographical importance for oregano species because it is the homeland for its known ancestor genus, *Satureja,* during the Pliocenic era (Ietswaart, 1980). Moreover, the country has a variety of species from eight of the ten *Origanum* sections spread throughout its geography. Among the eight oregano sections that naturally grow in Türkiye, species of sections *Amaracus, Majorana* and *Origanum* were suggested to be the origins of speciation within the genus *Origanum* (Ietswaart, 1980). The major factor that created the additional sections from these three ancestral groups is assumed to be hybridization (Ietswaart, 1980). Most species belonging to genus *Origanum* undergo frequently homoploid hybridization events that maintain the same chromosome number with the parental species and creates intermediate hybrid speciation (Arabacı et al., 2020; Dirmenci et al., 2021). In Türkiye, the hybridization events and the gene flow between natural oregano populations have not been previously assessed over species from the eight sections: *Amaracus*, *Anatolicon*, *Brevifilamentum*, *Chilocalyx*, *Longitubus*, *Majorana*, *Origanum* and *Prolaticorolla*.

In this dissertation, the genetic relationships among all eight sections were examined for the first time with SRAP markers (Chapter 2, published in Taşcıoğlu et al. (2018). In this part of the work, genetic diversity and gene flow were examined in herbarium material from Inönü University (Malatya) consisting of 22 native oregano species (24 taxa), 15 of which were endemic. The materials were investigated with 25 Sequence Related Amplified Polymorphism (SRAP) and six Expressed Sequence Tagged – Simple Sequence Repeat (EST-SSR) markers. In addition, the Turkish national oregano collection from the gene bank at the Aegean Agriculture Research Institute (AARI) was examined with ten SRAP markers that were selected for their high level of informativeness (Chapter 3). A total of 130 native oregano individuals from four sections (*Anatolicon*, *Brevifilamentum*, *Majorana*, and *Origanum*, and undefined oregano

specimens) were genetically characterized in this part of the work. In addition to analysis of genetic diversity and population structure, an abbreviated nomenclature for oregano sections and species is presented in the Appendix.

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LIST OF ACRONYMS, ABBREVIATIONS and SYMBOLS

B.C.E.	Before Common Era
E	Endemic
MAPs	Medicinal and Aromatic Plants
SM	Secondary metabolite
UNK	Unknown

Acronyms

AARI	Aegean Agriculture and Research Institute
ETAE	Ege Tarımsal Araştırma Enstitüsü
WHO	World Health Organisation
CCSCH	Codex Committee on Spices and Culinary Herbs
NCBI	National Center for Biotechnology Information
TUBIVES	Turkish Plant Data Service
SRA	Sequence Read Archive
IPGRI	The International Plant Genetic Resources Institute
WCSP	World Checklist of Selected Plant Families
IUCN	The International Union for Conservation of Nature

Morphological Determiners

RF	Reticulate-foveate (mericarp)
R	Ruminate (mericarp)
F	Foveate (mericarp)
P	Pusticulate (mericarp)
RR	Reticulate-ruminate (mericarp)
C	Colpate (pollen grain)
SO	Suboblate (pollen grain)
OSF	Oblate-spheroid (pollen grain)
SP	Subprolate (pollen grain)
PSF	Prolate-spheroid (pollen grain)

Sterility

CMS	Cytoplasmic Male Sterility
MS	Male Sterility
NMS	Nuclear Male Sterility

Red List Endangered Degrees

cd	conservation dependent (status)
CR	Critically Endangered (status)
EN	Endangered (status)
lc	least concerned (status)
nt	nearly threatened (status)
VU	Vulnerable (status)

Regional Indicators

A	Aegean (region)
M	Marmara (region)
MT	Mediterranean (region)
BS	Black Sea (region)
CA	Central Anatolia (region)
EA	Eastern Anatolia (region)
R	Region
SA	Southeastern Anatolia (region)
TR	Türkiye

Diversity Methods and Analyses Indicators

af	allele frequency
AMOVA	Analysis of Molecular Variance
bp	base pair
A.1.	Subcluster A.1. of Cluster A
B	Cluster B
BSA	Bovine Serum Albumin
C.2	Subcluster C.2. of Cluster C
CTAB	cetyltrimethylammonium bromide
DD	Data deficient
dNTP	deoxynucleotide triphosphate

FST	F-statistics
GD	genetic diversity
h	diversity
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
I	Shannon Index
K	Number of subpopulation (Population Structure)
MCMC	Markov Chain Monte Carlo
Na	Number of alleles
NGD	*Nei's* genetic distance
NGI	*Nei's* genetic identity
Ni	Number of individuals
NJ	Neighbor Joining
Nm	Number of migrants
Ns	Number of sections
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PerCov (%)	coverage percent (%) of the species in that province
PerLoc (%)	distribution of species per location in percentage (%)
PhiPT	Phi-statistics, *FST* (Wright's statistics) analogue
PIC	Polymorphism Information Content (analogue of GD value)
Pops	Populations
SE	Standard error
SP	Subpopulation (Population Structure)
SPI	Subpopulation I
SPII	Subpopulation II
SPIII	Subpopulation III
SPIV	Subpopulation IV
SPV	Subpopulation V
SPVI	Subpopulation VI
SPVII	Subpopulation VII
SPVIII	Subpopulation VIII

TAE	Tris-acetate-EDTA
Taq	*Thermus aquaticus*
UPGMA	Unweighted Pair Group Method with Arithmetic Means

Molecular Markers

AFLP	Amplified Fragment Length Polymorphism
CAPS	Cleaved Amplified Polymorphic Sequence
EST-SSR	Expressed Seqence Tag-Simple Sequence Repeat
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Spacer
RAPD	Random Amplified Polymorphic DNA
SCAR	Sequence Characterized Amplified Regions
SCoT	Start Codon Targeted
SRAP	Sequence Related Amplified Polymorphism
TU-DAMD	Touch-up Direct Amplification of Microsatellite region DNA

Sections

ANA	*Anatolicon*
AMA	*Amaracus*
BRE	*Brevifilamentum*
CHI	*Chilocalyx*
LONGI	*Longitubus*
MAJ	*Majorana*
CAMPA	*Campanulaticalyx*
ELON	*Elongatispica*
ORG	*Origanum*
PRO	*Prolaticorolla*
I	1
II	2
III	3
IV	4
V	5
VI	6
VII	7

VIII	8
IX	9
X	10

Species

OAC	*Origanum acutidens*
OAK	*Origanum akhderense*
OAM	*Origanum amanum*
OBA	*Origanum bargyli*
OBA	*Origanum bargyli*
OBI	*Origanum bilgeri*
OBO	*Origanum boissieri*
OBR	*Origanum brevidens*
OCA	*Origanum calcaratum*
OCO	*Origanum cordifolium*
OCO	*Origanum compactum*
OCY	*Origanum cyrenaicum*
ODA	*Origanum dayi*
ODI	*Origanum dictamnus*
ODU	*Origanum dubium*
OEH	*Origanum ehrenbergii*
OEL	*Origanum elongatum*
OFL	*Origanum floribundum*
OGR	*Origanum grosii*
OHA	*Origanum haussknechtii*
OHY	*Origanum hypericifolium*
OIS	*Origanum isthmieum*
OLA	*Origanum laevigatum*
OLE	*Origanum leptocladum*
OLI	*Origanum libanoticum*
OMA	*Origanum majorana*
OMI	*Origanum minutiflorum*
OMICRA	*Origanum micranthum*

OMICRO	*Origanum microphyllum*
OON	*Origanum onites*
ORA	*Origanum ramonense*
ORO	*Origanum rotundifolium*
OSA	*Origanum saccatum*
OSC	*Origanum scabrum*
OSI	*Origanum sipyleum*
OSO	*Origanum solymicum*
OSY	*Origanum syriacum*
OSYBE	*Origanum syriacum* subsp. *bevanii*
OVE	*Origanum vetteri*
OVG	*Origanum vulgare* subsp. *gracile*
OVGL/ or	
OVGLAN	*Origanum vulgare* subsp. *glandulosum*
OVH	*Origanum vulgare* subsp. *hirtum*
OVVI	*Origanum vulgare* subsp. *viride/viridulum*
OVVIR	*Origanum vulgare* subsp. *virens*
OVVU	*Origanum vulgare* subsp. *vulgare*

Symbols

† specimens require re-examination

α *Amaracus*

β *Anatolicon*

χ *Brevifilamentum*

ε *Chilocalyx*

δ *Longitubus*

ϕ *Majorana*

γ *Origanum*

λ *Prolaticorolla*

CHAPTER 1

INTRODUCTION

1.1. Medicinal and Aromatic Plants (MAPs)

Medicine is the treatment of human and animal diseases using knowledge and biological sources such as plants. Among these sources, plants are the most valuable because they consume CO_2 and create the oxygen humans need to survive, in addition to being nutritionally and chemically wealthy for many types of organisms. There is global concern about the survival of humankind mostly due to the fact that the ecosystem we interact with is currently threatened by many factors such as natural disasters, pollution and unbalanced usage of resources. These problems are worsened by the increasing human population and climate change. Eighty percent of African and Asian populations lack sufficient food and medicine supplies due to their poor economy and being unable to access health care because of geographical isolation. Thus, people in these developing countries use Medicinal and Aromatic Plants (MAPs) which contain bioactive as traditional remedies in their health practices (Luziatelli et al., 2010). The Plant Kingdom consists of four groups of plants: Bryophytes (mosses), Seedless vascular plants, Gymnosperms (cone bearing seed plants) and Angiosperms. Medicinal plants belong to Angiosperms (flowering plants) and are found in many plant families.

It has been reported that early civilizations like the Sumerians had written information about medicinal plants on clay tablets (Awuchi, 2019). Moreover, over 600 plants were being used as remedies in Ancient Greece (Saber, 1982; Pakdemirli et al., 2021). Early developments in folk medicine have been furthered by increased scientific research on plant species.

The Greek word "*phyt*(on)" refers to the English word "plant" and is used as the prefix "phyto" to mean "plant-related". Plants having phytochemical bioactive compounds such as phenolic acids and flavonoids which enable their use in perfume, spice, and medical industries are called medicinal aromatic plants – MAPs (Picos-Salas et al., 2021). Among MAPs, more than 30 plant genera including Orchidaceae, Liliaceae,

Papaveraceae, Fabaceae, Rubiaceae, Theaceae, and Lamiaceae have contributed to ethnobotanical studies for many years (Source: Url1). The bioactive constituents of MAPs such as essential oils and flavonoids can be used as powdered, extracted, purified, or partially purified active substances which create a delightful flavor and scent (Source: Url1; Picos-Salas et al., 2021). There are approximately 60,000 (Source: Url1) economically important MAP species including red pepper, poppy, rose, coffee, tea, oregano (also called "thyme"), cumin, mint, and lavender with tons of production per year (Table 1.1). The demand for growing these plants is due to accelerated developments in biotechnology and various fields such as cosmetics, medicine, gastronomy, and the ornamental plant industry. They also have been used as folk remedies for some cultures since the Mesopotamian age (Acıbuca and Budak, 2017).

Medicinal plant products are labelled and called medicinal herbal products – MHPs. MHPs include herbal teas, cosmetic products, or other dietary supplements. MHPs have been used especially in traditional medicine since ancient times. According to WHO (World Health Organization), 20,000 plants are used as medicinal plant species and the pharmaceutical effects of many MAPs have already been investigated and recorded (Pakdemirli et al., 2021).

Table 1.1. Production (in tons) amounts of some economically important MAPs adapted from Pakdemirli et al. (2021).

	Years	2018		Years	2018
Area	Species	Production (thousand tons)	Area	Species	Production (thousand tons)
WORLD	Cinnamon	222	TÜRKİYE	Anise	8664
	Coffee	10303		Cumin	24195
	Ginger	2938		Lavender	1040
	Hemp seed	143		Lemon balm	84
	Hops	149		Mint	14511
	Mint	107		Oregano	15895
	Poppy seed	76		Poppy capsule	26991
	Spices	2018		Red pepper	227380
	Tea	6338		Rose for oil	14773
	Vanilla	8		Sage	428

According to an *in silico* publication analysis (Salmerón-Manzano et al., 2020), MAPs were mostly studied in the fields of Pharmacology, Toxicology, and Pharmaceuticals with 21.7% of publications in these areas while Biochemistry,

Molecular Biology, and Genetics were studied in 16% of the total publications (Salmerón-Menzano et al., 2020).

1.1.1. Benefits of MAPs

Plants have a critical role in everyday life and contribute to advances in medical treatments for humans and other animals. The World Health Organization (WHO) reported that 30% of MAP phytochemicals have been used as a drug ingredient from more than 21,000 taxa (Pandey et al., 2020). Unfortunately, there is uncontrollable use of certain plant species while some undiscovered treasures are threatened with extinction (Pandey et al., 2020). MAP species carry important bioactive terpenoid compounds such as alkaloids, glycosides, and essential oils with anti-cancer, anti-inflammatory, anti-diabetic, and anti-microbial characteristics (Figure 1.1) (Zielińska-Błajet and Feder-Kubis, 2020). However, environmental conditions like light saturation and physical factors such as harvesting time have an impact on the quality of these bioactive compounds in nature as well as in cultivated field conditions (Yeşil and Özcan, 2021). Moreover, phytochemical uses such as the antimicrobial effects of essential oils are also dependent on the sampled fraction such as flowers, aerial parts, leaves or roots (Aydın and Aydın, 2016). Therefore, evaluation of the optimum conditions to grow MAPs and the phytochemical content of different tissues will help the development of important phytochemical-based drugs with nontoxic and effective levels of raw substances.

Although much is known about some MAPS, some chemicals have not yet been investigated. Thus, there is a need for determining the biochemical composition of native plants, wild relatives and cultivars. In a few decades, MAPs that were underrated will be lost forever due to threatening factors such climate change and uncontrolled collection. In addition, the collection of wild native MAPs for personal, medicinal, or trading purposes generates risks for endemic and genetically uncharacterized, but invaluable, plant species.

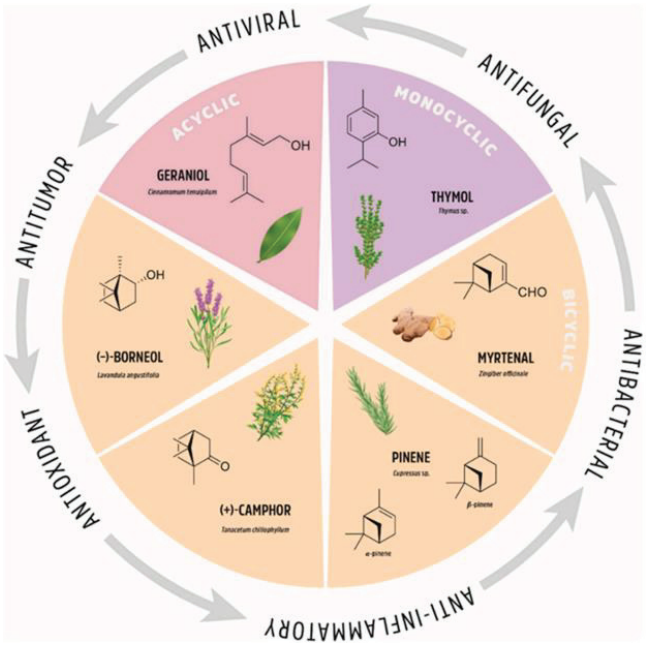


Figure 1.1. Representation of benefits of essential oils and the responsible chemicals in MAPs (Source: Zielińska-Błajet and Feder-Kubis, 2020).

1.1.2. Taxonomy of MAPs

Many MAP species have been morphologically characterized; however, almost the same number of uncharacterized species remains to be studied. Every year, the number of reported plant species increases due to scientific activity in discovering new plant species that may already face a risk of extinction (Antonelli et al., 2020). In 2019, 1942 new plants species were reported (Antonelli et al., 2020). Research on this abundance of plant species consumes a lot of energy, funding, and time. Correct systematics and naming of plant species will be achievable with easier and quicker approaches such as phytochemical analysis and utilization of DNA markers for uncharacterized species (Pandey et al., 2020). Naming and taxonomy are a major concern of conservation due to the local use and naming of many plant species.

Classification of MAPs is a requirement for their correct utilization and the sustainability of medicinal and aromatic plant genetic resources. Recent approaches to classify MAPs have been suggested by Alamgir (2017) and Joy and colleagues (2014) (Pandey et al., 2020). Alamgir (2017) suggested a comprehensive MAP classification for herbs and plants according to I) use, ii) active constituents, iii) period of life, iv) botanical taxonomy, v) habit, vi) mode of nutrition, and vii) habitat. Joy et al. (2014) used a

classification for managing the existence and conservation of exclusively MAPs based on i) importance, ii) aromatic parts, iii) growth habitat, iv) habitat, v) crop-duration, vi) method of propagation, and vii) botanical classification (Figure 1.2). Thus, criteria for classifying MAPs are based on subdivision of these seven criteria according to the plant species; type of tissue such as root, leaf, or flower that is utilize; neighboring plant types such as trees or grasses; climatic conditions such as tropical or temperate regions of the geographical location; life cycle (annual or perennial); reproductive system (vegetation or sexual); and consideration of taxonomical classification.

Figure 1.2. Seven major criteria in classification of MAPs (Source: Joy et al., 2014; Pandey et al., 2020).

1.2. The family, Lamiaceae (Labiatae) – Ballıbabagiller; one of the Most Important and Cosmopolitan Angiosperm Plant Family

The Angiosperms (flowering plants) contain 405 plant families; with 14,559 genera and 951,140 scientific plant names reported (Source: Url2). Among flowering plants, only 32% of the reported names refer to a certain accepted plant species and the rest are synonyms. Lamiaceae (Labiatae) is the sixth largest family of angiosperms and is the largest family within Lamiales (Zhao et al., 2021). The family Lamiaceae covers up to 30,745 scientific plant names including invalid, illegitimate, and spelling variants, in addition to accepted species (Source: Url2). Lamiaceae is the third most ethnobotanically utilized family with 25 species at the taxon level (Gras et al., 2021) after Astraceae (52) and Fabaceae (30). Currently, there are 13 defined main subfamilies in the Lamiaceae (Source: Url3). The largest subfamily, Nepetoideae, consists of four

subtribes: Elsholtzieae, Lavanduleae, Mentheae, and Ocimeae (Source: Url3). In Mentheae, there are many economically important medicinal plants such as *Salvia*, *Thyme*, *Melissa*, and *Origanum* (Figure 1.3) all of which have essential oils and flavones that are crucial for plant self-defense mechanisms. These bioactive chemicals are also the targets of many ethnobotanical studies.

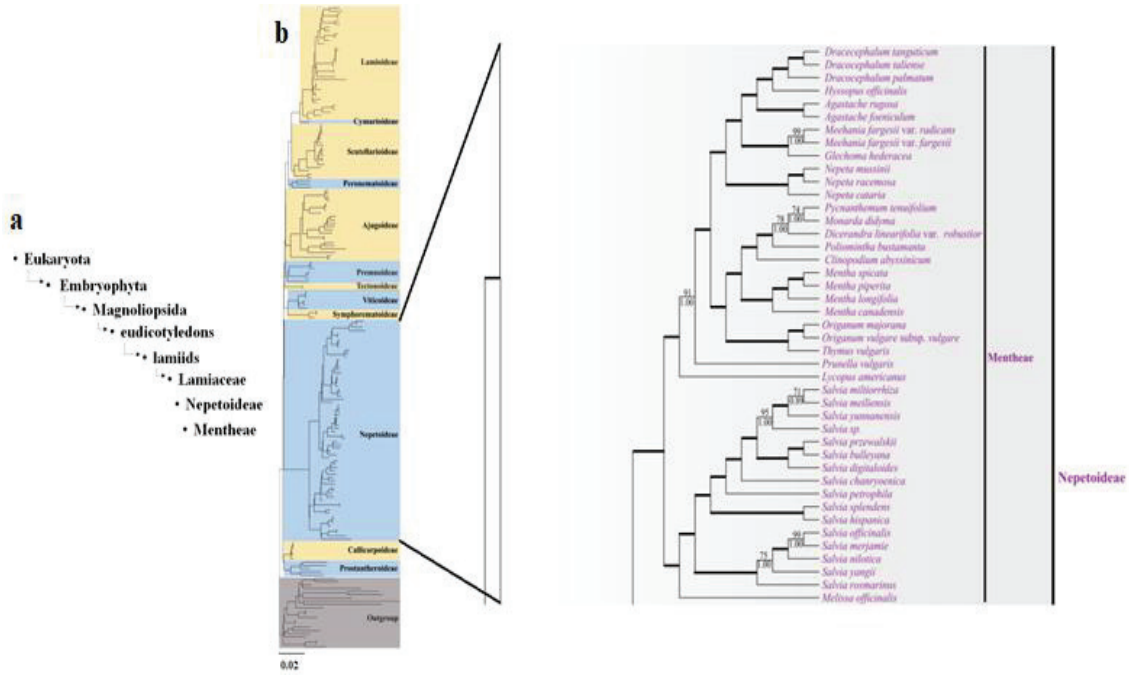


Figure 1.3. Lineage and phylogeny for the tribe Mentheae. a) Summary of lineage for the Lamiaceae family to the tribe Mentheae (Source: Schoch et al., 2020), b) Lamiaceae phylogenetic tree and closer look at tribe, Menthae, under subfamily Nepetoideae (Source: Zhao et al., 2021).

Plants from Lamiaceae have been of interest for six main areas of medicine: a) circulatory system and bloodstream disorders, b) pain and inflammation, c) digestion problems and nutritional deficiencies, d) dermatitis and other tissue disorders, e) respiratory problems, and f) tonic for medicinal and cosmetic uses (Gras et al., 2021).

1.3. Kekik/Oregano/Thyme

"Kekik", which is also commonly called oregano and marjoram in English, are MAP species that have been used extensively in folk medicine due to their high thymol and carvacrol levels. These "kekik" species belong to the Menthae tribe of the Lamiaceae

family and are represented mostly by the genera *Coridothymus*, *Origanum*, *Satureja*, *Thymus*, and *Thymbra* (Arabacı et al., 2019a, Bozdemir, 2019, Schoch et al., 2020). However, there are many other plant species such as *Coleosanthus* – Compositae, *Calamintha* – Lamiaceae, *Hedeoma* - Lamiaceae, *Hyptis* – Lamiaceae, *Monarda* – Lamiaceae, *Ocimum* - Lamiaceae, *Poliomintha longiflora* L. – Lamiaceae, *Satureja* – Lamiaceae, *Borreria* – Rubiaceae, *Limnophila* – Scrophulariaceae, *Eryngium* – Umbelliferae and *Lantana* – Verbenaceae that are known as oregano in different parts of the world because of their scent and flavor traits (Ietswaart, 1980).

Mother nature is a good source of different types of wild and hybrid "kekik" species and commercially traded oregano is mostly collected from *Corydothymus capitatus*, *Thymbra spicata*, *Origanum onites*, *Origanum syriacum*, *Origanum majorana*, *Origanum minutiflorum*, and *Origanum vulgare* subsp. *hirtum* from the Lamiaceae family (Arabacı et al., 2019b). According to the "Turkish Food Codex Spice Rescript", *Satureja* is legally accepted as "kekik" in labelled spices in addition to oregano, thyme, and coridathymus (Source: Url4) Although species belonging to the genus *Origanum* L. are called "oregano" while species in the genus *Thymus* are called "thyme", some scientists and traders use these terms incorrectly. Most of the time, especially in the spice and food industry, all "kekik" species are called "thyme" but they are not actually *Thymus*. In addition, *Lippia* ssp. from family Verbenaceae is known as Mexican oregano and has a stronger flavor than the oregano species from Lamiaceae (Kintzios, 2012). It is also known that species with "oregano-like" smell or aroma are being accepted as oregano. Every year, the Codex Committee on Spices and Culinary Herbs – CCSCH which is the international authority, updates standards for naming or classifying spices and herbs in collaboration with producer countries under the control of the European Union (Cilak et al., 2021; Source: Url5). Oregano from the genus *Origanum* L. is mostly called "European oregano" and is mainly used for medicinal research while most of the *Lippia* ssp. are used for culinary purposes as an aromatic for sauces or as a spice for pizzas (Kintzios, 2012).

In the early ages of systematics, plants were recognized and classified mainly according to their morphological and aromatic characteristics. In recent decades, it is understood that plants should be defined not only with morphological features but also their molecular genetic basis. However, economic restrictions make it hard to obtain the required knowledge about systematics. Fortunately, scientists never give up on working

hard to understand the evolutionary relationships among plant species. Every year, the number of publications is increasing gradually.

1.3.1. The genus *Origanum* L. (Linnaeus)

The position of the genus *Origanum* (L.) in the eudicotyledon lineage of angiosperms and its evolutionary network with the most critical hierarchical groups are shown in Figure 1.4 (de Vienne, 2016).

The species within the genus *Origanum* L. are shrubby and mostly bisexual or having only female organs (Ietswaart, 1980). These plants are perennial herbs that are mostly found growing naturally in rocky, high mountains, dry climates, and calcareous soils (Kintzios et al., 2012).

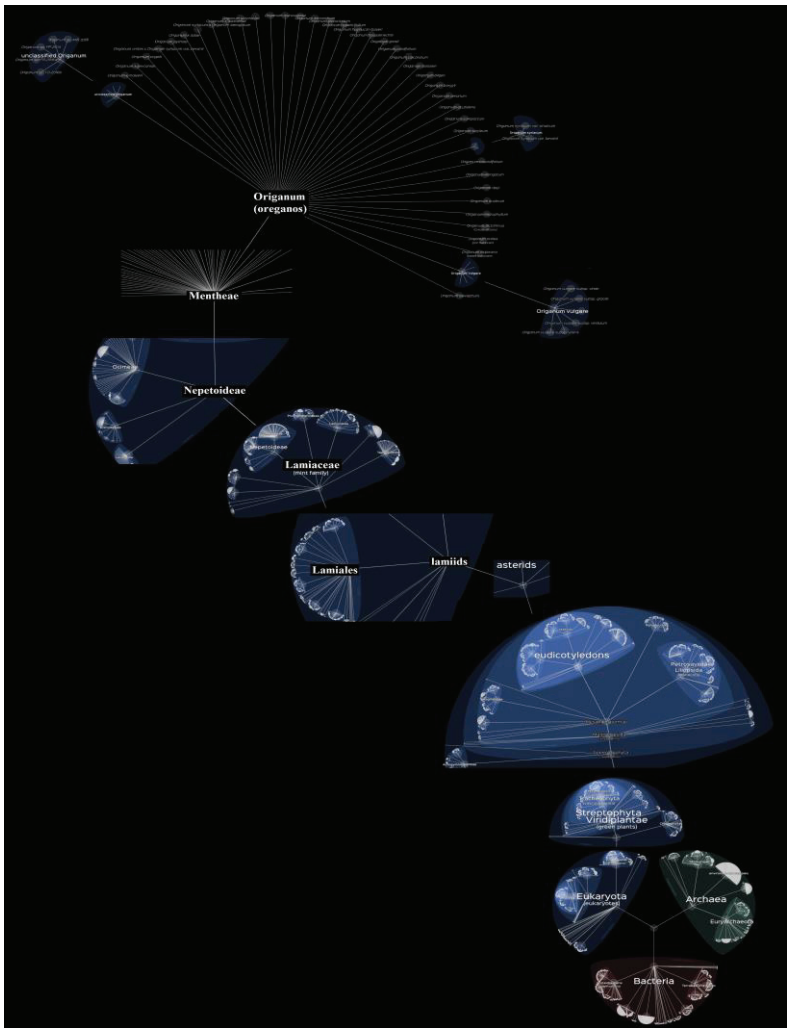


Figure 1.4. The family tree representation of *Origanum* L. genus (Source: de Vienne, 2016).

1.3.1.1. Food and livelihood security for herbal products containing oregano

There is concern about the toxicity and adverse effects of bioactive compounds in *Origanum* L. species that might create risks to human health (Jamshidi-Kia et al., 2018; Pakdemirli et al., 2021). These compounds are often extracted from specimens that are collected directly from the wild. Since oregano species have different levels of essential oils within the same species at different geographic locations and even from different collection years, there is a demand for estimating the annual measures of chemical content before using them in industry (Pakdemirli et al., 2021). Therefore, all oregano herbal products should be evaluated for authentication before release to markets in any form including teas or spices. For example, arbutin is an active compound of *O. dubium* that is utilized in cosmetic and drug industries but known for being toxic in the food industry (Lukas and Novak, 2020). Physical and chemical standards for spices and herbal products are determined by the Turkish Food Codex. According to the latest issue (2022/7), the standards for "kekik" species were updated for two groups of oreganos: 1st: *Thymus*, *Corydathymus*, *Satureja* and their admixtures, 2nd: *Origanum* (Source: Url4). The basic standard to industrialize oregano materials are determined by standardized drying and separation of leaf, flower, and shoots from the plant stems. The shoot and branch particles include oregano flowers and seeds (Source: Url4). The standards for leaf/flower oregano products and ground oregano spice are given in Table 1.2 (Source: Url4).

Table 1.2. Physical and chemical descriptive standards of leaf/ground spice for oregano-based herbal products (Source: Url4). *: *Thymus, Corydathymus, Satureja*; **: *Origanum* L.

	Leaf/flower	Ground
Maximum foreign materials (m/m%)	2	-
Maximum humidity (%)	10	12
Total ashes (%m/m)	12	12
Total ashes that is not soluble in HCl(%m/m)	2	2.5
Essential oils (least) (ml/ 100 gr)	1*	1*
	2**	1.8**
Stem and branch pieces (most) (%m/m)	10	-

1.3.1.2. Benefits of oregano

Oregano is a medicinal plant species used for thousand years to treat some diseases such as flu, diabetes, or leukemia by local people and in medical applications as a supportive agent (Tepe et al., 2016). It has also been used for culinary and cosmetic purposes in addition to enhancement/inhibition of plant-insect interactions as summarized in Figure 1.5 (Kumar and Bahardwaj, 2020). Essential oils such as thymol, carvacrol, γ-terpinene, *p*-cymene and linalool make oregano especially economically important due to their antimicrobial activities (Karagöz et al., 2020).

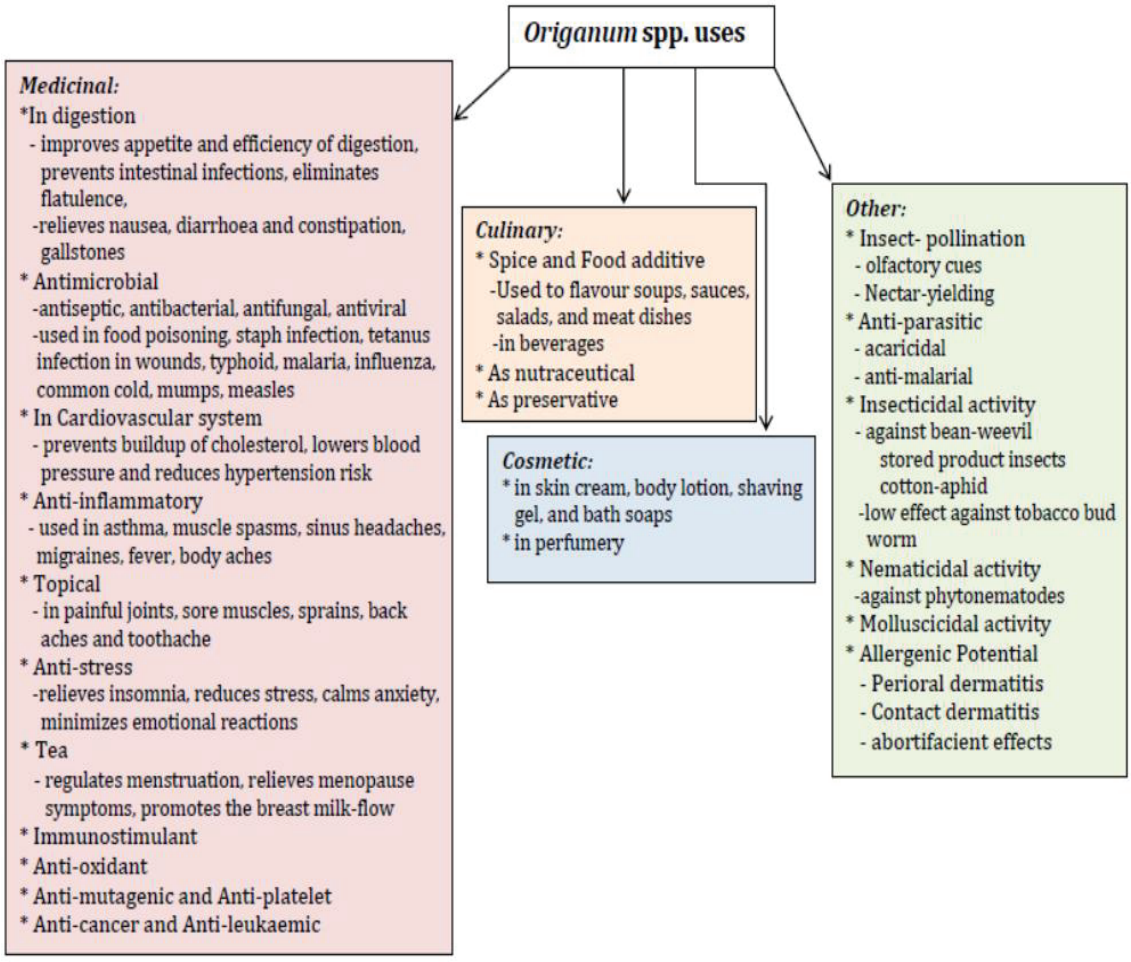


Figure 1.5. The current uses of oregano species (Source: Kumar and Bahardwaj, 2020).

1.3.1.3. Genome

In Lamiaceae, chromosome counts for a haploid genome vary between n=5 to 120 and the main reason for large genome size is polyploidy (Martin et al., 2020). The genome size of oregano species is relatively small compared to other genera in

Lamiaceae (Lukas and Novak, 2020). The genome size of *Origanum* is 1.50 pg which is similar to *Thymus* species (1.68 pg, 2C value) (Bakha et al., 2017). The most closely related genera to *Origanum* and *Thymus* are *Satureja*, *Micromeria*, and *Thymbra.* The biggest genome size among these genera belongs to *Satureja* with a mean 2C value of 3.9 pg.

Within *Origanum*, genome size varies depending on species. In the study of Bakha et al. (2017), the genome size variation of Moroccan oregano was assessed with 1.43 pg for *O. vulgare* subsp. *virens* (section *Origanum*) as the smallest genome and 1.53 pg for *O. compactum* (section *Prolaticorolla*) (Bakha et al., 2017). In the study of Jedrzejczyk (2018), *O. majorana*, *O. syriacum* and *O. vulgare* ssp. were investigated and the mean 2C value was observed as 1.48 pg for *O. vulgare* species and 1.70 pg for species from section *Majorana*.

In previous reports, the haploid number of chromosomes per taxon was reported as between *n*=14 and 16 for *Origanum vulgare* (Magulaev, 1984; Ayyagar and Vembu, 1985). According to the most recent findings, *Origanum* L. has a haploid genome of *n*=15 (*2n=2x*=30) for all *Origanum* L. taxa except *O. sipyleum* (n=14) and *O. rotundifolium* (n=14) (Harley et al., 2004; Martin et al., 2020; Dirmenci et al., 2021a). In addition, there are two exceptional haploid chromosome numbers of *n*=14 and *n*=18 due to aneuploidy for *O. vulgare* L. (Ayyangar and Vembu, 1985; Magulaev, 1984; Bakha et al., 2017). There are also recently identified and characterized oregano hybrid species *Origanum* x *adae*, *O.* x *intermedium*, *O.* x *haradjanii*, *O.* x *munzurense*, *O. sevcaniae*, *O.* x *font-queri* having haploid chromosome numbers of *n*=15 (Bakha et al., 2017, Dirmenci et al., 2019, Martin et al., 2020).

In *Origanum*, the main source of genetic variation is homoploid hybridization (Martin et al., 2020), and this results in conservation of the haploid genome size. For example, hybridization between *O. vulgare* subsp. *hirtum* (2n=30) with *O. onites* (2n=30) resulted in the formation of a new hybrid species, *O. x intercedens* with the same chromosome number, 2n=30 (Figure 1.6) (Arabacı et al., 2021a).

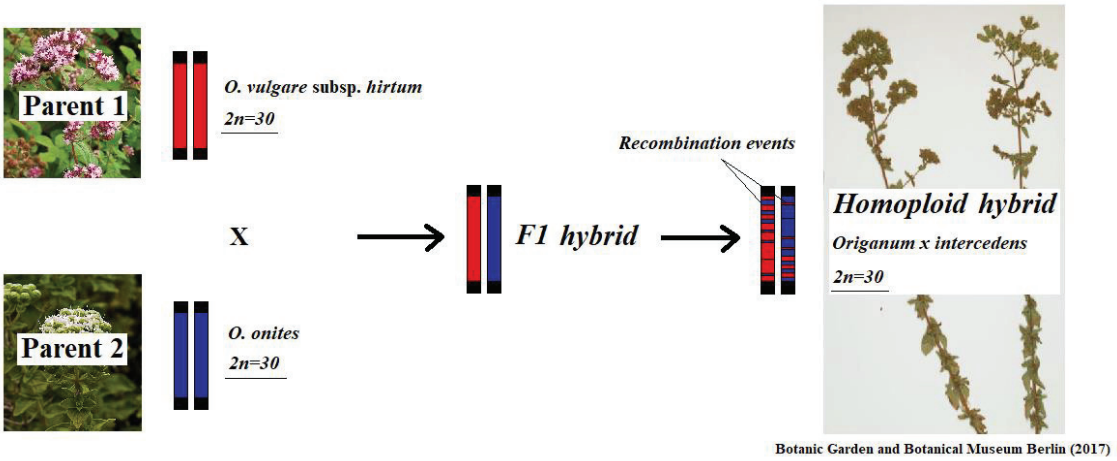


Figure 1.6. Hybridization between *O. vulgare* subsp. *hirtum* and *O. onites* leads to the appearance of the homoploid hybrid, *O. x intercendens* having the same number of chromosomes (2n=30) with its ancestors.

The complete chloroplast genome of *O. vulgare* L. has been determined (Lukas et al., 2013). Moreover, the whole genome shotgun sequence of *O. vulgare* L. (6.5 Gbp) and *O. majorana* L. (8.7 Gb) was investigated and is accessible in the SRA database of NCBI (Source: Url3).

1.3.1.4. Origin and distribution

Gene microcenters are an important concept in terms of understanding the evolution, origin, and distribution of plant species. The majority of oregano species are mostly located in Europe and the Mediterranean region, mainly in Türkiye, Greece and Cyprus; while a cosmopolitan group of oregano species called as "*O. vulgare* ssp." from sect. *Origanum* are placed throughout the world (Figure 1.7) (Dirmenci et al., 2021b). Oregano is also found in Syria, Lebanon; European countries such as France, Italy, and England; and also in Morocco and Western North Africa (Ietswaart, 1980). Among all oregano species, around 65% of species naturally grow in Türkiye (Figure 1.8) (Padulosi, 1997; Baser and Arslan, 2016).

Figure 1.7. Worldwide distribution map of *Origanum* species: most endemic oregano species are distributed throughout Europe and Mediterranean lands (red line), while species from section *Origanum* (*O. vulgare* ssp.) are widely distributed throughout the world (black dashed lines) (Source: Dirmenci et al., 2021b).

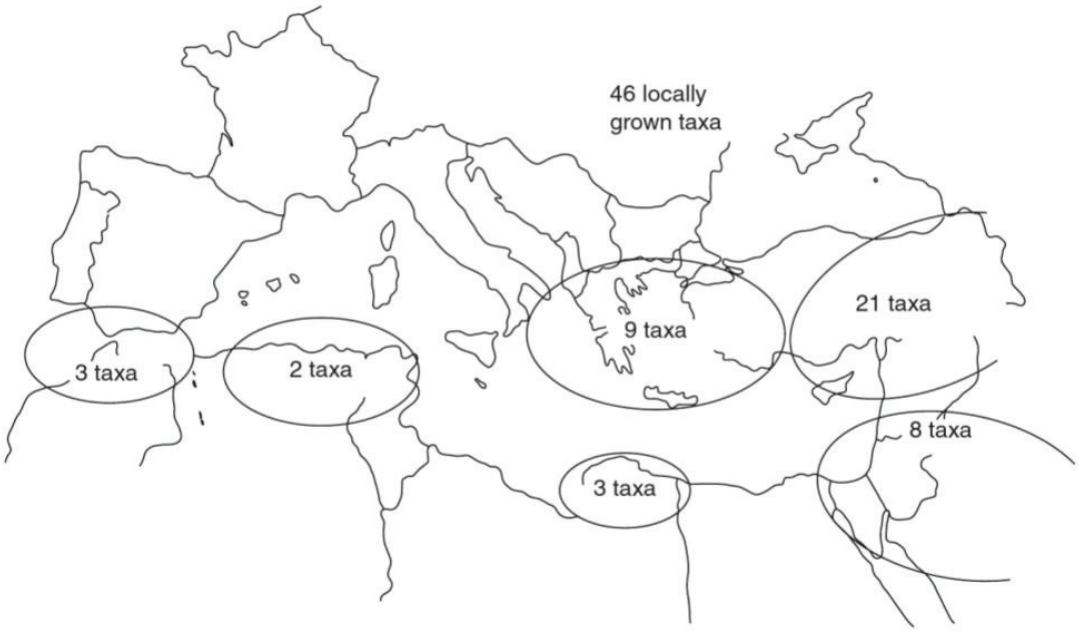


Figure 1.8. Locations of most native Mediterranean *Origanum* L. species, 65% of which are naturally found growing in Türkiye (Source: Baser and Arslan, 2016).

There are intermediate hybrid oregano species that are predominantly found in Türkiye (13 in number), while other hybrids are also found in Greece (5), Lebanon (2), Syria (1), Spain (1) and Italy (1) (Table 1.3) (Cattaneo et al., 2022; Dirmenci et al.,

2021a). According to the information in Table 1.3, hybrids between section *Anatolicon – Majorana*, *Anatolicon – Origanum*, *Amaracus – Anatolicon*, *Amaracus – Origanum*, *Brevifilamentum – Amaracus*, *Brevifilamentum – Majorana*, *Brevifilamentum – Origanum*, *Brevifilamentum – Prolaticorolla*, *Chilocalyx – Origanum*, *Longitubus – Prolaticorolla*, *Majorana – Amaracus*, *Majorana – Origanum*, and *Prolaticorolla – Majorana* were observed in nature. According to the native hybrids, the section *Brevifilamentum* had cross-sectional hybridizations among 50% of the sections, while *Chilocalyx* and *Longitubus* had hybridizations with only one section (12.5%).

The geographic distribution of some of the 21 oregano taxa in Türkiye can be monitored via an online database called "TUBIVES – Turkish Plant Data Service" (Babaç, 2004; Bakış et al., 2011). General information such as where they grow, endemism or elevations where they appear and geographical distribution is available online for the species: *Origanum boisseri*, *O. munzurense*, *O. saccatum*, *O. solymicum*, *O. hypericifolium*, *O. sipyleum*, *O. rotundifolium*, *O. acutidens*, *O. haussknechtii*, *O. bargyli*, *O. brevidens*, *O. husnucanbaseri*, *O. leptocladum*, *O. amanum*, *O. bilgeri*, *O. micranthum*, *O. minutiflorum*, *O. majorana*, *O. onites*, *O. syriacum* subsp. *bevanii*, *O. vulgare* subsp. *gracile*, *O. vulgare* subsp. *hirtum*, *O. vulgare* subsp. *viride*, *O. vulgare* subsp. *viride* (*O. vulgare* subsp. *viridulum*), *O. vulgare* subsp. *vulgare* and *O. laevigatum* (Figure 1.9, Figure 1.10). According to distribution maps, the most widely distributed oregano species is *O. vulgare* (subsp.) in Türkiye (Figure 1.10). In Figure 1.9, 21 species of *Origanum* L. are represented, and the diversity of oregano species mostly shows a restricted distribution area. For example, *O. sipyleum* is shown as one of the most predominant species in this map, however, it only grows in four regions of Türkiye (Black Sea, Marmara, Aegean and Mediterranean). In addition to this, *O. acutidens* is also displayed in many areas but only covering three regions (Central Anatolia, North-Eastern Black Sea, and South-Eastern Anatolia). This map also allows the recognition of endemic species such as *O. boisseri* which is located in only one province in the Mediterranean Region.

Table 1.3. Current natural hybrids of oregano (2022) (Source: Cattaneo et al., 2022; Dirmenci et al., 2021). #: Number; Sections; *AMA: Amaracus*,

ANA: Anatolicon, *BRE: Brevifilamentum*, *PRO: Prolaticorolla, MAJ: Majorana, ORG: Origanum, CHI: Chilocalyx, LONGI: Longitubus*.

#	Hybrid	Hybridization	Section	Identifier Scientists	Year	Distribution
1	*Origanum* x *adae*	*O. ayliniae** x *O. sipyleum***	*: *AMA* **: *ANA*	Dirmenci & Yazıcı	2018	Aydın, Aegean Region (A), Türkiye (TR)
2	*O.* x *adanense*	*O. brevidens** x *O. laevigatum***	*: *BRE* **: *PRO*	Baser & Duman	1998	Osmaniye, Mediterranean Region (MT), TR
3	*O.* x *adonidis*	*O. libanoticum** x *O. syriacum* subsp. *bevanii***	*: *ANA* **: *MAJ*	Mouterde	1935	Lebanon
4	*O.* x *aytacii*	*O. sipyleum** x *O. vulgare* subsp. *hirtum***	*: *ANA* **: *ORG*	Dirmenci, Yazıcı & Arabacı	2020	Denizli, A, TR
5	*O.* x *barbarae*	*O. ehrenbergii** x *O. syriacum* subsp. *bevanii***	*: *PRO* **: *MAJ*	Bornm.	1898	Lebanon
6	*O.* x *bilgili*	*O. saccatum** x *O. vulgare* subsp. *hirtum***	*: *AMA* **: *ORG*	Dirmenci, Yazıcı & Arabacı	2020	Antalya, MT, TR
7	*O.* x *dolichosiphon*	*O. amanum** x O. *laevigatum***	*:*LONGI* **: *PRO*	Davis	1951	Hatay, MT, TR
8	*O.* x *dumanii*	*O. husnucan-baseri** x *O. saccatum***	*: *BRE* **: *AMA*	Dirmenci, Yazıcı & Arabacı	2020	Antalya, MT, TR
9	*O. haradjanii*	*O. laevigatum** x *O. syriacum* subsp. *bevanii***	*: *PRO* **: *MAJ*	Rech.f. [Syn. *O. symeonis*, Mouterde, 1973]	1952	Hatay, MT, TR
10	*O.* x *intercedens*	*O. onites** x *O. vulgare* subsp. *hirtum***	*: *MAJ* **: *ORG*	Rech.f.	1961	Aegean Islands, West of Türkiye

(Cont. on the next page)

Table 1.3. (cont.)

11	*O.* x *intermedium*	*O. onites** x *O. sipyleum***	*: *MAJ* **: *ANA*	Davis	1949	Denizli, A, TR
12	*O.* x *lirium*	*O. scabrum** x *O. vulgare* subsp. *hirtum***	*: *ANA* **: *ORG*	Heldr. ex Halacsy	1899	Greece
13	*O.* x *majoricum*	*O. majorana** x *O. vulgare* subsp. *virens***	*: *MAJ* **: *ORG*	Cambess.	1827	Spain
14	*O.* x *malatyanum*	*O. acutidens** x *O. vulgare* subsp. *gracile***	*: *BRE* **: *ORG*	Yıldız, Arabacı, & Dirmenci	2020	Malatya, Eastern anatolian Region (EA), TR
15	*O.* x *malyeri*	*O. boissieri** x *O. vulgare* subsp. *hirtum***	*: *AMA* **: *ORG*	Dirmenci & Yazıcı	2018	İçel, MT, TR
16	*O.* x *minoanum*	*O. microphyllum** x *O. vulgare* subsp. *hirtum***	*: *CHI* **: *ORG*	Davis	1953	Greece
17	*O.* x *munzurense*	*O. acutidens** x *O. vulgare* subsp. *hirtum***	*: *BRE* **: *ORG*	Kit, Tan, & Sorger	1984	Tunceli, EA, TR
18	*O.* x *nebrodense*	*O. majorana** x *O. vulgare* subsp. *viridulum***	*: *MAJ* **: *ORG*	Tineo ex Lojac.	1907	Sicilia
19	*O.* x *pabotii*	*O. bargyli** x *O. syriacum* subsp. *bevanii***	*: *BRE* **: *MAJ*	Mouterde	1973	Syria
20	*O.* x *sevcaniae*	*O. vogelii** x *O. vulgare* subsp. *hirtum***	*: *CHI* **: *ORG*	Dirmenci, Arabacı, & Yazıcı	2018	Niğde, Central Anatolia Region (CA), TR
21	*O.* x *symes*	*O. onites** x *O. calcaratum***	*: *MAJ* **: *AMA*	Calström	-	Greece
22	*O.* x *karpathicum*	*O. onites** x *O. vetteri***	*: *MAJ* **: *ANA*	Dirmenci, Cattaneo & Dimarchou nothosp.nov.	2022	Greece

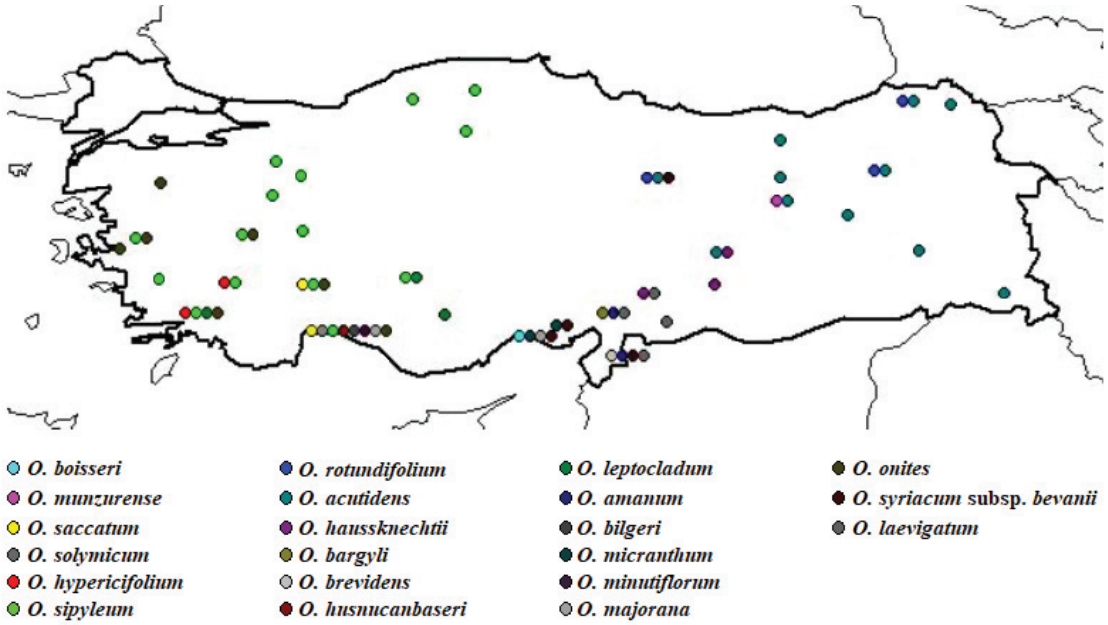


Figure 1.9. Geographical distribution of oregano species in Türkiye (Source: Babaç, 2004; Bakış et al., 2011).

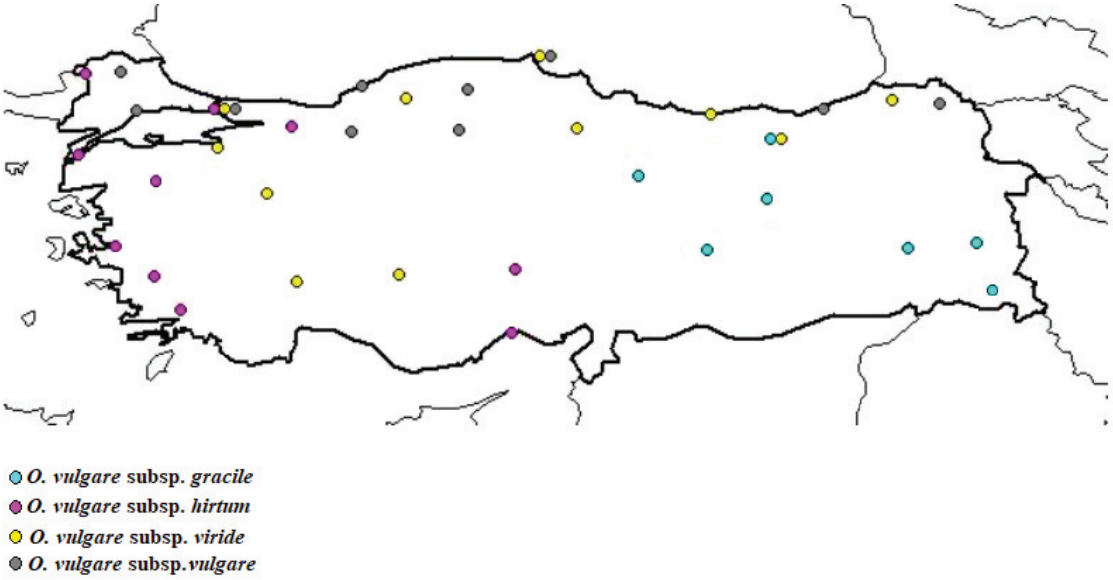


Figure 1.10. Geographical distribution of *O. vulgare* subspecies in Türkiye (Source: Babaç, 2004; Bakış et al., 2011).

In the map given in Figure 1.10, *O. vulgare* subsp. are shown to be distributed throughout Türkiye but within certain discrete regions. According to this subspecies map, *O. vulgare* subsp. *gracile* is mostly found in Central Anatolia, North-Eastern Black Sea, and South-Eastern Anatolia regions. *O. vulgare* subsp. *hirtum* appears to grow mostly in Western-Anatolia (Aegean-Marmara Regions). The subspecies *O. vulgare* subsp. *viride*

is the most widespread species in the map; it only seems to be absent from South-Eastern Anatolia. *Origanum vulgare* subsp. *vulgare* is located mostly in Northern provinces of Türkiye including the Marmara and Black Sea regions. However, the distributions of *O. vulgare* subsp. *hirtum* and other species are given differently in other maps (Figure 1.11) (Bizim Bitkiler®, 2022). As compared to the map generated by Bakıs et al. (2011), *O. vulgare* subsp. *hirtum* grows in five geographical regions of Türkiye but not in Central Anatolia and Southeastern Anatolia regions (Bizim Bitkiler®, 2022). This discrepancy might be due to a lack of updated information in the map of Bakış et al. (2011).

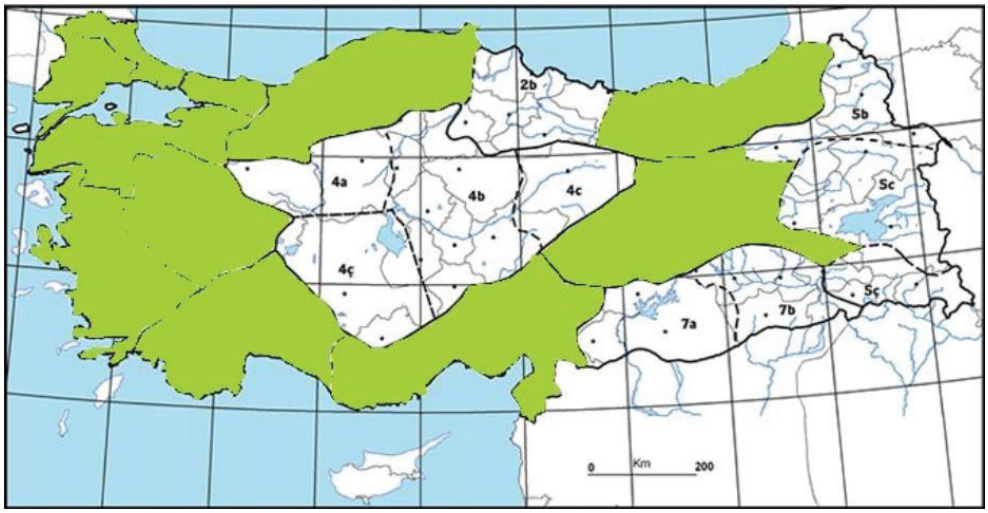


Figure 1.11. The distribution map of *O. vulgare* subsp. *hirtum* in Türkiye as shown by regions filled in green (Source: Bizim Bitkiler®, 2022).

In recent work, Behçet and Yapar (2021) reported that *O. vulgare* subsp. *viridulum* (OVVI) which is mostly known to be growing in the Black Sea region was found in a new area in Bingöl province (Iranian-Turanian element). This means that the overall distribution of oregano species is still expanding in nature. Thus, this expansion requires recognition and maps should be updated consistently.

1.3.1.5. Diversity

Genetic markers are representatives of any trait based on genetic variation (Kordrostami and Rahimi, 2015). These markers can depend on biochemistry, morphology, or DNA fingerprints (molecular).

1.3.1.5.1. Biochemical diversity

Plant primary metabolites are significant molecules directly affecting cell growth and division, immune response, respiration, storage, and reproduction (Hussein and El-Anssary, 2019). Secondary metabolites (SM) are derivatives of the primary metabolite pathways and, as bioactive agents target cellular molecules including enzymes, transcription factors, nucleic acids and mediators (Hussein and El-Anssary, 2019). SMs are utilized for therapeutic or prevention purposes to improve the health of many living organisms. The chemical richness of SMs has a significant impact on the function of plant self-defense mechanisms which facilitate several complex interactions with pollinators, insects, herbivores, pathogens, or other plants (Lichman et al., 2020). Phytochemicals are complex biological compounds produced by secondary plant metabolism that are especially used by plants as defense molecules (Gutiérrez-Grijalva et al., 2017). They are classified based on their functional groups under four main subgroups as phenolics (flavonoids and non-flavonoids), terpenes-steroids, alkaloids, and organosulfur compounds (Ülger and Ayhan, 2020). All these SMs have different roles in different MAPs.

Flavonoids are one of the most important bioactive derivatives of MAP secondary metabolism since they are water-soluble and have antioxidant effects on carcinogenic tissues (Picos-Salas et al., 2021). In *Origanum* L. species, there are two predominant flavones: luteolin and apigenin derivatives, both of which have high antioxidant, anticancer and anti-inflammatory features (Picos-Salas et al., 2021). Other than apigenin and luteolin, derivatives of these molecules such as lutein-7-O-glucoside (Figure 1.12) and compounds such as scutellarein molecules are also common in the genus *Origanum* L.

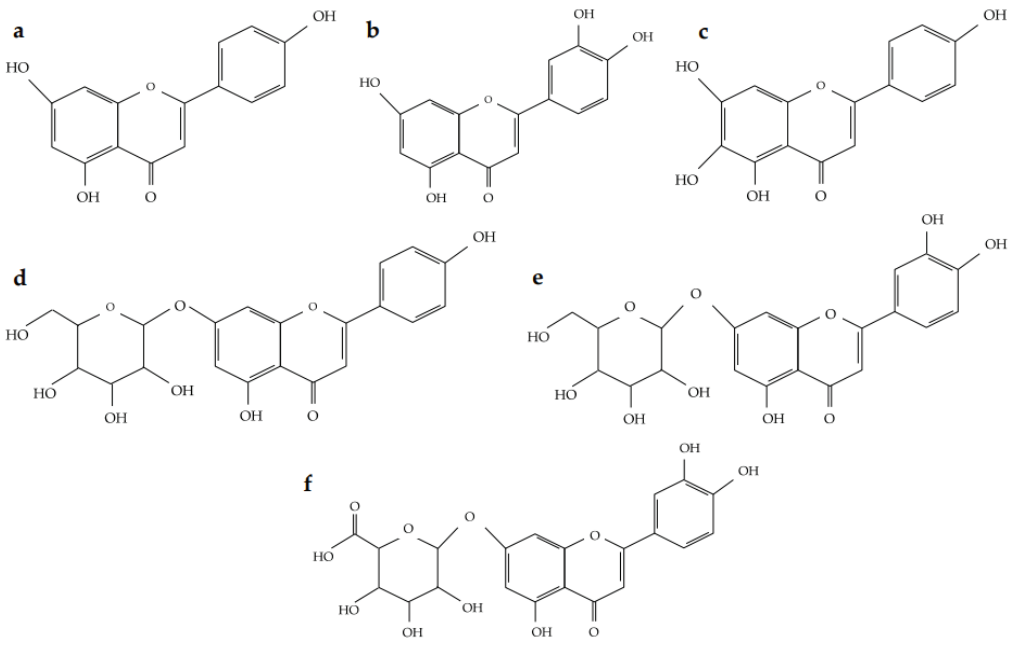


Figure 1.12. Most common flavones present in oregano species (a) apigenin, (b) luteolin, (c) scutellarein, (d) apigenin-7-O-glucoside, (e) luteolin-7-O-glucoside, (f) luteolin-7-O-glucuronide (Source: Gutiérrez-Grijalva et al. 2017).

The terpenes-steroids group of SMs are the most rich and diverse SMs in MAPs (Hussein and El-Anssary, 2019). Among angiosperms, mints have lineage-specific gene families to produce such metabolites. One example is the terpene synthase genes which affect the diversity of volatile oils in Lamiaceae ssp. (Lichman et al., 2020). In the genus *Origanum*, there are acyclic, monocyclic, and bicyclic monoterpenes, and sequiterpenoids that create a vast diversity of medicinally and aromatically important chemotypes. Among those, the most common terpenoid compounds among all species in the genus *Origanum* (Baser, 2002; Arabacı et al., 2019b) are thymol and carvacrol (Figure 1.13). The generation of these products might be positively or negatively affected by the different expression levels of terpene synthase genes in different oregano taxa. In the study of Jan et al. (2018), three *O. vulgare* and three *O. majorana* specimens were investigated for 14 terpene synthase genes and five cytochrome P450 genes. As a result, they concluded that each terpene synthase gene ia individually responsible for generation of certain mono- or sesquiterpene products and these genes can directly create almost all terpenes found in oregano when they work collectively (Jan et al., 2018).

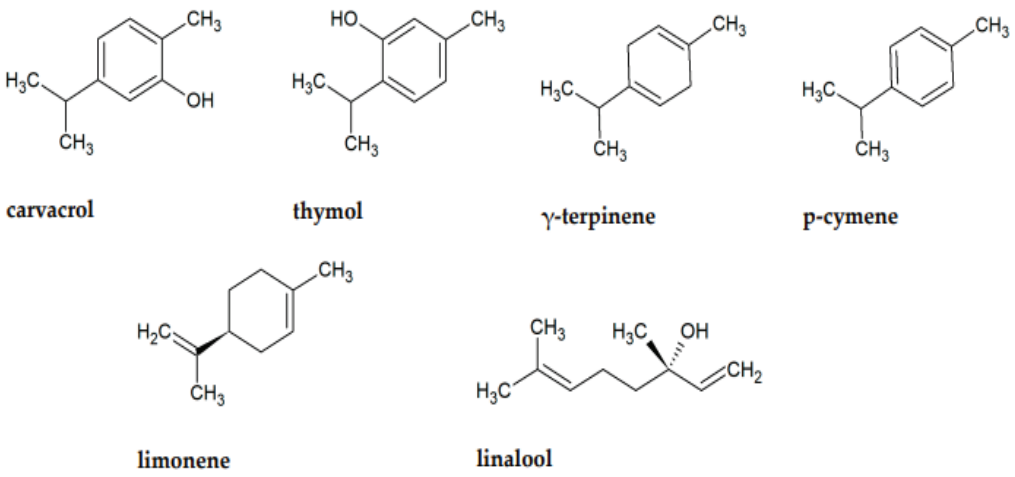


Figure 1.13. Most common chemicals present in essential oil content of oregano species.

The major volatile constituents for *O. majorana*, *O. onites*, *O. minutiflorum*, *O. vulgare* subsp. *hirtum*, *O. syriacum* subsp. *bevanii*, *O. acutidens*, *O. bilgeri*, *O. hypericifolium*, *O x intercedens*, *O xadanense*, and *O. bargyli* are **carvacrol** and **thymol**. *O. saccatum*, *O. solymicum*, *O. leptocladum*, *O. boissieri,* and *O. haussknechtii* are rich in **p-cymene**. *O. husnucan-baseri*, *O. onites*, *O. vulgare* subsp. *viride*, *O. micranthum*, *O. majorana*, *O x majoricum*, and *O. rotundifolium* are rich in **other monoterpenes** while *O. vulgare* subsp. *gracile*, *O. vulgare* subsp. *vulgare*, *O. vulgare* subps. *viride*, *O. laeviganum* and *O x dolichosiphon* are rich in **sequiterpenes** (Baser et al., 2002; Taş, 2010).

1.3.1.5.2. Morphological diversity

Morphological characters allow individuals to be distinguished at the species, section, or genus level for oregano species. The most important determiners of genus *Origanum* are mainly differences in spike, bract, calyx, calyx teeth, corolla, stamina, or indumentum size and shape (Ietswaart, 1980). These characters may overlap for some sections within the genus and their use leads to the determination of phenotypic clusters (Ietswaart, 1980; Antaloudaki et al., 2022). For example, species from the genus *Origanum* L. are grouped according to features like large/compact spike formation, number of branches, and the type of bracts into sections such as *Amaracus*, *Anatolicon*, *Brevifilamentum*, *Longitubus*, or *Prolaticorolla*. These sections all share a lack of

branches, while *Amaracus* has larger spikes and *Anatolicon* has compact spikes. Moreover, while both *Brevifilamentum* and *Longitubus* sections have lots of flowers, *Amaracus*, *Anatolicon*, two subspecies of section *Origanum*, and one individual from section *Prolaticorolla* have a common bract formation. Other sections like *Campanulaticalyx* and *Chilocalyx* can be clustered according to other features such as leaf structures or calyx teeth. Corollas are mostly similar between *Anatolicon, Origanum*, and *Elonggatispica*; *Prolaticorolla* and *Brevifilamentum*; *Longitubus* and *Brevifilamentum*; and *Majorana* and *Chilocalyx* (Ietswaart, 1980). All these minor differences allow the separation of major sections in the genus *Origanum*.

Recently, mericarp morphology of the genus was characterized in detail from Turkish national herbarium materials for the first time by Ecevit-Genç (2020). According to this study, mericarps were grouped into two main clusters: Type I and Type II. These two clusters had five different types in total: ruminate (R), reticulate-ruminate (RR), foveate (F), reticulate-foveate (RF), and pusticulate (P). In summary, sections *Chilocalyx* and *Amaracus* (*O. solymicum* – Antalya/2014) were R type. Sections *Brevifilamentum* (*O. brevidens*), *Longitubus*, and *Prolaticorolla* shared RR type of mericarp morphology. Sections *Amaracus*, *Anatolicon*, and *Majorana* (*O. onites*) had P type mericarps. Only *O. husnucan-baseri* (OHU) from section *Brevifilamentum* had RF type mericarps. This information supports the previous findings about OHU that it is different from other taxa in section *Brevifilamentum* with its reticulate pollen ornamentation (Akyalçın, 1998). The sections *Origanum* and *Brevifilamentum* had F type morphology in their mericarps. Thus, this new study added more detailed knowledge of mericarp morphology in the genus *Origanum*. As a result of this work, macro- and micro-mericarp variations were found to be useful markers for distinguish differences at species and section level among the genus *Origanum* and between other genera in Lamiaceae (*Mentha*, *Thymus*, *Satureja*) when used together with knowledge of geographical distribution and flower morphology. However, some individuals could not be distinguished at the species level by considering mericarp morphology but can be grouped together in sections such as *Chilocalyx*, *Anatolicon*, and *Origanum* with type II mericarp morphology because some singular species do not share the common mericarp features within their sections. For example, the accessions *O. acutidens* (Malatya/2013), *O. haussknechtii* (Erzincan/2013), and *O. rotundifolium* (Artvin/2013) have F-type of mericarp morphology and are also found in close proximity in nature. The major factor making them different from other individuals in the section *Brevifilamentum* is flower morphology variation. In contrast, *O.*

leptocladum (Karaman/2014) and *O. husnucan-baseri* (Antalya/2014) are morphologically similar and found together in nature, but they have different mericarp morphologies. In addition, the endemic and currently data-deficient species, *O. brevidens* (Osmaniye/2014), was suggested for the first time to be a hybrid between species *O. amanum* (Osmaniye/2014) and *O. laevigatum* (Hatay/2013) due to their common phenotypic features and shared growing locations (Ecevit-Genç et al., 2020).

As mentioned above, previous work by Akyalçın et al. (1998) also investigated pollen grains. In this work, pollen grains were characterized as colpate (C), suboblate (SO), oblate spheroid (OSF), subprolate (SP), prolate (P), and prolate spheroid (PSF). However, there was no certain correlation of pollen grain and ornament structures with the type of species although SP was mainly found in *Amaracus*, *Chilocalyx*, and *Origanum*; spheroid types (PSF and OSF) were observed in *Anatolicon*, *Brevifilamentum*, *Majorana*, and *Prolaticorolla*; SO formation was reported in *Brevifilamentum*, *Majorana*, and *Origanum*; and P structure was in *Brevifilamentum*. Although these indicators seem to be shared within sections, they can vary depending on the geographical origin or chemotypic structure. Therefore, it is important to have more than one individual per species to create informative taxa. For example, the study had different *O. sipyleum* specimens collected from different provinces and districts. The individuals collected from Manisa and Balıkesir had the same PSF structure, while accessions that were collected from different districts of Ankara (Kızılcahamam and Beypazarı), showed different pollen morphologies (PSF and OSF).

Based on this previous research, the power of the use of both geographical distribution and various combinations of morphological characteristics cannot be denied. The addition of molecular genetic tools will undoubtedly enhance the power of discrimination at the species level.

1.3.1.5.3. Molecular diversity

A plant population is a group of individuals of a given species that can intra or interbreed randomly due to chance. Tissue or species–specific diversity between and within plant populations is determined by genetic events that provide plasticity for each individual in a particular habitat. These genetic incidents can happen in nuclear or plastid regions (mitochondria or chloroplast genomes) in plant species (Zhang et al., 2020). All

changes within and between these genomes create polymorphisms and affect the adaptability of a plant to its environment. Variation within these genetic compartments can be manifested by substitutions, duplications, in/dels, inversions and translocations with at least five different flow patterns from mitochondrion to nucleus, plastid to nucleus, plastid to mitochondrion, nucleus to mitochondrion, and mitochondrion to plastid (Zhang et al., 2020). In gynodiecious plants such as species of the genus *Origanum*, female plants carry cytoplasmic male sterility (CMS) alleles in maternal compartments such as mitochondria making them male sterile while hermaphrodites do not have CMS genes or have nuclear restorer genes (Touzet and Meyer, 2014; Mollion et al., 2017). The presence of CMS genes guarantees the generation of new female individuals which is advantageous for the survival of the population (Mollion et al., 2017). Variants of these CMS alleles can be introduced to populations via large recurrent spontaneous mutations in low frequencies that create genetic sweep or with the entrance of different CMS genes into the population via migration (Frank, 1989; Mollion et al., 2017).

Diversity within or between gene pools includes all the alleles for a locus in a particular population of the species. Allele frequencies are only expected to remain constant under conditions of Hardy-Weinberg Equilibrium (HWE). In such cases there should be no mutation, no migration (no gene flow), no random mating, or no genetic drift (dramatic changes in allele frequencies from both parents leading to reduction of diversity). However, HWE is not applicable to natural plant populations even in the case of clonal populations since the size of the clonal population, the clone itself, sexual reproduction, migration, life cycle, gametic state (monoecious/diecious), and distribution of populations all affect allele frequencies (Douhovnikoff and Leventhal, 2016). According to Darwin (1872), a selective advantage of any gene variant among individuals in a population will lead to fixation of that allele in the population. This suggests that genetic variance will lead the population to evolve over evolutionary time. This variation might be due to mutation or genetic drift and leads to diversity at the DNA, individual, subpopulation (SP) or population (microevolutionary) levels (Ronfort et al., 2005; Mechergui et al., 2017) and contributes to macroevolution. According to Touzet (2012), genetic drift can dramatically affect the reduction of diversity in small populations. In larger populations, diversity reduction happens with fixation of a favorable rare allele with linkage drag/hitchhiking of linked genes through balancing selection. Gene flow (migration) is the tendency to reduce variation between different

gene pools at the molecular level by the exchange of genetic materials with homologous or non-homologous recombination. Hybridization among taxa through gene migration can enhance or decrease the pace of differentiation (Grünig et al., 2021). Moreover, a sympatric barrier can inhibit migration of individuals by geographical isolation and cause a single ancestral population to lead to the formation of two sister species (Foote, 2018). Therefore, determination of gene flow will help to determine genetic relatedness, speciation dynamics, and diversity in plant populations. It can also help to choose the best specimens for breeding applications.

The importance of gene flow was reflected in the cross-pollination among oregano species including *O. vulgare* subsp. *vulgare* and two out groups: *O. vulgare* subsp. *hirtum*, and *O. vulgare* subsp. *glandulosum* as determined with EST-SSR screening (Mechergui et al., 2017). According to their results, *O. vulgare* subsp. *glandulosum* taxa with all specimens are wild and from one location, Tunisia, showed high gene flow (27.34), while the gene flow between *O. vulgare* subsp. *vulgare* (OV) (Italy) – *O. vulgare* subsp. *hirtum* (OH) (USA) and *O. vulgare* subsp. *glandulosum* – OV/OH was low (0.47 and 0.17 – 0.28). In this work, genetic homogenization and high gene flow in discrete areas was observed and concluded to be the source of a single complex gene pool of their oregano population.

Genetic distance assessment has been used to collect substantial knowledge about kinship among *Origanum* species for their better characterization and taxonomic classification. However, to date, there are an insufficient number of molecular genetic marker-based diversity analyses.

1.3.1.6. Reproduction among oreganos and speciation hypothesis

Plants reproduce with sporophyte (2n; diploid; multicellular) and gametophyte (n; haploid; multicellular) cycles. Unlike animals, plants undergo mitosis to create male (sperm; n) and female (egg; n) organs. The fertilization of an egg and sperm generates a zygote (2n) and the zygote grows to a diploid mature plant (2n). Diploid genome size varies from species to species; and it is affected by the mating system as well as intergenic rearrangements and genome duplication. In addition, plant fertilization results in two types of ploidies (number of chromosome sets from both parents): polyploidy (more than two set of chromosome pairs) and homoploidy (the same number of chromosomes as in

the parents). For instance, *Brachypodium distachyon* is a small model organism known to be genetically close to wheat with a haploid genome of n=5 with 3.5 * 10^8 bp chromosomal content, while *Triticum aestivum* L. (bread wheat) has a haploid giant genome of n=21 with 1.6 * 10^{10} bp (Jones et al., 2013). This genome size variation was caused by polyploidization events in the evolution of bread wheat. Polyploids can be autopolyploid or allopolyploid (Jones et al., 2013). If the plant undergoes autopolyploidization, offspring will have two sets of each parent's chromosomes after mitosis. Allopolyploids have genetic material from two or more ancestral genomes to form a new species via interspecific hybridization (Jones et al., 2013). This feature is utilized by plant breeders because it provides heterosis for special traits of interest in domesticated species. However, plants naturally undergo hybridization to generate a new species and polyploidy can create male/female sterility. In contrast, homoploid speciation can occur among individuals unless there is a reproductive isolation barrier inhibiting gene flow. Homoploid speciation allows fixation of parental chromosome number in next generation (Abbott et al., 2000; Buerkle et al., 2000).

Hybridization is a driving force in both the generation of new species and in reverse speciation (Culicchi, 2022). There are many unclear hypotheses about the hybridization behavior of *Origanum* L. species. However, the longest accepted speciation theory was discussed by Ietswaart (1980). According to his hypothesis, hybridization is a common phenomenon and results in speciation in the genus *Origanum* (Ietswaart, 1980; Lukas and Novak, 2020). Hybridization may occur between both wild and cultivated oregano populations within a certain proximity and can lead to the generation of new species (Dirmenci et al., 2018). *Origanum* species were hypothesized and proven to have substantial homoploid hybridization among populations (Ietswaart, 1980; Arabacı et al., 2021a).

Speciation is not only affected by hybridization behavior, it is also affected by biotic (e.g., pathogens) and abiotic (e.g., global warming, day and night, human forces) factors, reproductive systems, genetic events, and geographical distribution. All of these factors have resulted in the diverse number and kinds of plant species on Earth. Plant diversification is mainly dependent on mating system and reproductive strategies (Barrett, 2010). Plants can produce an offspring via stolon/runner branch formation (asexual; clonal reproduction) or via fertilization (sexual reproduction; selfing or hybridization). Fertilization can occur gynogenetically (having both hermaphrodite and singular female organs in separate plants) or hybridogenetically (premeiotic elimination

of one parental genome creates a transition state of a new species divergence, unsettled) (Som et al., 2007). Asexual reproduction generates new plants with the exact same genotype as its progenitor while sexual reproduction may result in new genotypes with the exact number (homoploid) or enhanced number (polyploid) of parental chromosomes.

Angiosperms mostly undergo bisexual reproduction with flowers carrying both male and female reproductive organs. However, oreganos mainly have gynodioecious reproduction which means that female (male-sterile; MS) and hermaphrodite *Origanum* L. individuals coexist in a native population (Kheyr-Pour, 1980). This means that these plants can undergo both selfing and engage in bisexual reproduction. Sections *Chilocalyx*, *Elongatispica*, *Majorana*, *Origanum* and *Prolaticorolla* are most known for gynodioecy in the genus (Ietswaart, 1980). The percentage of possible male-sterile (female) and hermaphrodite individuals in a native population varies from 1 to 62% (Kheyr-Pour, 1980; Langbehn et al., 2001; Lukas and Novak, 2020). Male-sterile plant development in female plants might be due to polygenic or monogenic factors mainly based on cytoplasmic male-sterility genes (CMS genes converting hermaphrodites into females) and nuclear male-sterility restorer genes (NMS restoring alleles restoring a "male function") (Touzet, 2012). The introduction of rare CMS or NMS restorer alleles into a new population via migration or mutation may give rise to balancing selection within the population for that allele (Touzet, 2012). While sterility alleles are fixed, plant genomes might have genetic drift due to the reduction of population size. Reduction in population size can lead to increased genetic similaritys. In contrast, large populations maintain higher levels of diversity (Touzet, 2012). The effect of nuclear restorer genes was found to be responsible for thyme male sterility in female only plants (Belhassen et al., 1991; Charlesworth and Laporte, 1998; Touzet, 2012). Mollion et al. (2017) examined the *Thymus vulgaris* (*Thyme*) genome which is a close relative of *Origanum* and considered to be an ancestral species. The aim of this work was to understand the genetic basis of gynodiecy in their population and transfer of the chloroplastic genome to the mitochondrial genome was observed. In addition, their data showed that CMS is observed at different levels among or within *T. vulgaris* populations in nature (Mollion et al., 2017).

Gynodioecy is known to result from a "female advantage" because, in a population, female plants are better able to adapt to the environment by not wasting their energy to produce pollen (Touzet, 2012). Since the female plants can outcross, it becomes

advantageous for the plant to generate new viable hybrids with other pollen sources. For example, even though the homoploid hybrid species, *O. x intercedens*, had a lower percentage of viability (57%) when compared to its ancestors, *O. vulgare* subsp. *hirtum* (70%) and *O. onites* (81%), its reproductive flexibility remains as an advantage in the next generation (Bariotakis et al., 2016; Lukas and Novak, 2020). All these events affect the overall population size and structure differently depending on the size of the gynodioecious population.

Naturally isolated gene pools and their sizes affect the fate of endemic plant species since most plants of such species tend to hybridize with different taxa and therefore have higher recombination frequencies. The evolutionary importance of isolated native populations was first suggested by Wagner (1889) and was supported by Charles Darwin and Lamarck (Singh, 2022). This perception was supported by the scientist Savage (1963) as he suggested that the evolution of new species occurred from population fragmentation and genetic divergence (Singh, 2022). Habitat fragmentation results in the isolation of populations based on geographic restrictions (Singh, 2022) causing i) generation of native plant species which are genetically capable to reproduce in isolated populations or ii) enhanced cross-hybridization behaviors to produce new, genetically intermediate species from an admixed population. These are expected to occur unless there is a gene flow barrier among species in a local area. *Origanum* having around 67% endemism within species and 58% endemism among taxa in their own territories (Kuşaksız, 2019) is, therefore, susceptible to loss of genetic richness in this way. For oreganos, most endemic species such as *O. amanum* or *O. brevidens,* which are known to be growing locally only on Amanos Mountains, are found in restricted areas (Ietswaart, 1980).

In the beginning of the Ice Age from the Pliocene to the Pleistocene, oregano species were distributed on high hills and mountains where they had genetic connections with Saturejeae genera, and were geographically isolated from water (Ietswaart, 1980). In this evolutionary process, some of the species were lost, while new species arose. This speciation has provided the generation of hybrid species and resulted in loss of original species especially in the sections *Anatolicon*, *Brevifilamentum*, *Chilocalyx*, *Elongatispica*, *Longitubus*, and *Prolaticorolla* (Ietswaart, 1980) (Figure 1.14).

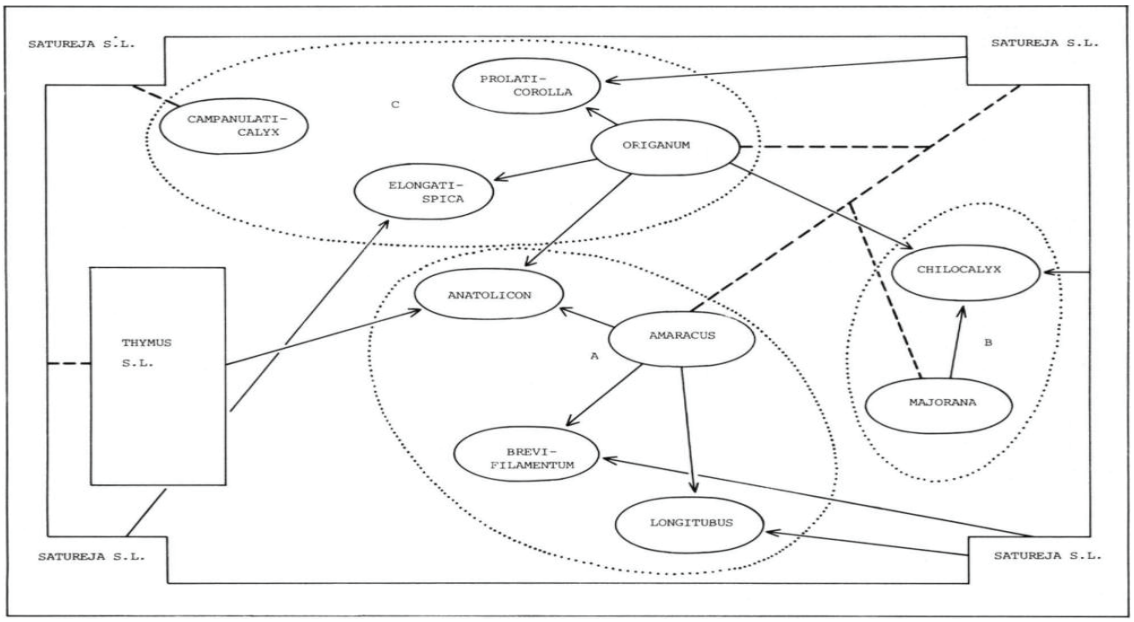


Figure 1.14. Schematic illustration of speciation between *Origanum* L. sections and other genera (Source: Ietswaart, 1980).

Cross-sectional and cross-taxa hybridization is possible between oregano species. Recently, transfer of the same set of chromosomes (homoploidization) within the genus was proven by karyological and molecular studies. In the study of Arabacı and colleagues (2021), three parental species from three distinct sections: section *Anatolicon* (*O. sipyleum*), *Majorana* (*O. onites*) and *Origanum* (*O. vulgare* subsp. *hirtum*) were compared with intermediate hybrid species (*O. x intercedens*: OON x OVH; *O. intermedium*: OON x OSI; *O. x aytacii*: OSI x OVH) (Arabacı et al., 2021). This comparison showed that the hybrid species are more closely related to one of their parents in each hybrid such that *O. x aytacii*, *O. x intercedens*, and *O. x intermedium* are closer to their OSI, OVH, and OON ancestors, respectively (Arabacı et al., 2021). Their molecular findings suggested that OSI was clustered alone while OON and OVH were in mixed-species clades. This finding suggests that homoploid hybridization is occurring in native oregano populations.

1.3.1.7. *Origanum* taxonomy

The genus *Origanum* (L.) was first suggested by Linnaeus in Labiatae in 1754. According to his definition, these species are found with flowers in various dense spikes, petals with colors, seed capsules with four types of lips: five equal teeth, two-lipped,

lower lip reduced, and corollas with two lips. The Greek word "*Origanum*" was first used by Hippocrates (460 – 370 B.C.E.), the physician who is known as "the father of medicine". The name "oregano" comes from the intersection between two Greek words: *oros* – hills and mountains and *ganos* – joyful ornament, showing knowledge about their growth habitat and appearance.

In the genus *Origanum*, the initial classifications were proposed by Miller (1754-1768, sections *Majorana* and *Origanum*), Gleditsch (1764, section *Amaracus*), Rafinesque and Scheele (1836 and 1843, section *Origanum*, never accepted), Kuntze (1867, 1891, about section *Origanum* as genus and *Thymus* as a section, then *Thymus* as genus and *Origanum* as section, never accepted), Bentham (1834, suggested *Amaracus*, *Majorana*, *Origanum* as three genera; then in 1848 – 1876, he accepted the Linnaeus concept and denied them being genera; assumed them as sections, and added the section *Anatolicon*), and Briquet (1895, sections *Schizocalyx*, *Holocalyx* and *Chilocalyx* in assumed genus *Majorana*). In later years, *Majorana* and *Origanum* were continued to be accepted as different genera. Other researchers started following the Linnaeus concept in Lamiaceae such as Moench (1794), Bornmiiller (1917), Hayek (1931). Rechinger (1943), Wolf (1954), Wunderlich (1967) and Zohary (1973). Other researchers who were interested in the taxonomical revision of *Origanum* L. species were Lamarck (1797), Sibthorp & Smith (1826), Willkomm & Lange (1868), Boissier (1879), Nyman (1881, 1890), Halacsy (1902), Post & Dinsmore (1933), Mouterde (1935), Davis (1949), Thiebaut (1953), El-Gazzar & Watson (1970), Fernandes & Heywood (1972) (Ietswaart, 1980). To date, Boissier (1879 – "Flora Orientalis") and Ietswaart (1981 – "Flora of Türkiye") are the most well-known classifications of *Origanum* L.

The most accepted taxonomic revision of the genus *Origanum* was written by Ieatwaart (1980) according to a large variety of morphological and chemical diversity. In the last few decades, there has been some debate on the classification of genus *Origanum* due to newer findings from molecular level studies (Lukas et al., 2010, Lukas et al., 2013). In addition, there are species such as *O. symes* (Govaerts, 2003) and *O. ayliniae* (Dirmenci et al., 2018) which were discovered after Ietswaart's morphological classification. Interestingly, *O. symes* is found in The Plant List (27.03.2022), IPGRI Proceeding (Padulosi, 1997), and Kew website (27.03.2022) but not in NCBI taxonomy browser (WCSP, 2022). Currently, the genus *Origanum* (Linnaeus, 1753) is represented by 50 taxa in the NCBI database including unclassified species (Table 1.4) (Source: Url3) and there are also 20 hybrids according to Arabacı et al. (2021).

In the taxonomical classification of the genus *Origanum*, there are still debates when a certain database is used as a resource due to the slowness of updates. All recent studies consider the taxonomical classification of *Origanum* species based on Ietswaart's (1980) morphological classification; however, the knowledge about the current number of species, remaining uncharacterized species, and taxonomical relationships among all oreganos is slow to be updated. Due to these factors, the taxonomical classification of the genus *Origanum* remains incomplete. For a better and more precise determination among oregano species, recent findings must be included equally in databases as they become published. Such updates must be clarified for better conservation of the oregano species in the genus and breeding applications.

Table 1.4. *Origanum* L. species names reported in NCBI database (08.11.2021).

NCBI list					
1	*Origanum acutidens*	26	*Origanum rotundifolium*		
2	*Origanum amanum*	27	*Origanum saccatum*		
3	*Origanum ayliniae*	28	*Origanum scabrum*		
4	*Origanum bargyli*	29	*Origanum sipyleum*		
5	*Origanum bilgeri*	30	*Origanum solymicum*		
6	*Origanum boissieri*	31	*Origanum syriacum*	*Origanum syriacum* subsp. *sinaicum*	
7	*Origanum calcaratum*	32		*Origanum syriacum* var. *bevanii*	
8	*Origanum compactum*	33		*Origanum syriacum* var. *sinaicum*	
9	*Origanum cordifolium*	34		*Origanum syriacum* x *Origanum laevigatum*	
10	*Origanum dayi*	35	*Origanum vogelii*		
11	*Origanum dictamnus*	36	*Origanum vulgare* (oregano)	*Origanum vulgare* subsp. *gracile*	
12	*Origanum elongatum*	37		*Origanum vulgare* subsp. *hirtum*	
13	*Origanum grosii*	38		*Origanum vulgare* subsp. *virens*	
14	*Origanum haussknechtii*	39		*Origanum vulgare* subsp. *viride*	
15	*Origanum husnucan-baseri*	40		*Origanum vulgare* subsp. *viridulum*	
16	*Origanum hypericifolium*	41		*Origanum vulgare* subsp. *vulgare*	
17	*Origanum laevigatum*	42	*Origanum* x *adae*		
18	*Origanum leptocladum*	43	*Origanum* x *adanense*		
19	*Origanum majorana*	44	*Origanum* x *malyerii*		
20	*Origanum micranthum (O. vogelii)*	45	*Origanum* x *sevcaniae*		

(Cont. on the next page)

Table 1.4. (cont.)

21	*Origanum microphyllum*		46	unclassified *Origanum*	*Origanum* sp. MIBzpl(1)
22	*Origanum minituflorum*		47		*Origanum* sp. MIBzpl(2)
23	*Origanum munzurense*		48		*Origanum* sp. PP-2014
24	*Origanum onites*	*Origanum onites* x *Origanum syriacum* var. *bevanii*	49		*Origanum* sp. TO-2016a
25	*Origanum ramonense*		50		*Origanum* sp. TO-2016b

1.3.1.8. Sections

The morphological taxonomic revision of the genus *Origanum* recommended by Ietswaart (1980) has been accepted for decades as most appropriate classification. In this revision, the most important driving force for speciation in oregano is assumed to be hybridization which is found to be mostly related to sexuality of flowers and male sterility of the plant itself (seen in *O. vulgare*). The facts that most oregano flowers are bisexual and some of them behave like they only have female organs means that genetic variations occur frequently in *Origanum* species due to hybridization. The complexity in the genus is also caused by ongoing natural cross-hybridization with species in closely related genera such as *Thymus*, *Satureja*, *Thymbra* and *Micromeria* (Jedzejczyk, 2018; Lukas and Novak, 2020).

There are ten main sections within the genus *Origanum* as follows*: Amaracus, Anatolicon, Brevifilamentum, Longitubus, Chilocalyx, Campanumaticalyx, Elongatispica, Majorana, Origanum,* and *Prolaticorolla*. Sections have been subdivided into ten, with three subkeys (A: *Amaracus, Anatolicon, Brevifilamentum, Longitubus*; B: *Chilocalyx, Majorana*; C: *Campanumaticalyx, Elongatispica, Origanum, Prolaticorolla*) due to their hybridization behaviors and evolutionary history in Ietswaart's classification (1980) (Table 1.5).

Table 1.5. Representation of three main clusters (sub keys) among ten sections in the genus, *Origanum* L. (Source: Sharifi-Rad et al., 2020). *Ni:* Number of individual species per section; *Nsub:* Number of subspecies; ∑*Ni*: Total number of individual species per section; and ∑*Nsub*: Total number of varieties of species.

SECTIONS	***Ni***	***Nsub***	**SPECIES**	**Phenotypic Clusters called as "Sub key"**
I. Section ***Amaracus***	8	-	*O. ayliniae; O. boissieri; O. calcaratum; O. cordifolium; O. dictamnus; O. saccatum; O. solymicum*	**Sub key A (*Amaracus, Anatolicon, Brevifilamentum*)** a) Large calyces, two or one lipped, 4 to 12 mm long, b) Bracts rather large, 4 to 25 mm long, c) membranous, usually purple, rarely yellowish green, d) Slight glabrous
II. Section ***Anatolicon***	8	-	*O. akhdarense; O. cyrenaicum; O. hypericifolium; O. libanoticum; O. scabrum; O. sipyleum; O. vetteri; O. pampaninii*	
III. Section ***Brevifilamentum***	7	-	*O. acutidens; O. bargvli; O. brevidens; O. haussknechtii; O. leptocladum; O. rotundifolium; O. husnucan-baseri*	
IV. Section ***Longitubus***	1	-	*O. amanum*	
V. Section ***Chilocalyx***	5	-	*O. bilgeri; O. microphyllum; O. minutiflorum; O. vogelii*	**Sub key B (*Chilocalyx, Majorana*)** a) Small calyces, two or one lipped, 1.3 to 3.5 mm long, b) Bracts small, 1 to 5 mm long, c)leaf-like in texture and color, more or less hairy
VII. Section ***Campanulaticalyx***	6	-	*O. dayi; O. isthmieum; O. ramonense; O. petraeum; O. punonense; O. jordanicum*	**Sub key C (*Campanulaticalyx, Elongatispica, Origanum, Prolaticorolla*)** a) Calyces with five (sub)equal teeth, two or one lipped
VIII. Section ***Elongatispica***	3	-	*O. elongatum; O. floribundum*	
IX. Section ***Origanum***	6	1	*O. vulgare* ssp. *vulgare*; *O. vulgare* ssp. *glandulosum*; *O. vulgare* ssp. *gracile*; *O. vulgare* ssp. *hirtum*; *O. vulgare* ssp. *viride/viridulum; O. vulgare* ssp. *virens*	
X. Section ***Prolaticorolla***	3		*O. compactum; O. ehrenbergii; O. laevigatum*	

∑*Ni*:50; ∑*Nsub*: 5

1.3.1.8.1. *Origanum* species

Origanum species are woody, perennial subshrubs and grow on various types of soils, rocky areas, or under pine trees with a white and purple flower. There are several unique morphological features such as stem, leaf, and stoma and distinct growing environment requirements in the genus affecting the genotype of the next generation, endemism, and speciation. They also have hybrid speciation. Some of these hybrid species have already been reported in the literature. Within the genus *Origanum*, sections *Amaracus*, *Majorana*, and *Origanum* are possible ancestral lineages (Ietswaart, 1980; Martin et al., 2020). According to Ietswaart's hypothesis, some morphological characters such as branched stems in *Thymus* were common in species under section *Anatolicon* of *Origanum*; however, other morphological features such as calyx teeth mean that species under that section cannot be classified as *Thymus* (Ietswaart, 1980). Most of the species are hypothesized to have been generated via intergeneric hybridization of *Origanum* species with individuals from *Satureja* (Ietswaart, 1980). In the genus *Origanum*, intrageneric hybridizations are also very common and the gynodioecious feature of the genus causes generation of new hybrids or loss of endemic species according to the selective advantage of allele frequencies for a given phenotypic trait.

Origanum species for which information was available are described in the following sections.

1.3.1.8.1.1. Section I: *Amaracus* (Gleditsch) Bentham, 1848 (*AMA*)

Flowers are usually large and bisexual in section *Amaracus* (Ietswaart, 1980). Among species, *O. x ayliniae*, *O. boissieri*, *O. saccatum*, *O. dictamnus* and *O. solymicum* are reported to be occur naturally in Türkiye.

1.3.1.8.1.1.1. *O. boissieri* Ietswaart, 1859 (OBO)

O. boissieri is found in southern Türkiye, and it grows at elevations of 500 to 2000 mt on rocks and in shady places. Its flowering season is from July to August. OBO is similar to *O. dictamnus,* but its calyces are different (Ietswaart, 1980).

1.3.1.8.1.1.2. *O. calcaratum* Jussieu, 1789 (OCA)

O. calcaratum is endemic to Amanos mountain but found in other places such as Kriti. This species grows at sea level up to 700 mt. However, the calcareous structure of the soil makes it inaccessible to reach the plant. It has flowering stage from April to October. Hybridization between OCA and *O. dictamnus* is reported (Ietswaart, 1980).

1.3.1.8.1.1.3. *O. cordifolium* Montbret et Aucher ex Bentham (Vogel) 1841 (OCO)

O. cordifolium is found in Cyprus and also in Syria. It grows from 500 to 1500 mt in rocky places and in shade under pine trees. OCO has some features not found in section *Amaracus*. OCO is present in the absence of OSA and OSO species in some areas (Ietswaart, 1980).

1.3.1.8.1.1.4. *O. dictamnus* Linnaeus, 1753 (ODI)

O. dictamnus is found in many places in Kriti, Greece and western Türkiye. It grows on calcareous rocks, from 300 to 1500 mt. Its flowering happens from June to October. ODI is the parent species for some hybrids such as *O. x hybridium* which is the probable parent of *O. sipyleum* (OSI) (Ietswaart, 1980).

1.3.1.8.1.1.5. *O. saccatum* Davis, 1949 (OSA)

O. saccatum grows in the Mediterranean area of Türkiye, especially in Isparta and Antalya. It grows in rocky, calcareous slopes and under pine trees at 1000 mt. Its flowering time is from July to August. OSA is related to OSO and OSI (Ietswaart, 1980).

1.3.1.8.1.1.6. *O. solymicum* Davis, 1949 (OSO)

O. solymicum grows in calcareous rocks and in the shade of *Pinus britia* trees. OSO perceptibly differs from OSA and OCO species in the same section (Ietswaart, 1980).

1.3.1.8.1.2. Section II: *Anatolicon* Bentham, 1848 (*ANA*)

Flowers are usually bisexual and medium sized in the section *Anatolicon* (Ietswaart, 1980). In section *Anatolicon*, species *O. hypericifolium* and *O. sipyleum* grow naturally in Türkiye.

1.3.1.8.1.2.1. *O. akhdarense* Ietswaart et Boulos, 1975 (OAK)

O. akhdarense grows in Libya and has bisexual flowers and grows in shady rock faces together with other endemic plant species. The flowering stage for OAK is September to October (Ietswaart, 1980).

1.3.1.8.1.2.2. *O. cyrenaicum* Beguinot et Vaccari, 1913 (OCY)

O. cyrenaicum is a bisexual endemic species which grows mostly on calcareous rocks in Libya. Generally, it is found together with other endemic plant species. Recently, it had been reported that it grows also in the Green Mountains in Vermont, USA (Elabbar et al., 2014).

1.3.1.8.1.2.3. *O. hypericifolium* Schwarz et Davis, 1949 (OHY)

O. hypericifolium has bisexual flowers and it grows in Türkiye at elevations of 1500 to 2000 mt. It mostly grows in rocky areas and black pine forests, flowering between July to August. It is known to be close ditributed with *O. sipyleum* (*Anatolicon*) in nature (Ietswaart, 1980).

1.3.1.8.1.2.4. *O. libanoticum* Boissier, 1844 (OLI)

O. libanoticum has bisexual flowers and grows close to dry areas, flowering from June to October at 700 to 2000 mt from sea level. It is endemic to mountains in Lebanon. This species is known to have natural hybridization with *O. syriacum* (Ietswaart, 1980).

1.3.1.8.1.2.5. *O. scabrum* Boissier et Heldreich, 1846 (OSC)

O. scabrum is known to have both bisexual flowers and only female flowers (male-sterile) and it grows endemically at around 1000 to 1800 mt in limestone soils and next to boulders in Greece. It is known to have natural hybrids with *O. vulgare* subsp. *hirtum*, and the hybrid is known as *O. lirium* (Ietswaart, 1980).

1.3.1.8.1.2.6. *O. sipyleum* Linnaeus, 1753 (OSI)

O. sipyleum has bisexual flowers and grows in a wide range of lands from the Black Sea Region to the Mediterranean Region in Western Türkiye. It prefers to grow at 100 to 1500 mt elevated from sea level, under pine trees in calcareous rocky areas, flowering from June to August. *O. sipyleum* naturally generates hybrids with *O. onites* and *O. vulgare* subsp. *hirtum* (Ietswaart, 1980).

1.3.1.8.1.2.7. *O. vetteri* Briquet et Barbey, 1895 (OVE)

O. vetteri has bisexual flowers and it is endemic to the Greek island, Karputhos. It grows close to calcareous rocks, flowering in a short period of time from June to July at 1100 mt above sea level. The overall characteristics are more likely similar to the genus *Thyme* (Ietswaart, 1980).

1.3.1.8.1.3. Section III: *Brevifilamentum* Ietswaart, 1980 (*BRE*)

There are several flower characteristics such as being bisexual, subsessile, verticillaster, and large in section *Brevifilamentum* (Ietswaart, 1980). In Türkiye, all of the species belonging to section *Brevifilamentum* naturally grow in certain geographic locations.

1.3.1.8.1.3.1. *O. acutidens* Handel – Mazzetti, Ietswaart, 1913 (OAC)

O. acutidens has verticillaster flowers. It grows on limestone, sunny slopes (Karagöz et al., 2022) and non-calcareous areas in Northeastern Türkiye at 1000 to 3000 mt elevation and flowers from June to August. Although it is known to be similar to *O. rotundifolium* and *O. haussnechtii* from the same section *Brevifilamentum*, they are not likely to grow in close proximity (Ietswaart, 1980). OAC has fewer stomata than OBI, OMI, OVO and OVVU (Taş, 2010).

1.3.1.8.1.3.2. *O. bargvli* Mouterde, 1973 (OBA)

O. bargyli is a rare species known as "Yarpuzmercanı" in Turkish and specifically grows in Adana province in areas rich in pine and also in Syria. It is similar to *O. amanum* in its appearance. It is also known to be closely related with *O. brevidens* and both have the same corolla structure as *O. leptocladum*. OBA generates hybrids with OSY in nature (Ietswaart, 1980).

1.3.1.8.1.3.3. *O. brevidens* Bornmüller, Dinsmore, 1933 (OBR)

O. brevidens has verticillaster and subsessile flowers. It was previously reported as "hasn't been recorded since 1933" after it was found in Amanum Mountain in Türkiye (Ietswaart, 1980). Luckily, ISTE and TD herbarium collections include specimens reported as *O. brevidens* that were collected in 2014 from Osmaniye near the Amanos Mountains (Ecevit-Genç et al., 2020; Martin et al., 2020).

1.3.1.8.1.3.4. *O. haussknechtii* Boissier, 1879 (OHA)

O. haussknechtii has verticillaster flowers and grows at mountains from 1000 to 1650 mt elevation and flowers from June to September in Eastern Türkiye. Although they are in the same section, OHA differs from OAC and ORO (Ietswaart, 1980).

1.3.1.8.1.3.5. *O. leptocladum* Boissier, 1879 (OLE)

O. leptocladum has verticillaster and subsessile flowers and grows on chalky soils. It grows in Southern Türkiye, flowering in the two-month period from July to August at 1500 mt. OLE from section *Brevifilamentum* is a phenotypic reminiscent of *O. laevigatum* from the section *Prolaticorolla* (Ietswaart, 1980). OLE from the section *Brevifilamentum* and OBI from section *Chilocalyx* have the most dense secondary metabolite crystals in their secretory glands when compared to other species that naturally grow in Türkiye (Taş, 2010).

1.3.1.8.1.3.6. *O. rotundifolium* Boissier, 1859 (ORO)

O. rotundifolium has verticillar flowers and it grows at 400 to 1500 mt above sea in Northeastern Türkiye, flowering from June to September. ORO is known as being related to OAC (Ietswaart, 1980).

1.3.1.8.1.4. Section IV: *Longitubus* Ietswaart, 1980 (*LONGI*)

Flowers are bisexual, very large and long corollas in section *Longitubus* (Ietswaart, 1980). Only one species belongs to section *Longitubus*, *O. amanum*.

1.3.1.8.1.4.1. *O. amanum* Post, 1985 (OAM)

O. amanum has an isolated distribution area located on Amanos Mountains at 1500 to 2000 mt. It grows on calcareous soils, flowering over a relatively long season from June to September. Even though OAM is reminiscent of OBA and OBR, it differs from these species with its unique corolla structure. Therefore, it was classified into a separate section. It has natural hybrids with another endemic species to Amanos Mountains, OLA (Ietswaart, 1980).

1.3.1.8.1.5. Section V: *Chilocalyx* (Briq.) Ietswaart (*CHI*)

Flowers are both bisexual or female (male-sterile) and very small in section *Chilocalyx* (Ietswaart, 1980). In this section, species *O. bilgeri*, *O. micranthum*, and *O. minituflorum* grow in Türkiye.

1.3.1.8.1.5.1. *O. bilgeri* Davis, 1949 (OBI)

O. bilgeri has both bisexual and female flowers. This endemic species grows close to Cedrus forests at Geyik Mountain in Antalya (Taş, 2010). OBI is known to be close to OMI. OBI also share a common number of stomata with OVO, OMI, and OVVU.

1.3.1.8.1.5.2. *O. micranthum* Vogel, 1841 (OMICRA)

O. micranthum is also being called *O. vogelii* (OVO) and *O. vulgare* (OVH) creating misclassification (The Plant List, 01.12.2022). It has bisexual and female only flowers. It grows in the Taurus Mountains of Türkiye. It grows at 1500 mt elevation flowering from July to September. OMICRA is mostly found having hybrids with OVH in nature. OBI and OMI have been accepted to be related to OMICRA (Ietswaart,1980).

1.3.1.8.1.5.3. *O. microphyllum* (OMICRO)

O. microphyllum has bisexual flowers and grows in rocky environments at 500 to 1700 mt flowering from June to September. This species does not have a similar appearance as the other three species from the same section (Ietswaart, 1980).

1.3.1.8.1.5.4. *O. minutiflorum* (OMI)

O. minutiflorum has individuals with bisexual and female only flowers. It is found in Southern regions in Türkiye and it grows on rocky areas at 1600 mt elevation with flowers from June to August. It is known to be closely related to OBI (Ietswaart, 1980).

1.3.1.8.1.6. Section VI: *Majorana* (Miler) Benth. (*MAJ*)

The flowers are both bisexual and female in section *Majorana*. Within section *Majorana*, species *O. dubium* (ODU), *O. onites* (OON), and *O. syriacum* subsp. *bevanii* (OSYBE) naturally grow in Türkiye.

The genetic relationships of species belong to section *Majorana* were revised by Lukas et al. (2013) (Figure 1.15). According to ITS sequence analyses from specimens including clones from native lands Italy, Greece, Türkiye, Cyprus, Syria and from greenhouse plants from Austria, *O. onites* was proposed as hybridizing with *O. dubium* which itself has been reported as a hybrid of *O. syriacum*, *O. onites*, and one other unknown species (Lukas et al., 2013). In addition, OON specimens collected from Greece and Sicily were defined as "pure" with no significant gene flow (F_{ST}) among other taxa due to their geographical isolation from other members of section *MAJ* (Lukas et al., 2013). The Turkish OON population had higher gene flow between Sicilian oreganos than Greek taxa. Turkish OON species having an admixed genetic structure was attributed to the gene flow from ODU taxa (Lukas et al., 2013).

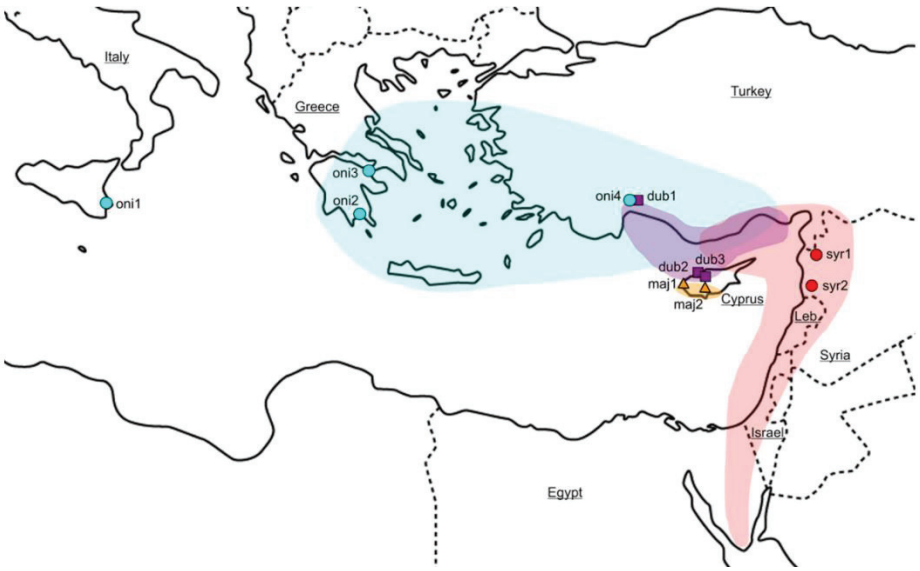


Figure 1.15. Collection locations of *O. onites*, *O. dubium*, *O. majorana*, and *O. syriacum* species from taxonomically revised section *Majorana* in the study of Lukas et al. (2013) .(Source: Lukas et al., 2013)

1.3.1.8.1.6.1. *O. majorana* (OMA)

O. majorana called as "marjoram or sweet marjoram" has bisexual and female only flowers (Lukas and Novak, 2020). It is cultivated in several districts including the Mediterranean region and the Asian, American, and European continents. It is known to grow naturally in Cyprus (Lukas et al., 2013). It grows on rocky soils at 100 to 1500 mt flowering from May to September. OMA is closely related to OSY but differs in some morphological characters such as leaves. There are artificial hybrids of OMA with OVVIR and OVVU (Ietswaart, 1980).

1.3.1.8.1.6.2. *O. dubium* (ODU)

O. dubium is a chemotype of OMA with cymyl content (linalool type) while OMA has sabinyl essential oil (Lukas et al., 2013). In the study of Lukas et al. (2013), an ODU-specific gene, 1-deoxy-D-xylulose 5-phosphate synthase (DXS), was determined in Turkish OON taxa recommending appearance of a recent hybridization from ODU towards OON in Türkiye and the gene flow was observed from ODU (dub1) to OON (oni4) (Lukas et al., 2013). No molecular data reflected gene flow from OON towards ODU.

1.3.1.8.1.6.3. *O. onites* (OON)

O. onites is the species that is accepted as the most phenotypically distinguishable species in the genus (Lukas et al., 2013). It has a large geographical distribution over the world but especially grows in Greece, the Greek Islands, and Türkiye. According to Ietswaart (1980), it has an isolated population on Sicily in Mediterranean Sea. It grows in limestone and rocky mountains up to 1400 mt from sea level and flowers from April to August. It has natural hybrids with OSI and OVH which led to the generation of hybrids *O. x intermedium* and *O. x intercedens* (Arabacı et al., 2021). Moreover, OON is one of the earliest ancestors in the section *MAJ* (Lukas et al., 2013) and it has an admixed genetic structure in Türkiye which might be due to hybridization between ODU taxa or geographical location (Lukas et al., 2013).

1.3.1.8.1.6.4. *O. syriacum* (OSY)

O. syriacum grows in limestone and rocky places up to 2000 m from sea level and flowers over a relatively long term from May to October in Mediterranean countries including Türkiye. It is known to have hybrids with *O. libonaticum* (section *Anatolicon*), *O. bargyli* (*Brevifilamentum*), *O. ehrenbergii* (*Prolaticorolla*), and *O. prolaticorolla* (*Prolaticorolla*) (Ietswaart, 1980). For example, *O. leptocladum* from the section *Brevifilamentum* was suggested to be a hybrid between *O. syriacum* (*Majorana*) and *O. bargyli* (*Brevifilamentum*). *O. syriacum* has three variants: *O. syriacum* var. *syriacum* (OSYSY) (Israil, Urdun, and Syria), *O. syriacum* var. *bevanii* (OSYBE) (Türkiye, Cyprus, Syria, and Lebanon), and *O. syriacum* var. *sinaicum* (OSYSI) (Sina peninsula). Türkiye only has OSYBE species.

1.3.1.8.1.7. Section VII: *Campanulaticalyx* (*CAMPA*)

Flowers are usually bisexual, very small or medium sized in section Campanulaticalyx. There is no species belonging to section *CAMPA* that naturally grows in Türkiye (Ietswaart, 1980).

1.3.1.8.1.7.1. *O. dayi* (ODA)

O. dayi has bisexual and female only flowers and has pillosellous staminal filaments. It grows on hard limestone and deserts up to 800 mt flowering from July to August.

1.3.1.8.1.7.2. *O. isthmieum* (OIS)

O. isthmieum has bisexual flowers and it is endemic. It grows on hard limestones on the Sinai Peninsula up to 500 m with flowers in June.

1.3.1.8.1.7.3. *O. ramonense* (ORA)

O. ramonense has bisexual flowers and has pillosellous staminal filaments. It grows on hard limestone in Israel at 800 to 1000 mt and flowers in September.

1.3.1.8.1.8. Section VIII: *Elongatispica* (*ELON*)

The section *Elongatispica* has very small bisexual and female only flowers.

1.3.1.8.1.8.1. *O. elongatum* (OEL)

O. elongatum grows in Morocco between 400 to 1500 mt and flowers from June to October. It has a close relationship with species *O. floribundum* and *O. grosii*; however, they have some different morphological features like stem and leaf characteristics (Ietswaart, 1980).

1.3.1.8.1.8.2. *O. floribundum* (OFL)

O. floribundum grows in Algerian mountains at 300 to 1600 mt and flowers from July to November (Ietswaart, 1980).

1.3.1.8.1.8.3. *O. grosii* (OGR)

O. grosii grows in Morocco at 650 to 1000 mt and flowers from June to July (Ietswaart, 1980).

1.3.1.8.1.9. Section IX: *Origanum* (*ORG*)

O. vulgare ssp. have bisexual or female only flowers and are one of the most traded and widely distributed oregano species in most of Europe, Asia and North Africa (Ietswaart, 1980; Lukas and Novak, 2020). Ietswaart (1980) classified *O. vulgare* species into six groups according to variation in morphological characters such as indumentum,

sessile glands, bract and flower color and size (Figure 1.16) (Ietswaart, 1980; Lukas and Novak, 2020).

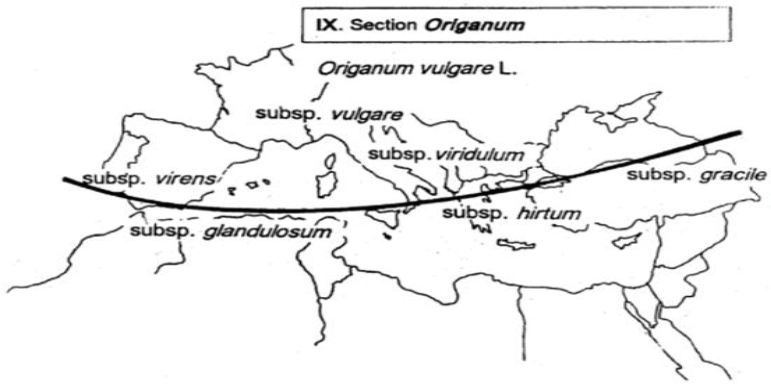


Figure 1.16. Distribution of individuals of the section *Origanum* throughout the world (Source: Ietswaart, 1980).

In a recent study by Soltani et al. (2021), the distribution of *O. vulgare* ssp. was divided into two groups according to antimicrobial essential oil contents as rich and poor regions. A map was constructed to display the geographical distribution of the groups of *O. vulgare* ssp. According to this map, *O. vulgare* subsp. *hirtum*, *O. vulgare* subsp. *glandulosum*, and *O. vulgare* subsp. *gracile* grow in Mediterranean regions and have poor monoterpenoid volatile content while *O. vulgare* subsp. *vulgare*, *O. vulgare* subsp. *virens*, and *O. vulgare* subsp. *viride* grow in Southern Europe regions and have rich monoterpenoids such as thymol and carvacrol (Figure 1.17) (Lotti et al., 2019; Soltani et al., 2021).

Figure 1.17. Current distribution of *O. vulgare* ssp. in the world: Regions above the black line are the essential oil rich Southern origin while those below the black line are the Mediterranean region having poor essential oil (Source: Soltani et al. 2021).

1.3.1.8.1.9.1. *O. vulgare* ssp. *vulgare* (OVVU)

O. vulgare subsp. *vulgare* grows on calcareous non-limyl soils from sea level up to 1400 mt and flowers from May to October in a wide range of areas including Europe and Asia (Ietswaart, 1980). It has been reported that OVVU naturally hybridizes with OMA and has artificial hybrids with ODU from section *Majorana.* It also hybridizes with OSI from *Anatolicon* and OVH from section *Origanum* (Ietswaart, 1980).

1.3.1.8.1.9.2. *O. vulgare* ssp. *glandulosum* (OVGL/or OVGLAN)

O. vulgare subsp. *glandulosum* grows in Algeria and Tunisia on rocky areas up to 1200 mt and flowers from May to August. It is closely related to *O. vulgare* subsp. *hirtum* (Ietswaart, 1980).

1.3.1.8.1.9.3. *O. vulgare* ssp. *gracile* (OVG)

O. vulgare subsp. *gracile* grows in Eastern Türkiye and Arabic countries such as Iraq, Iran, and Afghanistan. It grows on calcareous and stone rich places which can be non-limyl, moistured, or dried soils and flowers bloom from June to September (Ietswaart, 1980).

1.3.1.8.1.9.4. *O. vulgare* ssp. *hirtum* (OVH)

O. vulgare subsp. *hirtum* grows on calcareous, non-limyl, grassy abandoned hills and it loves both shade and sunny places up to 1500 mt flowering from May to December. It grows on the Balkan Penninsula of Türkiye. OVH is the most investigated species in terms of its antimicrobial benefits. However, there are still concerns about the correct usage of volatile oils from *O. vulgare* in medicine to avoid toxicity despite the fact that thymol and carvacrol have been approved for human usage by the US Food and Drug Administration (FDA) (Soltani et al., 2021). In nature, it has been reported that this subspecies has natural hybrids with OMIC, OON, OSC, and OSI (Ietswaart, 1980).

1.3.1.8.1.9.5. *O. vulgare* ssp. *virens* (OVVIR)

O. vulgare subsp. *virens* grows in the Canary and Baleric Islands, and North Africa. It grows on calcareous non-limyl, partially shaded soils at 100 to 200 mt above sea level and flowers from May to August. In France, there is a hybrid zone for OVVIR, OVVI, and OMA (*MAJ*) (Ietswaart, 1980).

1.3.1.8.1.9.6. *O. vulgare* ssp. *viride/viridulum* (OVVI)

O. vulgare subsp. *viride* grows on dry/fully sunny places up to 300 mt and flowers from May to October in a wide geographic area including China. It is mostly similar to OVVU and OVVIR (Ietswaart, 1980).

1.3.1.8.1.10.Section X: *Prolaticorolla* (*PRO*)

In section *Prolaticorolla*, flowers are large and bisexual or female only.

1.3.1.8.1.10.1. *O. compactum* (OCO)

O. compactum grows on dry hills up to 700 mt flowering from June to August in Morocco (Ietswaart, 1980).

1.3.1.8.1.10.2. *O. ehrenbergii* (OEH)

O. ehrenbergii grows under pine trees from sea level up to 1500 mt and flowers over a short time period, June to July. It has natural hybrids with OSY (Ietswaart, 1980).

1.3.1.8.1.10.3. *O. laevigatum* (OLA)

O. laevigatum grows locally inside maqui forests and open woods up to 300 to 2000 mt in Amanos Mountains of Türkiye. It has similar morphological characteristics to OLE and some other species. It can hybridize with OAM (*LONGI*) and OSY (*MAJ*) in nature (Ietswaart, 1980).

1.3.1.9. *Origanum* in Türkiye

In Türkiye, the genus *Origanum* is represented by 21 species including 24 taxa of which 13 are endemic and five are in endangered status (Kuşaksız, 2019). There are also 13 named hybrids with 12 of them endemic (Arabacı et al., 2021) in Türkiye.

1.3.1.10. Trade, production and cultivation

The fact that there are various chemotypes of oregano species increases their economic importance. According to Tuik reports, Türkiye has become one of the leading countries in oregano trade and production (Source: Url6). The production rate of oregano in 2020 increased greatly when compared to 2019 (Figure 1.18; Boztas et al., 2021).

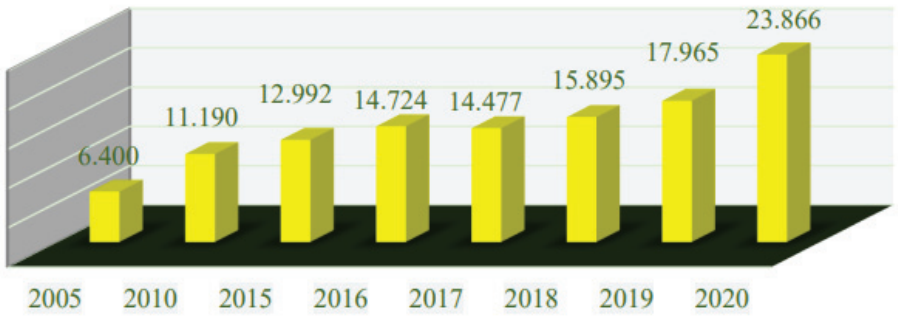


Figure 1.18. Production amount of oregano in Türkiye according to Tuik (Source: Boztas et al., 2021)

The external trade of oregano over the world is currently 17 thousand tonnes and Türkiye accounted for 88% of the trade capacity by 2018 (Source: Url6). Türkiye has recently made remarkable profit with oregano exports by increasing the income from 10 million dollars to 52 million dollars in a decade (2008 – 2018) (Source: Url6; Sokat, 2021). The traded oregano species are mostly from genus *Origanum* and the most traded species is known to be *O. onites* (Sarı et al., 2002; Sokat, 2021).

1.3.1.11. Endemism and threatened status of oregano species

Endemism is the appearance of a species in a restricted land area at a given time under certain ecological and climatic conditions. The endemism of a plant species can be due to geographical fragmentation in a province. For example, in Egypt, there are 11 endemic oregano taxa such as the infraspecific taxon *O. syriacum* subsp. *siniacum* (Endangered Status) which is thought to be in "extinction" category due to deficient data in herbarium banks.

In Türkiye, there are around 12,000 taxa of plant species and 1/3 of them are endemic species (Şenkul and Kaya, 2017) (Figure 1.19). There are at least 12 endemic plant species in Türkiye that have become extinct in recent decades (Ekim et al., 2000; Karaer et al., 2022).

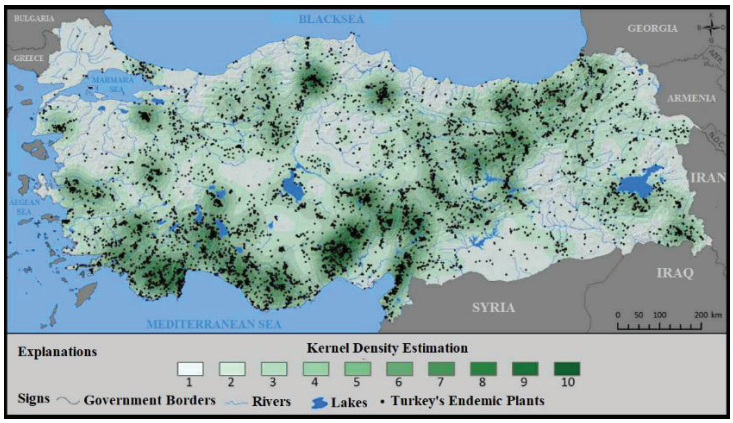


Figure 1.19. Locations of Türkiye's endemic plant species (Source: Şenkul and Kaya, 2017).

Türkiye's endemic plant flora predominantly consists of species from the families Asteraceae, Lamiaceae, and Fabaceae with the following number of locations: 1497 (15.5% of all species), 1069 (11%), and 971 (10%) within a total of 9677 locations in Türkiye (Şenkul and Kaya, 2017). Specifically, there are 2557 (26.4%), 1177 (12.2%) and 5943 (61.4%) endemic locations in the Mediterranean, European-Siberia, and Irano-Turanian phytogeographic regions in Türkiye, respectively (Şenkul and Kaya, 2017). In the latest reports, there are 79, 63, 5 and 11 rare and endemic Lamiaceae species distributed in the Mediterranean, Irano-Turanian, Euro-Siberian and unknown/multiregional phytogeographic regions in Türkiye, respectively (Kuşaksız, 2019).

The endemic species of oregano are categorized under the IUCN red list of threatened species (IUCN, 2022). Currently, the most threatened species of oregano is taxon *O. boissieri* from section *Amaracus* which under is critically endangered (CR) category in Türkiye (Gürbüz et al., 2010; Kuşaksız, 2019). The oregano species that are classified under endangered (EN) category are *O. munzurense* (OMU; hybrid of *O. acutidens* x *O. vulgare* subsp. *gracile*) which was recently removed from section *Brevifilamentum* (Dirmenci et al., 2019a) and *O. solymicum* from section *Amaracus*. The taxa *O. bargyli* from section *Brevifilamentum*, *O. laevigatum* from section *Prolaticorolla*, and *O. micranthum* from section *Chilocalyx* are classified as vulnerable (VU). Within the least risk category, *O. amanum* from section *Longitubus*, *O. bilgeri* from section *Chilocalyx*, *O. saccatum* from section *Amaracus*, *O. sipyleum* from section *Anatolicon*, in addition to *O. haussknechtii* and *O. leptocladum* from section *Brevifilamentum*, are considered to be conservation dependent. Also, in this category, there are *O. minutiflorum* from section *Chilocalyx* which is nearly threatened. *O. acutidens* from section *Brevifilamentum*, *O. saccatum* from section *Amaracus*, and *O. hypericifolium* from section *Anatolicon* belong to the least concerned category (Gürbüz et al., 2011).

1.3.1.12. Conservation strategy: Interdiciplinary characterization and cultivation of native oregano species

Türkiye is rich in endemic species in the genus *Origanum*. To date, there are 13 endemic oregano species out of 24 taxa and 12 endemic hybrid oreganos out of 13 natural hybrid species reported in Türkiye (Taşcıoğlu et al., 2018; Martin et al., 2020; Arabacı et al., 2021) including: *O. boissieri, O. saccatum, O. solymicum, O. hypericifolium, O. sipyleum, O. acutidens, O. haussknetchii, O. husnucan-baseri, O. leptocladum, O.x munzurense, O. x aytacii, O. amanum, O. bilgeri, O. vogeli,* and *O. laevigatum*.

There is an ongoing loss of diversity and increased demand for sustainable plant biodiversity due to threatening factors such as agricultural activities like burning stubble, industrialization, fires affecting natural forests, climate change, tourism activities natural habitats; and rapid increase in the human populations (Karagöz, 2001; Maxted et al., 2012). There are three main global plant genetic diversities to conserve: a) wild genetic

resources, b) genetic resources for food and agriculture, and c) plants for non-food utilization applications as given in Figure 1.20 (Maxted et al., 2021).

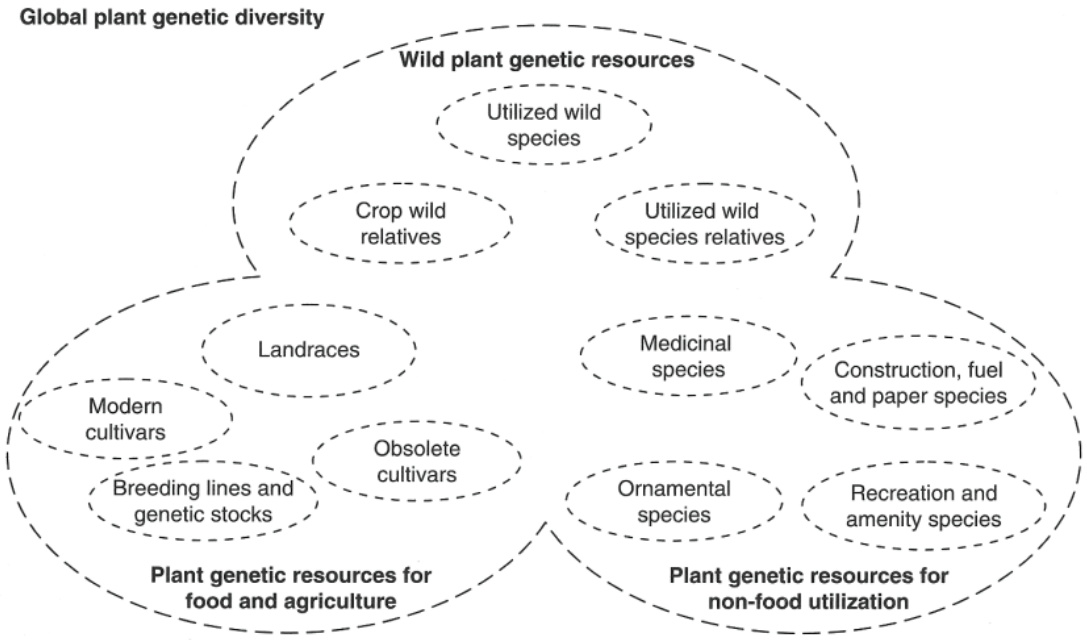


Figure 1.20. Scheme for grouping of global plant genetic diversity (Source: Maxted et al., 2012).

Although there are many recommended conservation methods including both *in situ* and *ex situ* applications such as natural protection sites, botanical gardens, or gene banks, there is an obstacle to conventional *in situ* conservation in practice (Shukrullo, 2020). There are ten main crop wild relative *in situ* conservation hot spots and eight of them are in Türkiye (Castañeda-Álvarez et al., 2016; Zair et al., 2021). Türkiye is a hot spot for plant genetic resources such as wheat, sugar beet, poppy, tea, and oregano by providing various types of pollinators and climates. Türkiye is situated at the intersection of the European-Siberia, Iranian-Turanian (Irano-Anatolicon) and Mediterranean bioclimatic regions which is placed between 26–45° and 36–42° longitude and latitude in the Northern hemisphere. This region hosts ten sub climates that make its geography special for the origin and speciation of wild relatives and the appearance of hybrid species for many plants (Yıldırım et al., 2021). These features ensure richness for endemic plant species since the bioclimate and geomorphology are highly variable and allow extraordinary species diversification (Parolly, 2004; Noroozi et al., 2019) (Figure 1.21).

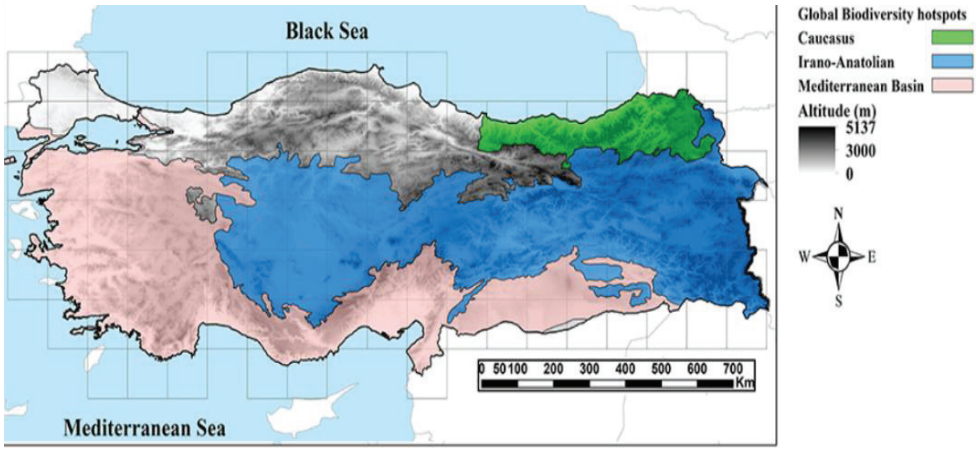


Figure 1.21. Topographic regions of Türkiye according to climatic hotspots: Caucasus, Irano-Anatolicon, and Mediterranean phytogeographic locations of Türkiye (Source: Noroozi et al., 2019).

Since many plant species originated from the "fertile crescent" which covers a large area of lands near Türkiye's eastern borders, there are several conservation centers for wild crops concentrated in the country. Thus, a map showing the overlapping models for distribution of crop wild relatives shows Türkiye as a hot spot (Figure 1.22) and every year 60 more plant species are added to the Flora of Türkiye (Özhatay et al., 2017; Akalin et al., 2020).

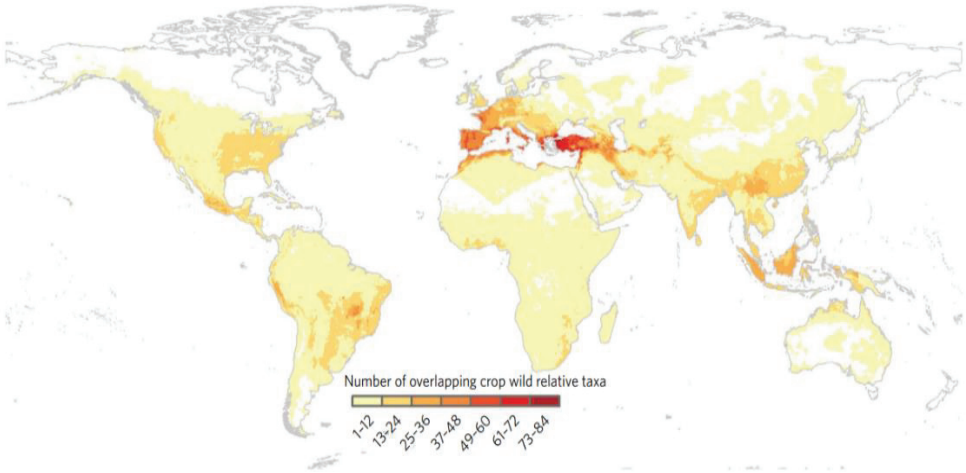


Figure 1.22. Distribution map of overlapping crop wild relatives throughout the world (Source: Castañeda-Álvarez et al., 2016). Transition from yellow to red implies that red represents the highest number of wild relatives in a geographic area, while yellow to white indicates fewer taxa.

Oregano species are mainly wild varieties that grow naturally and are mostly utilized directly from nature for culinary uses in addition to their usage in both medicinal and scientific research. The conservation of wild oregano species is important for sustainability and protection since most are endemic and grow locally in certain soil and climate conditions. According to Ietswaart (1980), oregano can include individuals from *Thymus*, *Satureja* and *Micromeria*. Thus, "kekik" is represented by 57 *Thymus*, 24 *Origanum*, 14 *Satureja*, 4 *Thymbra* and 1 *Coridothymu*s taxon in Türkiye (Bozdemir, 2019). The rich diversity of *Thymus* species in our country and Türkiye being a hot spot for *Origanum* L. supports Ietswaart's hypothesis about natural speciation and evolutionary relationships. His hypothesis claims that the hybridization between *Thymus* and *Origanum* might cause generation of natural hybrid species that have the potential to become new species. This information is also supported by the conserved number of chromosomes for *Thymus*, *Origanum*, and *Micromeria* from *Satureja* (2n=30) (Martin et al., 2020).

The sections *Amaracus*, *Majorana* and *Origanum* are the earliest ancestors of species in the genus *Origanum* (Martin et al., 2020). Moreover, the section *Anatolicon*, shares similarities between the sections *Amaracus* and *Origanum*. Observations about these types of knowledge will lead to better conservation of the oregano gene pool for cultivation and breeding. Knowledge will also be gained by screening native oregano populations and interdisciplinary studies of agromorphologic, biochemical (chemotypes), and molecular traits.

1.3.1.13. Literature on molecular studies in *Origanum*

The utilization of molecular markers generates data to classify, utilize and conserve multiple plant species in a faster and easier way than morphological traits. There are several molecular markers such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Internal Transcribed Spacer (ITS), Expressed Sequenced Tag – Simple Sequence Repeat (EST-SSR), Sequence Characterized Amplified Regions (SCAR), and Start Codon Targeted (SCoT), and Touch-up Direct Amplification of Microsatellite region DNA (TU-DAMD) markers that have been used in genetic diversity analyses for oregano species (Klocke et al., 2002; Ayanoğlu et al., 2006; Novak et al., 2008; Akeel et al., 2009;

Katsiotis, 2009; Marieschi et al., 2010; Amar and Wahab, 2013; Karaca et al., 2013; Lukas et al., 2013; Ince et al., 2014; Mutu et al., 2014; Zaghloul et al., 2014; Azizi et al., 2016; Aboukhalid et al., 2017; Jedrzejczyk, 2018; El-Demerdash et al., 2019; Taş, 2019; Arabacı et al., 2021; Karagöz et al., 2022; Antaloudaki et al., 2022; Saleh, 2022; Tan, 2022). Sequence Related Amplified Polymorphism (SRAP) markers were studied for the first time for *Origanum* species in this dissertation (Chapter 2).

In 2006, Ayanoğlu et al. (2006) screened Turkish 43 *O. onites* and one *O. vulgare* specimens that were conserved at Aegean Agriculture and Research Institute (AARI) with ten AFLP primer combinations and observed 81% polymorphic loci. Their clustering analysis resulted in two main clusters in UPGMA analysis. According to similarity values, Or12 (TR 54483) and Or42 (TR 54549) specimens were genotypically most distant (40%), while Or27 (TR 54514) and Or29 (TR 54523) accessions were most similar (72%). Their results indicated that there was no direct correlation between genetic diversity and collection area except for some accessions from Izmir, Muğla and Çanakkale provinces. Moreover, their study reflected a low genetic similarity observation between different *O. onites* specimens conserved in the gene bank. Since *O. onites* is growing widespread around Türkiye, they concluded that the genetic differentiation might be related to their adaptation to various environmental conditions at different locations or their mating behaviors (selfing or cross pollinating).

In the study of Van Looy and colleagues (2009), AFLP markers were used to evaluate the genetic dissimilarities in 21 taxa to identify the correlation between flooding events and genetic diversity (Van Looy et al., 2009). Genetic diversity within *O. syriacum* subsp. *sinaicum* (OSYSI) was investigated with AFLP markers and resulted in an average genetic polymorphism of 57% among accessions (Zaghloul et al., 2014). AFLP markers were also used to screen *O. vulgare* species by Azizi et al. (2016). As a result, 75% (214/285) allelic polymorphism was observed within their *O. vulgare* population. In 2019, El-Demerdash et al. (2019) investigated the genetic diversity among cultivated *O. vulgare* and wild *O. syriacum* specimens using four AFLPs. As a result, 53% of 193 loci were observed as polymorphic.

In the study of Akeel et al. (2009), genetic diversity between *O. syriacum* and *O. majorana* taxa was investigated with RAPD markers. As a result, among 1359 loci, only 10% were polymorphic which might indicate that 90% of alleles are conserved between those species from the same section, *Majorana*. RAPD markers were used to assess genetic diversity between *O. vulgare* subsp. *hirtum* taxa and individuals from seven other

Origanum taxa (Katsiotis et al., 2009). In the study of Mutu et al. (2014), seven native/wild *O. vulgare* subsp. *vulgare* specimens from a single location (Orheiul Vechi, Moldova) were screened via RAPD primers. A total of 257 RAPD fragments were obtained. As they indicated, high genetic variability was observed within population.

Marieschi et al. (2010) generated SCAR markers to investigate the genetic relatedness among ten Italian, American and German *Origanum* species. To our knowledge, this is the only study in *Origanum* that used this type of markers.

The first development of oregano specific molecular markers was conducted by Novak et al. (2008) and they developed 13 expressed sequence tag single sequence repeat (EST-SSR) markers for taxa *O. vulgare* and *O. majorana*. In later years, more EST-based SSR markers were developed by Karaca et al. (2013) for 12 genera of the Lamiaceae family collected from Antalya, Türkiye. In addition, 30 new SSR markers and cleaved amplified polymorphic sequence (CAPS) markers were developed for eight *Origanum* species (Antalya province, Türkiye) (Ince et al., 2014).

Aboukhalid et al. (2017) studied 670 Moroccan oregano accession collected from 59 different locations from a single taxon (*Origanum compactum*) and screened then with 15 SSRs to determine their genetic diversity. In the study of Jedrzejczyk (2018), ISSR markers were used to determine genetic diversity among four taxa: *O. heracleoticum*, *O.majorana, O. vulgare* subsp. *hirtum,* and *O. vulgare* subsp. *gracile*. Internal transcribed spacer (ITS) sequencing was also used to determine genetic relatedness between three parental species and their hybrids (Arabacı et al., 2021).

In a more recent study, Saleh (2022) characterized a *O. syriacum* (section *Majorana*) population composed of 37 accessions that were collected as wild specimens from three main regions of Syria (coastal, central, and southern) with TU-DAMD markers. Allelic diversity was 81% polymorphic with a total number of 188 bands. Average number of polymorphic alleles was 9.5 and PIC value was observed between 0.051 to 0.322 with an average of 0.225. *O. syriacum* accessions were divided into two main clusters in UPGMA analysis. They concluded that the observed genetic diversity can be related to the putative hybrid species and cross hybridizations (Ietswaart, 1980).

Molecular genetic variability can also be investigated with the SRAP marker system. SRAP markers target open reading frames with the primers initially having CCGG and AATT in the forward and reverse directions, respectively (Li and Quiros, 2001). These markers are advantageous due to their cross genera transferability and

highly polymorphic nature. SRAP markers have been used to screen genera from the Nepetoideae subfamily including those from genus *Origanum* .

Zagorcheva and colleagues (2020) investigated genetic diversity of ten Bulgarian lavender species (*Lavandula angustifolia* Mill.). Lavender is in the same subfamily (Nepetoideae) as oregano. The study also included five unknown samples and breeding lines and utilized 51 SRAP marker combinations. In this work, 4697 alleles with high (77%) polymorphic allele frequency and an average of 27% polymorphism information content (PIC) value were observed. Their two different populations had high within group diversity (97%) and had low variance between two populations (3%) according to AMOVA analysis.

In the study of Aghaei et al. (2017), 54 accessions from five *Salvia* species (*Salvia virgata* Jacq., *S. nemorosa* L., *S. officinalis* L., *S. cereal* L. and *S. sclarea* L.) were screened with 14 SRAP primer combinations. As a result, 265 loci with 96% polymorphism were observed. The average PIC value was 31% for their germplasm. There was high genetic differentiation (F_{ST}= 0.34) and a low number of migrants (*Nm*= 0.750). Their findings supported differentiation into five taxa at the species level.

Moein and colleagues (2019) investigated the relationship between ecology and genetic diversity among sage specimens. They screened 25 *Salvia aristata* specimens with 16 SRAP primer pairs. As a result, eleven SRAP markers were selected to be scored and generated a total number of 242 loci with 98% polymorphism. The minimum number of alleles was observed for me2-em1 (16) and the maximum number of alleles was observed for me3-em1 (27) marker pairs. The highest PIC value was 0.49 for the marker pair me4-em2. The best number of subpopulations for the species was determined as *K*=2. As a result of their genotypic model for ecological fragmentation of this endemic species, they concluded that these species are under dynamic speciation due to selection for divergent floral traits which resulted in reproductive isolation.

In a recent study, genetic diversity in the genus *Thymus* was investigated in 11 species with 77 accessions and eight SRAP combinations. As a result, the population was divided into five main subpopulations and three clusters. In addition, intra and inter-generic variation was observed with SRAP markers. The within population diversity was 63% in the *Thymus* population (Sarfaraz et al., 2021).

In the study of Amar and Wahab (2013), four *Origanum* taxa (*O. vulgare* subsp. *vulgare*, *O. vulgare* subsp. *hirtum*, *O. syriacum* subsp. *siniacum*, and *O. majorana*) were screened with a total number of 20 ISSRs and 36 SRAP marker combinations. Among

ISSRs, 16 were polymorphic, while 12 primer pairs were polymorphic for SRAP markers. In ISSR analysis, 177 polymorphic alleles were observed, while for SRAP analysis, a total number of 199 loci were polymorphic. The PIC values were considerably close as being 0.96 for ISSRs and 0.97 for SRAP markers. The average number of individuals per marker was 11 for ISSR and 16.6 for SRAP markers.Expected heterozygosities were 54% and 53% for ISSR and SRAP markers. According to their NJ results, there were two main clusters including close placement of specimens from *O. syriacum* subsp. *siniacum* (OSYSI) and *O. majorana* (OMA) from the same section (*Majorana)* and accessions from *O. vulgare* subsp. *vulgare* (OVVU) and *O. vulgare* subsp. *hirtum* (OVH) from section *Origanum*, respectively.

In the study of Tanhaş (2019), 21 herbarium specimens belonging to 18 taxa (21 species) were screened with 11 EST-SSRs and 16 SRAP combinations that generated 91 alleles. As a result of this study, the genetic diversity value was observed as 0.28, while Neighbor Joining dissimilarities were between 0.13 and 0.89. The population was divided into three subclusters and populations in NJ and population structure analyses. The dendrogram had three main clusters and structure had three main sub populations with groups of sections including i) *BRE, MAJ, CHI, LONGI, AMA*; ii) *ANA, MAJ, BRE, PRO, AMA, CHI*; and iii) *BRE, MAJ, CHI*, and *ORG* with the assignment of each specimen into sub populations at an identity threshold higher than 0.90. Analysis showed that OSYBE (*MAJ*) and OMA (*MAJ*) accessions were the most distant genotypes, while OON (*MAJ*) and OVH (*ORG*) was the closest. In this work, it was concluded that the geographical proximities of OSA (*AMA*), OBI (*CHI*), OAM (*LONGI*), OLA (*PRO*), and OSYBE (*MAJ*) species might be due to cross-hybridization and its outcome was genetic diversity.

In the study of Alexseeva et al. (2021), native Bulgarian *O. vulgare* subsp. *hirtum* was collected from two districts and consisted of 239 accessions with eight populations was screened with 11 SSR and eight SRAP markers. F_{ST} values were observed between 0.0047 – 0.11. Their genetic diversity results were combined with GC/MS data and hierarchical clustering was conducted. As a result, the dendrogram resulted in two main clusters reflecting two main geographical territories. Geographical isolation was proposed to affect metabolic richness in OVH species.

Antaloudaki et al. (2022) investigated the structure of Greek oregano populations with 193 specimens from ten taxa in eight species (OON, OSYME, OVVI, OVVU, OVH, OSC, ODI, OVE, OCA, OMICRO) using three nuclear and five chloroplast genes. A monophyletic structure was observed for all taxa except ODI (*AMA*). However,

intergeneric diversity was still unclear within taxa and among sections. According to their model which combined genetic, geographic and morphologic features, only species OCA reflected geographical isolation. Moreover, their morphological character analyses suggested that sections *AMA, ANA, CHI,* and *MAJ* have intermixing characters and there was no single section with unique morphological determiners which supports Ietswaart's suggestion (1980) about sections sharing at least two characters.

In the study of Karagöz et al. (2022), 70 *O. acutidens (*OAC) accessions from 70 different shires from five provinces of Türkiye including Artvin, Bayburt, Erzincan, Gümüşhane, and Erzurum were studied. They screened their OAC population with ten SCoT markers that created 109 alleles in total. This germplasm was divided into three sub populations and subclusters in both population structure analysis and NJ dendrogram analyses. The highest differentiation was observed in sub population C (F_{ST}: 0.21) with a mean of 0.14 in the population. Within OAC, the *Nei*'s genetic distance (*NGD*) was observed as 0.38 and genetic diversity was observed as 0.36. According to their previous paper (Karagöz et al., 2020), they stated that geographic and ecological conditions have great impact on gene flow between cross-pollinating hybrid oregano species.

In the study of Tan (2022), five (A, B, C, D, E) *O. vulgare* subsp. *hirtum* (OVH) vegetative clonal populations collected from different locations in Kaz Mountains were agromorphologically, chemotypically, and molecularly characterized. According to the physical and morphological measurements, the 12 most drought tolerant and susceptible clones were selected. These genotypes were screened with nine EST-SSR markers. The highest genetic distance was observed between a drought tolerant individual from population C and a drought susceptible accession from population A. Two main clusters were observed for genetic dissimilarity analysis in the NJ dendrogram, while the combination of genotypic data and all other measureable characters resulted in three main clusters including four subclusters. In these subclusters, the tolerant accessions clustered together. The two susceptible genotypes also formed an exclusive subcluster. As a result the OVH clones that were tested for drought tolerance were divided into three subpopulation, which may indicate their tolerance levels (tolerant, susceptible, alternative/partial).

1.4. Scope

The scope of this dissertation includes i) adding molecular knowledge to taxonomical classification of the genus *Origanum*, ii) understanding the molecular basis of gene flow, hybridization, and speciation behaviors of oregano species, iii) adding to current molecular genetic knowledge for the development of new breeding and conservation strategies for the genus *Origanum* and its wild endemic or uncharacterized relatives, iv) reviewing and comparing current records of oregano species. This dissertation includes evaluation of the molecular genetic structure of native Turkish *Origanum* taxa that were collected over different years from various locations and conserved in two different forms (as dried leaves and as *ex situ* sowed seeds) at national collections. This evaluation was based on genetic relatedness of oregano species determined with molecular marker data analyses. Determination of population structure and diversity provides vital data that can be added to the conservation strategies for endemic oregano genetic resources (Figure 1.23).

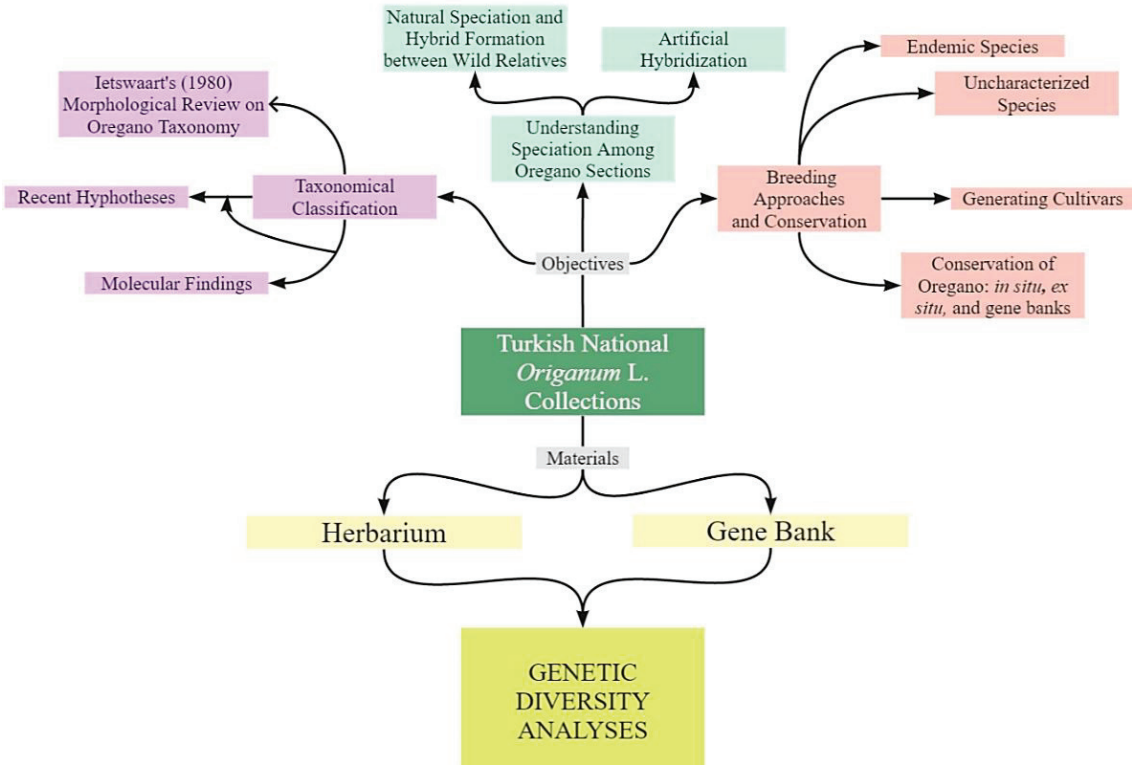


Figure 1.23. The objectives and materials investigated in the Turkish *Origanum* collections in this dissertation.

In this dissertation, the following analyses were conducted: (i) determination of the level of genetic diversity and the population structure among Turkish *Origanum* taxa in the sampled herbarium and gene bank material, (ii) assessment of gene flow in Turkish oregano germplasm collections, (iii) discussion about the recent findings in our work in the context of the applied classification of *Origanum* as described by Ietswaart (1980) and recent findings in the literature for a better conservation, and iv) suggestion of a new abbreviation strategy for global oregano nomenclature.

CHAPTER 2

MOLECULAR CHARACTERIZATION OF NATIVE TURKISH *Origanum* L. HERBARIUM COLLECTION

2.1. Introduction

Due to their enriched biodiversity, countries like Türkiye are known as gene centers for endemic plant species (Noroozi et al., 2019). Nearly 60 species are added to the Turkish Flora every year (Özhatay et al.,2017). The diverse climatic conditions and ecosystem in Türkiye generates hybridization zones for various plant populations. At the same time, factors such as terrorism, natural disasters, and non-reported wild plant collectors make it difficult to reach plant species for conservation purposes as our country becomes an area of endemism for different species. Fortunately, there are herbarium collections in which species are kept as dried specimens. Herbarium collections are one of the most popular conservation methods for plant species.

Throughout the world, there are 3100 herbaria and 390 million botanical specimens that are under conservation within these centers (Source: Url7). Amongst these, there are seven popular and important herbaria for plant species: KEW Herbarium (which is the richest collection of the world – London), Edinburg Herbarium (Scotland), Berlin Herbarium (Germany), Leningrad Herbarium (Russia), Paris Herbarium (France), Geneva Herbarium (Switzerland), and Genova Herbarium (Italy). According to Index Herbarium, there are a total of 57 herbarium collection centers in Türkiye including the cities: Bolu, Aydın, İzmir, Antalya, Aksaray, Istanbul, Eskişehir, Ankara, Artvin, Mersin, Erzurum, Çanakkale, Hatay, Adana, Sivas, Kütahya, Düzce, Kayseri, Trabzon, Elazığ, Tokat, Hakkari, Şanlıurfa, Malatya, Kırıkkale, Samsun, Denizli, Sakarya, Konya, Siirt, Isparta, Tekirdağ, Bursa, Diyarbakır, Van, and Zonguldak (Source: Url7). The most important herbarium collections for our country are in three main provinces of Türkiye: Istanbul, Ankara and İzmir. They are: Herbarium of Istanbul University, Faculty of Pharmacy (ISTE); Herbarium of Istanbul University, Faculty of Science (ISTF); Herbarium of Ankara University, Faculty of Science and Literature (ANK); Herbarium of Gazi University, Faculty of Science (GAZI); and Aegean University Botanical

Garden, Herbarium Research and Application Center. For example, the ISTE herbarium has a total of 130,000 specimens from *Papaver*, *Salvia*, *Allium*, *Rosa*, *Ferula* and *Origanum* taxa (Akalın et al., 2020). In Türkiye, it is common to travel and create personal herbaria for endemic/nonendemic plant species such as oregano. For example, scientists Tuncay Dirmenci (TD), Turan Arabacı (Arabacı), Türker Yazıcı (Yazıcı), and Narin Sadıkoğlu (Sadıkoğlu) individually created their own herbaria and worked together for the genus *Origanum*. Their herbaria have been used in morphological, panalogical, karyological, and genetic characterization of the oregano species that naturally grow in Türkiye.

Reconstructing phylogenies using old specimens with a small amount of cloneable DNA has been a concern of molecular taxonomists (Besnard et al., 2018). However, improvements in sequencing technology make such work easier. Since herbarium materials are invaluable sources of knowledge about genetic identities for a given time, location, and amount of specimen, there is a need to determine the genetic similarity/distance within and among herbarium collections to understand genetic and evolutionary relationships. Herbarium materials are very valuable for understanding the speciation behaviors of oregano taxa since the specimens were collected from several locations and over different years.

In this chapter, we examined a herbarium collection of 46 specimens that was created at Inonu University. These materials included 22 Turkish *Origanum* species representing 24 taxa, collected from 2005 to 2014. This work was published as: Taşcıoğlu, T., Sadıkoğlu, N., Doğanlar, S., & Frary, A. 2018. "Molecular genetic diversity in the *Origanum* genus: EST-SSR and SRAP marker analyses of the 22 species in eight sections that naturally occur in Turkey." *Industrial Crops and Products*, *123*, 746-761.

2.2. Plant Material

The herbarium belonging to the Faculty of Pharmacy (Inonu University, Malatya) provided all the plant materials used in this section of the dissertation. Native specimens from eight sections of the genus *Origanum* were represented with 46 accessions (accs.) including 24 taxa with 22 species as herbarium materials (registration years: 2005 – 2014) (Figure 2.1; Table 2.1). The specimens included all oregano species except *O.*

brevidens. The specimen number per discrete location ranged between one to 12. Antalya was the predominant location with samples from four sections: *Amaracus, Brevifilamentum, Chilocalyx*, and *Majorana* (Figure 2.1). Six accs. from sects. *Longitubus*, *Brevifilamentum*, *Prolaticorolla*, and *Origanum* were from Osmaniye. Mersin was represented with five accs. belonging to sects. *Amaracus*, *Majorana*, and *Origanum*. The three accs. from Tunceli were from sections *Brevifilamentum* and *Origanum*. Two accs. were from Isparta and Artvin each. Karaman, Hatay, Adana, and Erzincan provided one specimen each. Thus, the *Origanum* species were represented by a wide range of sampling areas throughout Türkiye.

Table 2.1. *Origanum* materials used in this study, their collection coordinates and other details. Endemism (E).

Sample Code		Species Name	Subspecies Name (Herbarium Identifier)	Section	Year	E	Location	Coordinates
OBO	OBO1	*O. boissieri* Ietsw.	*O. boissieri*/Narin/kekik/141	*Amaracus* (Gleditsch) Bentham	DD	E	Mersin	N37° 14′ 282″ E34° 37′ 740″
OSA	OSA1	*O. saccatum* P.H. Davis	*O. saccatum*/2012/6		2012	E	Burdur & Antalya	N36° 49′ 652″ E31° 56′ 797″
	OSA2		*O. saccatum*/2012/5		2012			N36° 46′ 426″ E31° 46′ 905″
	OSA3		*O. saccatum*/2009/17		2009			N36° 35′ 494″ E30° 27′ 624″
	OSA4		*O.saccatum*/2009/15		2009			N37° 37′ 965″ E30° 41′ 926″
	OSA5		*O. saccatum*/2009/33		2009			N36° 32′ 145″ E32° 14′ 892″
	OSA6		*O. saccatum*/2009/35		2009			N36° 33′ 237″ E32° 19′ 467″
	OSA7		*O. saccatum*/2009/20		2009			N36° 39′ 137″ E32° 07′ 694″
	OSA8		*O. saccatum*/2009/6a		2009			DD
OSO	OSO1	*O. solymicum* P.H.Davis	*O. solymicum*/2009/18		2009	E	Antalya	N36° 35′ 494″ E30° 27′ 624″
OHY	OHY1	*O. hypericifolium* O. Schwarz et P.H. Davis	*O. hypericifolium*/2009/16	*Anatolicon* Bentham	2009	E	Burdur	N37° 42′ 057″ E30° 18′ 812″
OSI	OSI1	*O. sipyleum* L.	*O. sipyleum*/2009/14		2009	E	Isparta	DD
OAC	OAC1	*O. acutidens* (Hand.-Mazz.) Ietsw.	*O. acutidens*/2014/1	*Brevifilamentum* Ietswaart	2014	E	Tunceli	N39° 21′ 864″ E39° 12′ 538″
OBA	OBA1	*O. bargyli* Mouterde	*O. bargyli*/2009/41		2009	DD	Osmaniye	N37° 01′ 373 ″E36° 26′ 982″
OHA	OHA1	*O. haussknechtii* Boiss.	*O. haussknechtii*/2009/2		2009	E	Erzincan	N39° 09′ 750″ E38° 37′ 167″
OHU	OHU1	*O. husnucan-baseri* H. Duman, Aytac & A. Duran	*O. husnucan-baseri*/2009/34		2009	E	Antalya	N36° 33′ 237″ E32° 19′ 467″

(Cont. on the next page)

Table 2.1. (cont.)

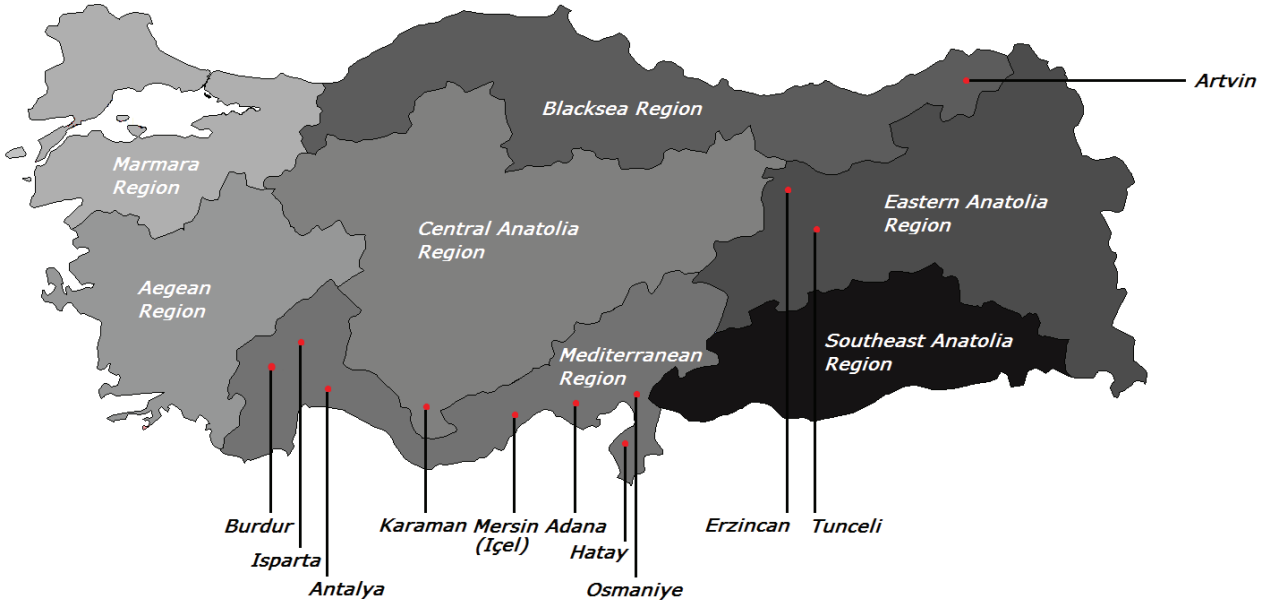
	OHU2		*O. husnucan-baseri*/2009/21		2009			N36° 33′ 100″ E32° 19′ 023″
OLE	OLE1	*O. leptocladum* Boiss	*O. leptocladum*/2005		2005	E	Karaman	DD
	OLE2		*O. leptocladum*/2009/22		2009			N36° 38′ 084″ E32° 41′ 564″
OMU	OMU1	*O. munzurense* Kit Tan & Sorger	*O. munzurense*/2014/2		2014	E	Tunceli	N39° 21′ 864″ E39° 12′ 538″
ORO	ORO1	*O. rotundifolium* Boiss	*O. rotundifolium*/2012/4		2012	DD	Artvin	N41° 18′ 972″ E41° 44′ 138″
	ORO2		*O. rotundifolium*/2012/3		2012			N40° 50′ 901″ E41° 32′ 411″
OAM	OAM1	*O. amanum* Post	*O. amanum*/Narin/kekik/143	*Longitubus* Ietswaart	DD	E	Osmaniye	DD
OBI	OBI1	*O. bilgeri* P.H. Davis	*O. bilgeri*/2012/7	*Chilocalyx* (Briq.) Ietswaart	2012	E	Antalya	N36° 45′ 948″ E32° 08′ 606″
OVO	OVO1	*O. vogelii* Greuter & Burdet	*O. vogelii*/Narin/kekik/80		DD	E	Adana	DD
OMI	OMI1	*O. minutiflorum* O. Schwarz et P.H. Davis	*O. minutiflorum*/2009/13		2009	E	Antalya	N37° 22′ 798″ E30° 56′ 427″
OMA	OMA1	*O. majarona* L.	*O. majarona*/2009/32	*Majorana* (Miler) Bentham	2009	DD	Antalya	DD
	OMA2		*O. majarona*/2009/19		2009			N36° 34′ 326″ E32° 00′ 942″
OON	OON1	*O. onites* L.	*O. onites*/2009/12		2009	DD	Isparta	N37° 37′ 866″ E30° 52′ 287″
	OON2		*O. onites*/2009/11		2009			DD
OSY	OSY1	*O. syriacum* L. subsp. *bevanii* (Holmes) Ietsw.	*O. syriacum*/2009/37		2009	DD	Mersin & Osmaniye & Hatay	N37° 12′ 595″ E34° 38′ 081″
	OSY2		*O. syriacum*/2009/6		2009			DD
	OSY3		*O. syriacum*/2009/24		2009			DD

(Cont. on the next page)

Table 2.1 (cont.)

OVVU	OVVU1	*O. vulgare* L. subsp. *vulgare* Linnaeus	*O. vulgare* subsp. *vulgare*/2009/8	*Origanum*	2009	DD	Osmaniye & Mersin	N37° 15′ 081″ E34° 45′ 830″
	OVVU2		*O. vulgare* subsp. *vulgare*/2009/7		2009			N37° 21′ 100″ E36° 27′ 680″
OVG	OVG1	*O. vulgare* L. subsp. *gracile* (K. Koch) Ietsw.	*O. vulgare* L. subsp. *gracile*/2014/3		2014	DD	Tunceli	N39° 21′ 864″ E39° 12′ 538″
OVH	OVH1	*O. vulgare* L. subsp. *hirtum* (Link) Ietsw.	*O. vulgare* L. subsp. *hirtum*/2009/36		2009	DD	Mersin	N37° 08′ 345″ E34° 41′ 560″
	OVH2		*O. vulgare* L. subsp. *hirtum*/2009/9		2009			DD
OVVI	OVVI1	*O. vulgare* L. subsp. *viridulum* (Martrin-Donos) Nyman	*O. vulgare* L. subsp. *viridulum*/2009/38		2009	DD	Isparta & Mersin	N37° 14′ 282″ E34° 37′ 740″
	OVVI2		*O. vulgare* L. subsp. *viridulum*/2009/10		2009			N37° 34′ 848″ E31° 10′ 560″
OLA	OLA1	*O. laevigatum* Boiss.	*O. laevigatum*/2009/23	*Prolaticorolla* Ietswaart	2009	E	Osmaniye & Hatay	N36° 52′ 685 E36° 16′ 770″
	OLA2		*O. laevigatum*/2009/39		2009			N37° 05′ 290″ E36° 20′ 116″
	OLA3		*O. laevigatum*/2009/42		2009			N37° 03′ 795″ E36° 23′ 131″
	OLA4		*O. laevigatum*/2009/5		2009			N37° 20′ 003″ E36° 27′ 320″
	OLA5		*O. laevigatum*/2009/40		2009			DD
	OLA6		*O. laevigatum*/St.Simean/27.11.2009		2009			DD

Notations are as follows: E = endemic; DD = data deficient



Regions

Provinces	Mediterranean	SECTION
	Burdur OHY1, OSA4	*Amaracus* *Anatolicon*
	Isparta OON1, OSI1, OVVI2	*Anatolicon* *Majarona* *Origanum*
	Antalya OBI1, OHU1, OHU2, OMA2, OMI1, OSA1, OSA2, OSA3, OSA5, OSA6, OSA7, OSO1	*Amaracus* *Brevifilamentum* *Chilocalyx*
	Mersin OBO1, OSY1, OVH1, OVVI1, OVVU1	*Amaracus* *Majarona* *Origanum*
	Adana OVO1	*Chilocalyx*
	Hatay OLA1	*Prolaticorolla*
	Osmaniye OAM1, OBA1, OLA2, OLA3, OLA4, OVVU2	*Brevifilamentum* *Longitubus* *Prolaticorolla* *Origanum*

Provinces	Central Anatolia	SECTION
	Karaman OLE2	*Brevifilamentum*

Provinces	Eastern Anatolia	SECTION
	Erzincan OHA1	*Brevifilamentum*
	Tunceli OAC1, OMU1, OVG1	*Brevifilamentum* *Origanum*

Provinces	Blacksea	SECTION
	Artvin ORO1, ORO2	*Brevifilamentum*

Materials with unknown sampling locations	SECTION
OLA5, OLA6, OLE1, OMA1, OON2, OSA8, OSY2, OSY3, OVH2	*Amaracus* *Majarona* *Origanum* *Prolaticorolla*

Figure 2.1. Collection locations of herbarium specimens and their sections.

2.3. Methods

2.3.1. DNA extraction

CTAB DNA isolation protocol was utilized to extract DNA from dried specimens (Doyle and Doyle, 1987) and combined with another procedure which added DNA purification steps for herbarium materials (Costa and Roberts, 2014). Genomic DNA quality and quantity were measured by SkanIt software for Multiscan Go 3.2 spectrophotometer (Thermo Scientific) and the presence of isolated DNA fragments was screened with ethidium bromide (Et-Br) on 0.8 % agarose gel. Each stock DNA was diluted to 50 ng/µl and kept at -20℃.

2.3.2. SRAP analyses

Twenty-five SRAP primer combinations (Li and Quiros, 2001) were used to produce random amplicons **(**Table 2.2**).** PCR reactions were performed with 10 X PCR Buffer (with BSA), 3 mM $MgCl_2$, 0.125 mM deoxyribonucleotide triphosphates (dNTPs), 1 U *Taq* DNA polymerase, 2 pmol forward and reverse primers and 50 ng template DNA in a total of 25 µl final volume. The PCR reaction was an initial denaturation step at 94 ºC for 5 min, which was followed with 5 cycles of 94 ºC for 1 min, 35 ºC for 1 min, 72 ºC for 1 min, then 35 cycles of 94 ºC for 1 min, 50ºC for 1 min, 72 ºC for 1 min were run with a final elongation step at 72 ºC for 10 min. PCR fragments were screened under UV light with BioRad (Universal Hood II) system after 2% agarose gel electrophoresis (solution and agarose were prepared with 1 X TAE Buffer) (110V – 3 hours).

Table 2.2. SRAP primer combinations and their sequence information

Primer Type	Pair Code	Forward	Sequence of Forward (5' to 3')	Reverse	Sequence of Reverse (5' to 3')
SRAP	**1**	**me2**	TGAGTCCAAACCGGAGC	**em1**	GACTGCGTACGAATTAAT
	2			**em2**	GACTGCGTACGAATTTGC
	3			**em3**	GACTGCGTACGAATTGAC
	4			**em5**	GACTGCGTACGAATTAAC
	5			**em6**	GACTGCGTACGAATTGCA
	6			**em7**	GACTGCGTACGAATTATG
	7			**em8**	GACTGCGTACGAATTAGC

(Cont. on the next page)

Table 2.2. (cont.)

SRAP	8			em11	GACTGCGTACGAATTTCG
	9			em15	GACTGCGTACGAATTCGT
	10	me3	TGAGTCCAAACCGGAAT	em1	GACTGCGTACGAATTAAT
	11			em2	GACTGCGTACGAATTTGC
	12			em3	GACTGCGTACGAATTGAC
	13			em4	GACTGCGTACGAATTTGA
	14			em5	GACTGCGTACGAATTAAC
	15			em6	GACTGCGTACGAATTGCA
	16			em7	GACTGCGTACGAATTATG
	17			em11	GACTGCGTACGAATTTCG
	18			em13	GACTGCGTACGAATTGGT
	19			em15	GACTGCGTACGAATTCGT
	20	me4	TGAGTCCAAACCGGACC	em1	GACTGCGTACGAATTAAT
	21			em2	GACTGCGTACGAATTTGC
	22			em3	GACTGCGTACGAATTGAC
	23			em5	GACTGCGTACGAATTAAC
	24			em7	GACTGCGTACGAATTATG
	25			em8	GACTGCGTACGAATTAGC

2.3.3. EST-SSR analyses

EST-SSR markers that were developed for the first time specifically for oregano species (Novak et al., 2008) were screened in the population, and six of them (OR09, OR12, OR13, OR27, OR32, OR40) were successfully scored and used in statistical analyses (Table 2.3).

Table 2.3. EST-SSR primers and their sequence information

Primer Type	Name	Primer	Sequence (5' to 3')	Repeat Pattern
EST - SSR	OR09	Forward	TTGAAGCATTGTTGGAGGTAGATG	$(TTTTTC)_4(T)_5(TTTTTC)_1$
		Reverse	TCCCAACTAGGGAGAAATGTGC	
	OR12	Forward	GCCCCTGCAGTGACTCCTAC	$(AG)_7G(AG)_3$
		Reverse	AAAAAGGCTTCGGACTCGATC	
	OR13	Forward	GAGAGAATCCAAGCCTCCGC	$(AAC)_7AGC(AAC)_1$
		Reverse	TGAAGGAGTCCGATGTTGACG	
	OR27	Forward	TCAGAAACAATGAAGGCCGC	$(CCT)_6$
		Reverse	CCGTACAGGTCAAACACCGG	
	OR32	Forward	TCTTGCCAATTTATGCGTGTTC	$(AG)_6GG(AG)_2GA(AG)_5GG(AG)_1$
		Reverse	GAAACAAGCATCTTTTCCTGAATT	
	OR40	Forward	G̃CCCAAGGACATCCAACTTG	$(GGT)_4GTT(GGT)_1$
		Reverse	CAACTGAACACCTCCCACAATG	

PCR was conducted with 10 X reaction buffer, $MgCl_2$ (3 mM) dNTPs (0.125 mM), *Taq* DNA polymerase (1 U), forward and reverse primers (2 pmol each) and diluted DNA template (50 ng) in a total of 25 µl. PCR cycles were applied as: initial denaturation (94 ^oC; 3 minutes), followed by 40 cycles with i) 94 ^oC, 1 minute, ii) Tm [61 to 66 ^oC], 1 minute, iii) 72 ^oC, 1 minute), final elongation step was completed with a single step at 72 ^oC for 10 minutes. PCR products were visualized under UV light after running on 2% agarose gel as described in SRAP analyses.

2.3.4. Statistical analyses

Scoring was carried out dominantly with "1" referring to band presence and "0" referring to absence for both marker types (SRAP and EST-SSR). The missing data were coded as "9" in each analysis. Genetic diversity at the section level was determined for seven sections with 45 individuals out of 46 with GenAlEx 6.5 plugin. *O. amanum* (AOM; section *Longitubus*) was excluded because it was represented by only one individual (Peakall and Smouse, 2006, 2012). In addition, F_{ST} analysis analogue *PhiPT* value was calculated with the species represented by more than one individual in 11 taxa (*O. saccatum*, OSA; *O. husnucan-baseri*, OHU; *O. leptocladum*, OLE; *O. rotundifolium*, ORO; *O. majorana*, OMA; *O. onites*, OON; *O. syriacum*, OSY; *O. vulgare* subsp. *vulgare*, OVVU; *O. vulgare* subsp. *hirtum*, OVH; *O. vulgare* subsp. *viridulum*, OVVI; and *O. laevigatum*, OLA) with the construction of a binary data matrix in GenAlEx 6.5 plugin. Analysis of Molecular Variance (AMOVA) was conducted to determine genetic diversity among sections/taxa for those accessions with more than one individual in the same plugin. Significant gene flow between taxa was accepted with *PhiPT* values less than 0.15 (Frankham et al., 2002). The pairwise permutations were selected as "9999" and *P* values were accepted as significant below 0.001. The GenAlEx plugin was also used to determine the number of effective alleles (*Ne*), Shannon's Index (*I*), and the mean diversity (*h*) with random binary data. *Nei's* genetic distances (*NGD*) and identities (*NGI*) were estimated for the taxa and section levels and the number of migrants per generation (*Nm*) among populations was calculated with the formula:

$$Nm = 0.25 * \left[\left(\frac{1}{PHIPT}\right) - 1\right]$$

(Source: Wood and Gardner, 2007).

2.3.5. Population structure and dendrogram analyses

GDdom software (Abuzayed et al., 2016) was used to calculate genetic diversity (*GD*) values for the 31 dominant markers. DARwin 6.0.8 Software was used to construct an unweighted Neighbor Joining (NJ) dendrogram (Perrier and Jacquemoud, 2006) with Jaccard dissimilarity index. The distances among accessions between units on Euclidean planes were represented graphically with a Principle Coordinate Analysis (PCoA) plot that was constructed with Eigen vectors for the first two axes using DARwin 6.0.8 software and GenAlEx 6.5 plugin (Perrier and Jacquemound, 2006; Peakall and Smouse, 2006).

The structure of the population was evaluated by a model-based Bayesian clustering method with STRUCTURE 2.3.4 software (Pritchard et al., 2000). The number of subpopulations (*K*) was tested for 1 to 10 groups and each *K* had 10 iterations. Initial burn in replications were set as 100,000 and followed by 100,000 Markov Chain Monte Carlo (MCMC) replications. These numbers of replications were determined to be sufficient thresholds to determine populatin structure in the literature (Sakiroglu et al., 2010). Structure Harvester was used to assign the best fitting number of *K* by calculating $\Delta(K)$ values which were used to determine the highest likelihood value from Structure Software analysis results (Earl and vonHoldt, 2012). An optimal identity threshold was assumed as 0.70 for sub population membership. This threshold is optimal and acceptable according to Scutari and Denis since they stated that any value between 0.5 and 0.85 yields the same pattern (Scutari and Denis, 2014). For each subpopulation, accessions with identity values less than 0.70 were assumed to be admixed individuals.

2.4. Results

2.4.1. Genetic diversity analyses

The genetic dissimilarities were determined at species/taxon levels with 25 SRAP and six EST-SSR markers. The total number of polymorphic alleles was determined as 325 (Figure 2.2). For SRAP markers, the number of polymorphic alleles ranged between 3 to 20 with a mean of 12.04 (Table 2.2). Genetic diversity (GD) was 0.35 at its highest for SRAPs with the me3-em4 marker pair. For the EST-SSR markers, the number of

polymorphic alleles ranged between 2 to 6 with a mean of 4 (Table 2.4). The highest GD was observed as 0.49 for OR09 marker. The higher average GD (0.36) was obtained from EST-SSR primers. Although SRAP markers had many more fragments, their GD was 0.27. All polymorphic markers had mean allele frequencies (af) ranging from 0.11 to 0.38 while af per individuals ranged between 0.02 and 0.96 (Figure 2.3).

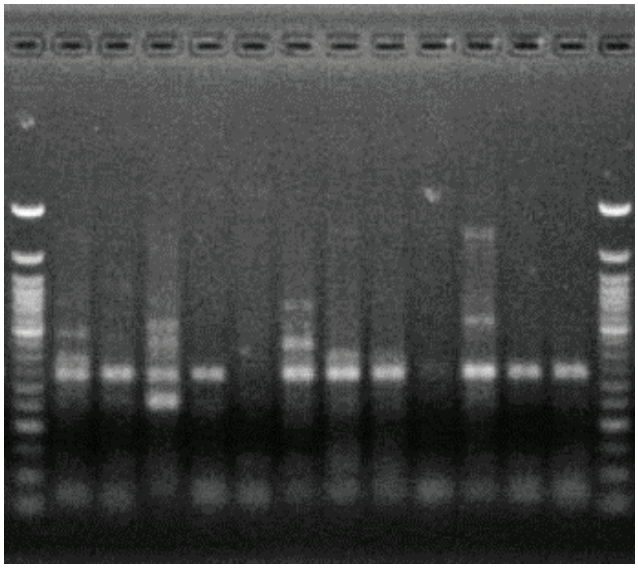


Figure 2.2. Allelic pattern of SRAP marker combination, em4-me3, on an agarose gel image.

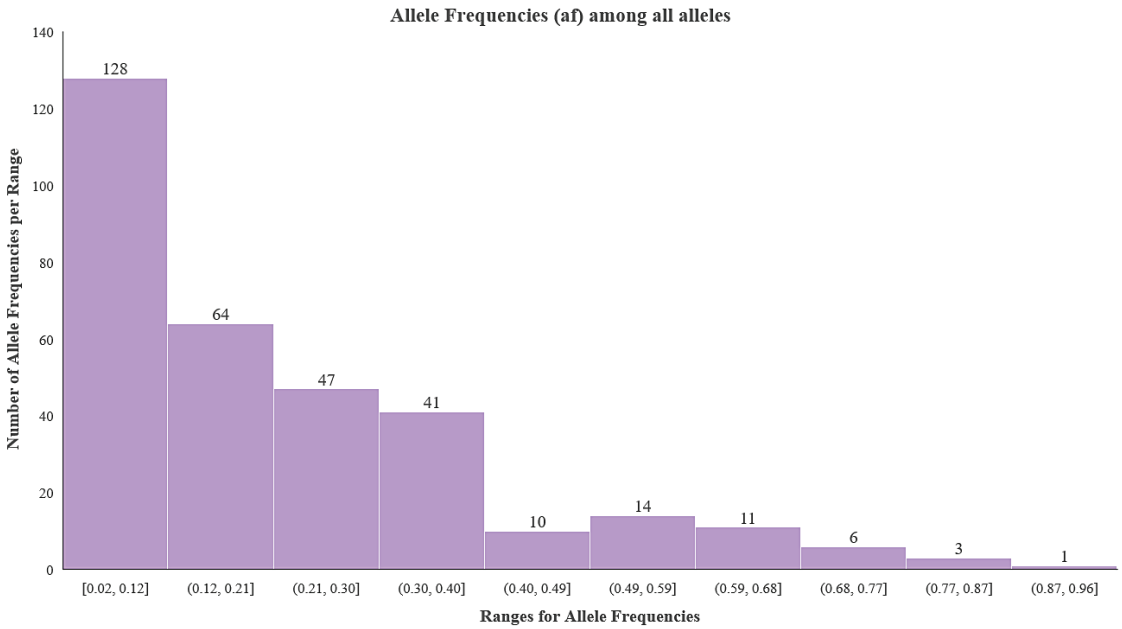


Figure 2.3. Distribution of individual allele frequencies (af) among all alleles for both SRAP and EST-SSR markers.

Table 2.4. Allele frequencies and genetic diversity values of herbarium oregano accessions for SRAP and EST-SSR markers. Definitions are as follows: total number of polymorphic alleles (∑*Na* =), average number of polymorphic alleles (*Na*), allele frequency and genetic diversity (GD) for each marker listed from the lowest to highest value. SE: Standard error.

Type	Marker	*Na*	Allele Frequency	GD ± SE	Average GD
SRAP	em11-me3	11	0.14	0.14 ± 0.04	0.27
	em6-me2	3	0.38	0.18 ± 0.09	
	em1-me2	19	0.18	0.20 ± 0.03	
	em13-me3	8	0.13	0.21 ± 0.05	
	em5-me2	19	0.16	0.22 ± 0.03	
	em2-me2	11	0.11	0.24 ± 0.04	
	em5-me4	19	0.15	0.25 ± 0.03	
	em2-me4	8	0.26	0.26 ± 0.05	
	em7-me2	13	0.24	0.26 ± 0.05	
	em15-me3	7	0.23	0.26 ± 0.06	
	em3-me4	14	0.21	0.27 ± 0.04	
	em7-me4	16	0.24	0.27 ± 0.04	
	em6-me3	20	0.21	0.29 ± 0.03	
	em1-me3	16	0.18	0.29 ± 0.04	
	em7-me3	5	0.2	0.29 ± 0.07	
	em1-me4	11	0.23	0.30 ± 0.05	
	em8-me4	9	0.24	0.30 ± 0.06	
	em3-me3	13	0.25	0.31 ± 0.03	
	em5-me3	10	0.27	0.31 ± 0.05	
	em2-me3	14	0.2	0.32 ± 0.04	
	em3-me2	7	0.19	0.32 ± 0.05	
	em11-me2	10	0.28	0.33 ± 0.05	
	em15-me2	7	0.21	0.33 ± 0.05	
	em8-me2	15	0.28	0.34 ± 0.04	
	em4-me3	16	0.3	0.35 ± 0.03	
EST-SSR	Or12	6	0.16	0.25 ± 0.06	0.36
	Or32	4	0.27	0.33 ± 0.04	
	Or40	4	0.28	0.33 ± 0.07	
	Or27	4	0.25	0.37 ± 0.05	
	Or13	4	0.31	0.40 ± 0.04	
	Or09	2	0.32	0.49 ± 0.00	

∑*Na*=325

AMOVA analysis determined that within section diversity was high (83%) and the remnant 17% of diversity was observed among sections. GD per section was between 0.40 and 0.42 (*h*). At the taxon level, the highest diversity was observed within taxa

(80%) while among taxa diversity was 20% (Table 2.5). Moreover, sections *Majorana* and *Origanum* appeared to be the most similar at the molecular level (0.88) according to the *NGI* values while sections *Majorana* and *Brevifilamentum* had least congruence (0.83) (Table 2.6).

Table 2.5. AMOVA results for Turkish *Origanum* L. species at taxon level

Source	df	SS	MS	Est. Var.	%
Among Pops	10	75.852	7.585	1.098	20%
Within Pops	22	97.451	4.430	4.430	80%
Total	32	173.303		5.528	100%

Definitions are as follows: df = degrees of freedom; SS = sum of squares; MS = mean of squares; Est. Var = estimated variance; % = percentage of variation.

Table 2.6. *Nei's* genetic distances (below diagonal) and *Nei's* genetic identity values (above diagonal) for the eight oregano sections. Bold font indicates the highest *Nei's* genetic distance between sections *Majorana* and *Brevifilamentum,* while *italic* font displays the lowest genetic distance between sections *Origanum* and *Majorana.*

***Nei's* Genetic Distance vs. *Nei's* Genetic Identity**	***Amaracus***	***Anatolicon***	***Brevifilamentum***	***Chilocalyx***	***Majorana***	***Origanum***	***Prolaticorolla***
Amaracus	-	0.84	0.86	0.86	0.85	0.87	0.85
Anatolicon	0.17	-	0.86	0.84	0.84	0.86	0.84
Brevifilamentum	0.15	0.15	-	0.84	0.83	0.85	0.84
Chilocalyx	0.15	0.18	0.17	-	0.85	0.87	0.86
Majorana	0.16	0.17	**0.19**	0.16	-	0.88	0.85
Origanum	0.14	0.15	0.16	0.14	*0.13*	-	0.87
Prolaticorolla	0.16	0.18	0.17	0.15	0.17	0.14	-

The pairwise combinations for the seven sections for which gene flow and number of migrants (*Nm*) could be calculated showed that the most gene flow appeared between sects. *Anatolicon* and *Brevifilamentum* (Table 2.7) while the least gene flow was observed between sects. *Anatolicon* and *Prolaticorolla*.

At the taxon level, *Ne*, *I* and *h* were determined as 1.6, 0.48, and 0.33 for the eleven taxa containing more than one individual. OHU and OSY individuals had the

highest *I* as 0.50 and had the highest *h* (0.35) among all species. Lower levels of diversity were observed for the taxa OLE, OMA, OVVU and OVVI. OVH accessions had the lowest diversity with an *I* of 0.44 and *h* of 0.31. The average polymorphic locus percentage was 75% among the eleven *Origanum* taxa. The maximum level of polymorphic loci was 79% for OHU. No private band was observed for all taxa (data not shown).

Table 2.7. Gene flow in the eight sections of *Origanum* L. *PhiPT* values are shown below the diagonal. Values accepted as significant (< 0.15) are in bold characters indicating relatively low allelic differentiation between taxa. Number of migrants per generation (*Nm*) for sections of *Origanum* L. genera are shown above the diagonal and bold characters indicate relatively high gene flow between taxa (*PhiPT* < 0.15).

***PhiPT* values vs. *Nm* values**	***Amaracus***	***Anatolicon***	***Brevifilamentum***	***Chilocalyx***	***Majorana***	***Origanum***	***Prolaticorolla***
Amaracus	-	**2.97**	**1.91**	1.09	0.98	0.81	0.68
Anatolicon	**0.08**	-	**6.08**	**1.67**	1.34	0.82	0.62
Brevifilamentum	**0.12**	**0.04**	-	**3.48**	**1.64**	**1.51**	1.17
Chilocalyx	0.19	**0.13**	**0.07**	-	**1.51**	0.88	0.81
Majorana	0.20	0.16	**0.13**	**0.14**	-	**2.18**	0.97
Origanum	0.24	0.23	**0.14**	0.22	**0.10**	-	0.77
Prolaticorolla	0.27	0.29	0.18	0.24	0.20	0.25	-

The highest *NGI* was observed between OLE and ORO (0.80), and OSA and OMA (0.79) (Table 2.8). The least gene flow was found between taxa OHU and ORO (0.31) (*PhiPT*, Table 2.9**).** The highest gene flow was 0.04 between the OON and OMA populations (*NGD* of 0.24). As expected from these results, the highest *Nm* value, 6.04, was observed between the OON and OMA populations. The *Nm* values were also high between the following taxon pairs: OVVU-OSY (3.27), OVH-OVVU (3.07), and OVVI-OVH (3.06).

Table 2.8. Pairwise population matrix with *Nei's* genetic distance (*NGD*) below the diagonal and *Nei's* genetic identity (*NGI*) above the diagonal at taxon level

	OSA	**OHU**	**OLE**	**ORO**	**OMA**	**OON**	**OSY**	**OVVU**	**OVH**	**OVVI**	**OLA**
OSA	-	0.77	0.74	0.7	0.79	0.76	0.76	0.76	0.72	0.75	0.76
OHU	0.27	-	0.76	0.7	0.76	0.74	0.75	0.78	0.75	0.77	0.77
OLE	0.30	0.28	-	0.8	0.75	0.77	0.74	0.76	0.73	0.75	0.75
ORO	0.32	0.29	0.27	-	0.76	0.76	0.73	0.73	0.73	0.75	0.73
OMA	0.24	0.27	0.29	0.3	-	0.78	0.77	0.74	0.73	0.76	0.76
OON	0.28	0.30	0.27	0.3	0.24	-	0.74	0.75	0.70	0.73	0.74
OSY	0.27	0.29	0.30	0.3	0.26	0.30	-	0.78	0.76	0.75	0.76
OVVU	0.28	0.24	0.27	0.3	0.30	0.28	0.25	-	0.75	0.75	0.77
OVH	0.33	0.29	0.32	0.3	0.31	0.36	0.28	0.29	-	0.74	0.76
OVVI	0.28	0.26	0.28	0.3	0.28	0.32	0.29	0.29	0.30	-	0.75
OLA	0.28	0.26	0.29	0.3	0.27	0.30	0.27	0.27	0.28	0.28	-

Table 2.9. Gene flow in the oregano accessions. *PhiPT* values are shown below the diagonal. *PhiPT* values that were accepted as significant (< 0.15) are in bold characters indicating relatively low allelic differentiation between taxa. Number of migrants per generation (*Nm*) for *Origanum* L. populations are shown above the diagonal. Bold characters indicate relatively high gene flow between taxa (*PhiPT* < 0.15)

	OS	**OH**	**OLE**	**ORO**	**OMA**	**OON**	**OSY**	**OVV**	**OVH**	**OVVI**	**OLA**
OSA	-	1.18	1.38	0.91	0.87	0.89	0.89	**1.45**	1.06	0.97	1.09
OHU	0.18	-	1.16	0.56	0.6	0.67	0.64	0.91	0.64	0.79	0.87
OLE	0.15	0.18	-	1.15	**2.66**	1.36	0.89	**1.48**	**1.81**	1.33	1.1
ORO	0.22	0.31	0.18	-	0.62	0.64	0.65	0.73	0.58	0.65	0.67
OMA	0.22	0.29	**0.09**	0.29	-	**6.04**	0.72	0.91	0.96	**1.51**	1.03
OON	0.22	0.27	0.16	0.28	**0.04**	-	0.81	1.23	1.33	**2.43**	1.09
OSY	0.22	0.28	0.22	0.28	0.26	0.24	-	**3.27**	0.89	0.92	0.84
OVV	**0.15**	0.22	**0.14**	0.26	0.22	0.17	**0.07**	-	**3.07**	**2.17**	**1.52**
OVH	0.19	0.28	**0.12**	0.30	0.21	0.16	0.22	**0.08**	-	**3.06**	0.98
OVVI	0.20	0.24	0.16	0.28	**0.14**	**0.09**	0.21	**0.10**	**0.08**	-	1.29
OLA	0.19	0.22	0.19	0.27	0.19	0.19	0.23	**0.14**	0.20	0.16	-

2.4.2. Dendrogram analysis

Jaccard pairwise dissimilarity indices were calculated among individuals and resulted in a mean dissimilarity of 0.76 with values ranging from 0.30 to 0.90 with a cophenetic r of 0.95. NJ dendrogram analysis indicated three main clusters: A, B, and C

(Figure 2.4**).** Sections *Majorana* (ϕ), *Prolaticorolla* (λ), and *Longitubus* (δ) were distributed throughout cluster A. Individuals belonging to section *Origanum* (γ) were placed mainly in cluster A with one accession in cluster B. All but one of the 10 *Amaracus* (α) accessions were distributed to cluster B with one accession in cluster A. The two *Anatolicon* (β) accs. (*O. hypericifolium*, OHY1 and *O. sipyleum*, OSI1) were separately placed in clusters B and C. Section *Brevifilamentum* (χ) was dispersed throughout all clusters of the dendrogram. All three of the *Chilocalyx* (ε) accessions were found in subcluster C1.

Cluster A was composed of 22 individuals from six sections. All but one (*O. vulgare* subsp. *gracile*; OVG1) of the accessions belonging to section *Origanum* were found in subcluster A1. Among these individuals, two OVH individuals subclustered within A1. OVVU and OVVI were most closely related to these OVH individuals. OVVU2 was placed with individuals from section *Majorana* in cluster A1. OON and OSY, species in section *Majorana,* were also found in cluster A1 with OON1 and OON2 most closely related to the the *Origanum* individuals. OSY1, OSY2, and OSY3 formed their own subcluster within A1. All six *Prolaticorolla* individuals (OLA) were found in cluster A2. Cluster A3 had an interesting distribution of single individuals belong to *Majorana* (OMA1 and OMA2), *Brevifilamentum* (OLE1), *Longitubus* (*O. amanum*; OAM1), and *Amaracus* (*O. boissieri*; OBO1).

Cluster B was composed of 17 individuals from four sections. Subcluster B1 contained all but one individual (*O. boissieri*, OBO1) of section *Amaracus* which included the species OSA, OBO and *O. solymicum* (OSO). All but one of the OSA (OSA3) individuals were found in this subcluster. OSA3 was most closely related to OSO1 and OHY1 (section *Anatolicon*). OHU individuals clustered together in subcluster B2 which also included OLE2 from the same section, *Brevifilamentum*. Three more *Brevifilamentum* taxa were found in cluster B3: *O. munzurense* (OMU), *O. acutidens* (OAC), and *O. haussknechtii* (OHA). This cluster also contained OVG1, the only *Origanum* accession in cluster B.

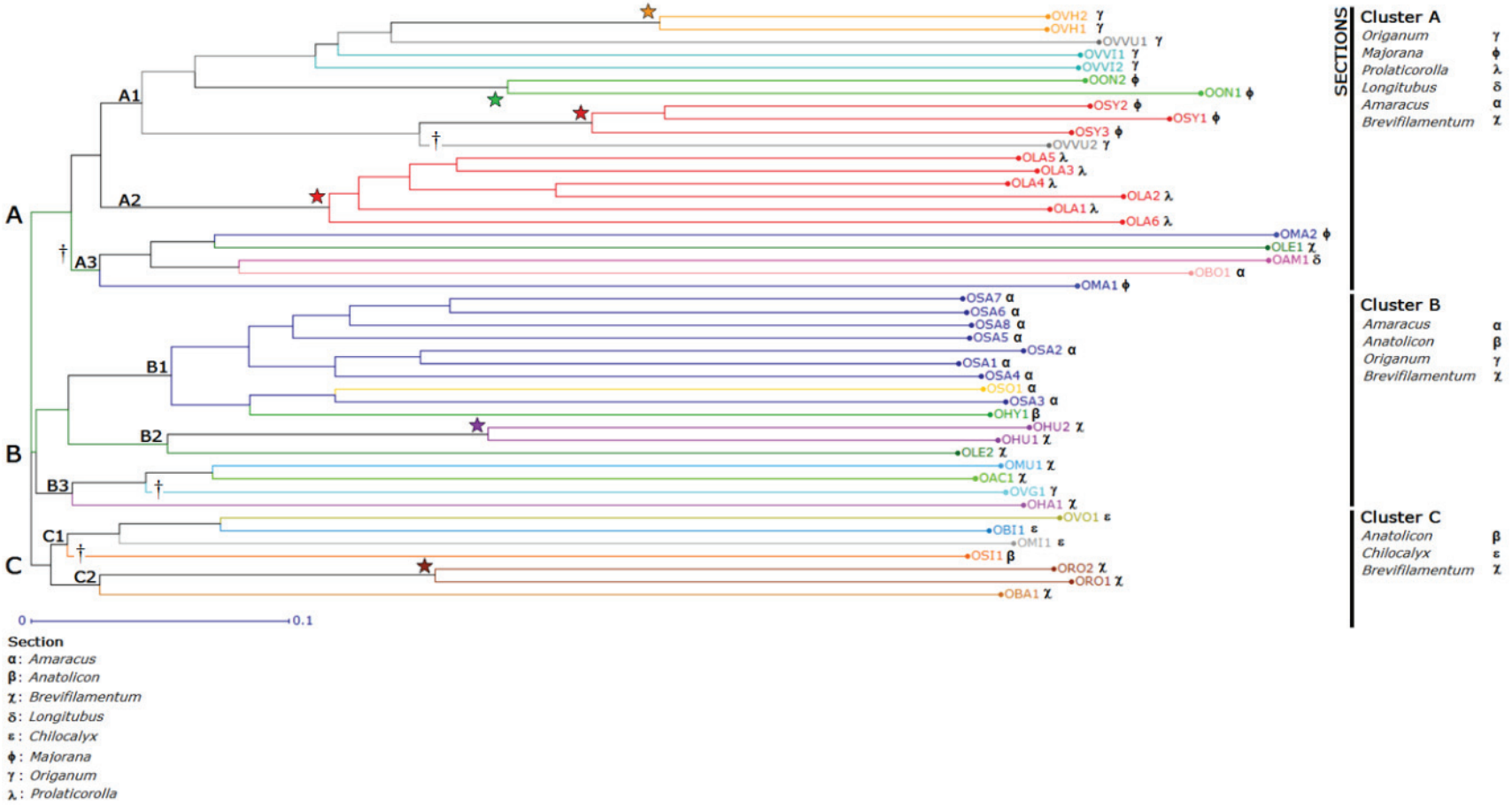


Figure 2.4. The NJ dendrogram of herbarium material for the genus *Origanum* L. Accessions/clusters which may require re-examination are represented by symbol: †.

Cluster C was composed of seven individuals in three sections. The three individuals [*O. vogelii* (OVO), *O. bilgeri* (OBI), and *O. minutiflorum* (OMI)] from section *Chilocalyx* were distributed together in subcluster C1. This subcluster also included the OSI individual from section *Anatolicon*. Subcluster C2 included ORO and the *O. bargyli* (OBA) individual from the same section, *Brevifilamentum*.

2.4.3. Principle coordinate analysis (PCoA)

The PCoA graph was plotted and the eigen values explained 11.93, 8.49, and 6.52 % of the variance, respectively. The PCoA graph resulted in three major clusters for the 24 taxa (Figure 2.5). The distribution of sections was as follows: *Prolaticorolla* (cluster I); *Origanum*, *Majorana*, and *Longitubus* (cluster II); and *Amaracus, Anatolicon*, *Brevifilamentum*, and *Chilocalyx* (cluster III). Interestingly the individuals OLE1 (*Brevifilamentum*) and OAM1 (*Longitubus*) were placed at the intersection of clusters II and III. Cluster I consisted of only OLA individuals which belong to section *Prolaticorolla*. As indicated, cluster II contained four different sects.: *Majorana* with all seven individuals from three different taxa (OMA, OON, and OSY); *Origanum* with its seven individuals from different taxa (OVVU, OVG, OVH, and OVVI); *Longitubus* with its single OAM individual and *Brevifilamentum* with one of the ten individuals from the section. The third cluster was composed of four different sects.: *Amaracus*, *Anatolicon*, *Brevifilamentum*, *Chilocalyx*. Overall, most of the taxa that had more than one individual per population, namely, OLA, OSY, OVVU, OVVI, OVH, OON, OHU, OSA and ORO were clustered as expected in two dimensions. In contrast, OMA (OMA1 and OMA2) and OLE (OLE1 and OLE2) individuals were not closely clustered in the plot.

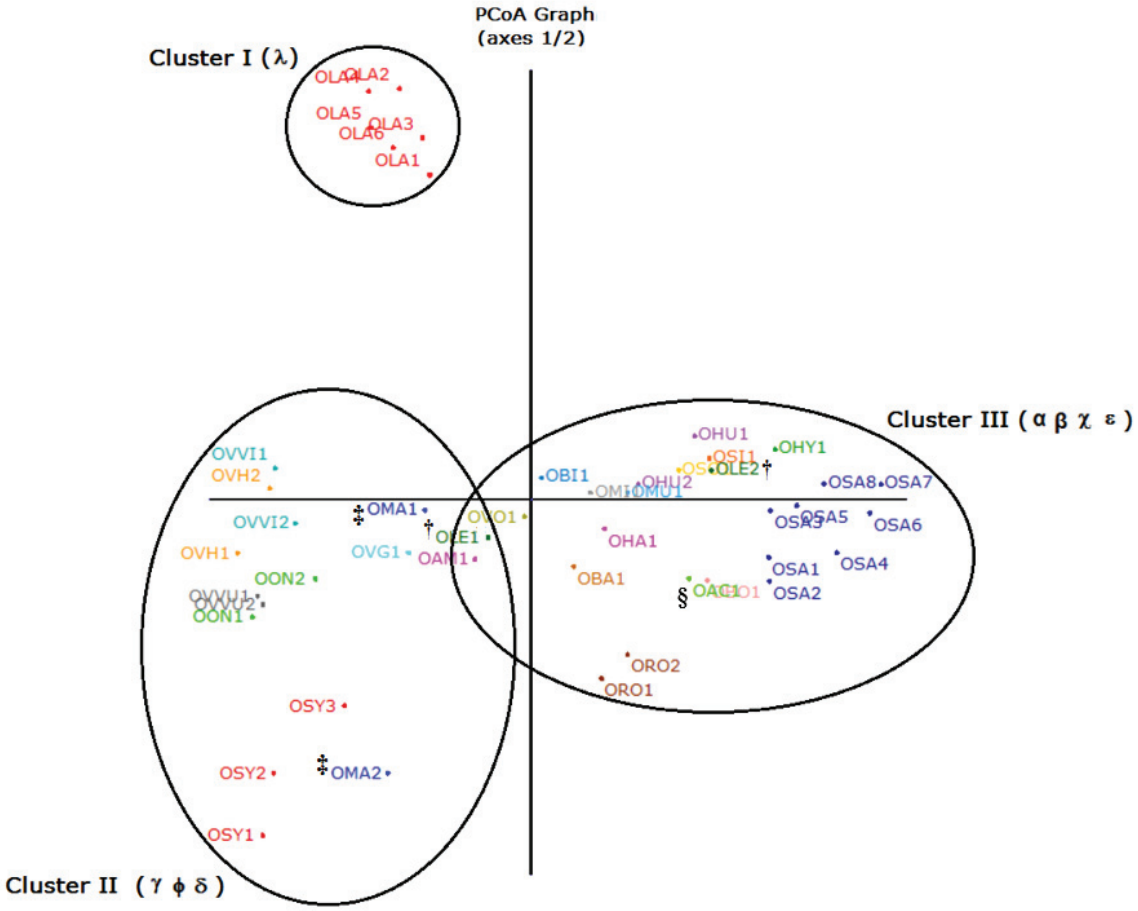


Figure 2.5. PCoA plot representing the three main subclusters for the herbarium collection. Sections are shown in bold with following symbols: **α:** *Amaracus*, **β:** *Anatolicon*, **χ:** *Brevifilamentum*, **δ:** *Longitubus*, **ε:** *Chilocalyx*, **ϕ:** *Majorana*, **γ:** *Origanum*, and **λ:** *Prolaticorolla*.

2.4.4. Population structure analysis

The population structure analysis resulted in two possible optimal numbers of subpopulations (*Ks*) because the most significant delta *K* [Δ(*K*)] values were observed for *K*=3 and *K*=8 (Figure 2.6). An identity threshold value greater than 0.70 was used for classifying individuals into subpopulations or as admixed (Table 2.11). Because the oregano material encompassed eight sections and *K=8* gave the highest likelihood value, the hypothesis of eight subpopulations was examined first (Figure 2.7). The graph for *K*=8 showed that *Prolaticorolla* formed its own subpopulation (SPVIII). In addition, eight of the 10 *Amaracus* individuals, specifically those belonging to OSA, fell into a

single subpopulation (SPV). Section *Brevifilamentum* individuals fell into four subpopulations with the ORO and OHU individuals forming exclusive subpopulations (SPIV and VI, respectively). Section *Majorana* accessions fell almost equally into two subpopulations: SPII and III. Five of the seven accessions from section *Origanum* were admixed while the remainder (OVH individuals) formed their own subpopulation, SPVII. The individuals from sects. *Longitubus* and *Chilocalyx* fell into subpopulations that they shared with other sections (SPI and III) while both *Anatolicon* accessions were admixed.

Overall, six of the eight (75%) subpopulations consisted of a single section (Table 10). Of these exclusive subpopulations, SPIV with two ORO accessions had the highest expected heterozygosity (*He*) value, 0.24 with moderate gene flow (F_{ST} = 0.49) (Table 2.10). The lowest heterozygosity and gene flow were observed in SPVII which contained only OVH. Overall, SPI with two sects. (*Brevifilamentum* and *Chilocalyx*) and five different taxa (OAC, OMI, OHA, OMU, OLE) had the highest *He* and gene flow, values of 0.34 and 0.02, respectively. All but one of these species (OLE) was represented by only one accession.

Table 2.10.Results of Bayesian clustering for *K*=8 and *K*=3. *Ns*: number of sections, ∑*Ni*: total number of individuals, *He*: expected heterozygosity, F_{ST}: fixation index.

K=8				
Sub population	***Ns***	**∑*Ni***	***He***	**F_{ST} values**
SP I	2	5	0.34	0.02
SP II	1	3	0.15	0.65
SP III	4	8	0.23	0.32
SP IV	1	2	0.24	0.49
SP V	1	8	0.22	0.39
SP VI	1	2	0.16	0.66
SP VII	1	2	0.09	0.8
SP VIII	1	6	0.15	0.58
Admixed	4	10		

(Cont. on the next page)

Table 2.10 (Cont.)

K=3				
Sub population	***Ns***	***∑Ni***	***He***	***F_{ST}* values**
SP I	2	7	0.36	0.0008
SP II	1	8	0.21	0.42
SP III	5	19	0.21	0.39
Admixed	5	12		

Notations are as follows: SP = Sub population; *Ns* = Number of sections for that cluster; *∑Ni* = Total number of individuals; *He* = Expected heterozygosity; F_{ST} = Fixation index

An alternative result from the population structure analysis was that the oregano accessions fell into three subpopulations. This hypothesis was supported by the highest Δ(*K*) for all tested values of *K* (Figure 2.6). Based on these results (Figure 2.6), section *Amaracus* fell into an exclusive subpopulation, SPII, containing all OSA individuals but excluding OSO1 and OBO1 individuals which were admixed. Accessions from sects. *Prolaticorolla*, *Origanum*, *Majorana*, *Longitubus* and *Brevifilamentum* fell into SPIII. The remaining *Brevifilamentum* individuals were found in SPI (six individuals) and in the admixed group (three accs.). One accession from section *Chilocalyx* was in SPI and the other two were admixed. The two *Anatolicon* accessions were admixed. As seen with *K* = 8 (Figure 2.7), the SP containing *Brevifilamentum* and *Chilocalyx* accessions had the highest gene flow and heterozygosity (Table 2.11).

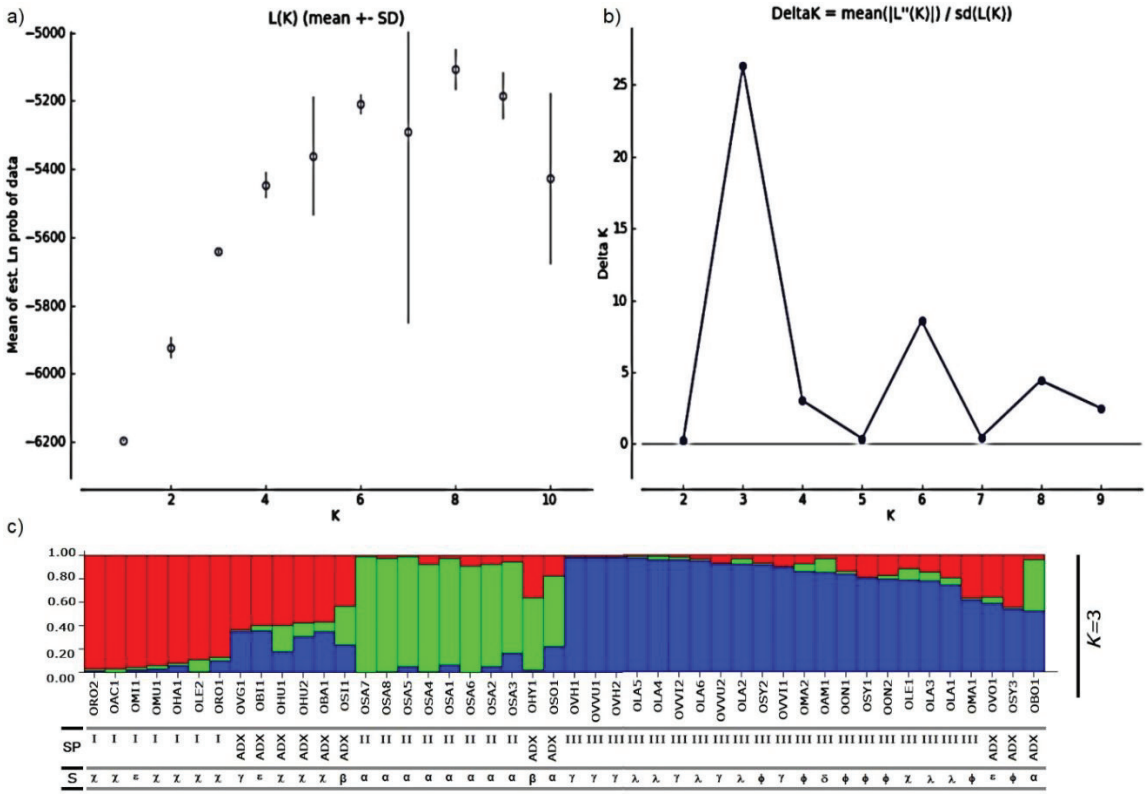


Figure. 2.6.Ln likelihood, Δ*K*, and population structure graph for *K*=3. (a) Likelihood values from Structure Harvester analysis are plotted for the ten different subpopulations tested in the analysis. (b) Delta *K* is plotted for each *K* with peaks indicating a high probability that the number of subpopulations was *K*=3 or *K*=8. (c) Structure graph for *K*=3. Each accession is coded and listed along the x-axis, the proportion of identity to a given subpopulation is indicated by the different colored bars in the y-axis.

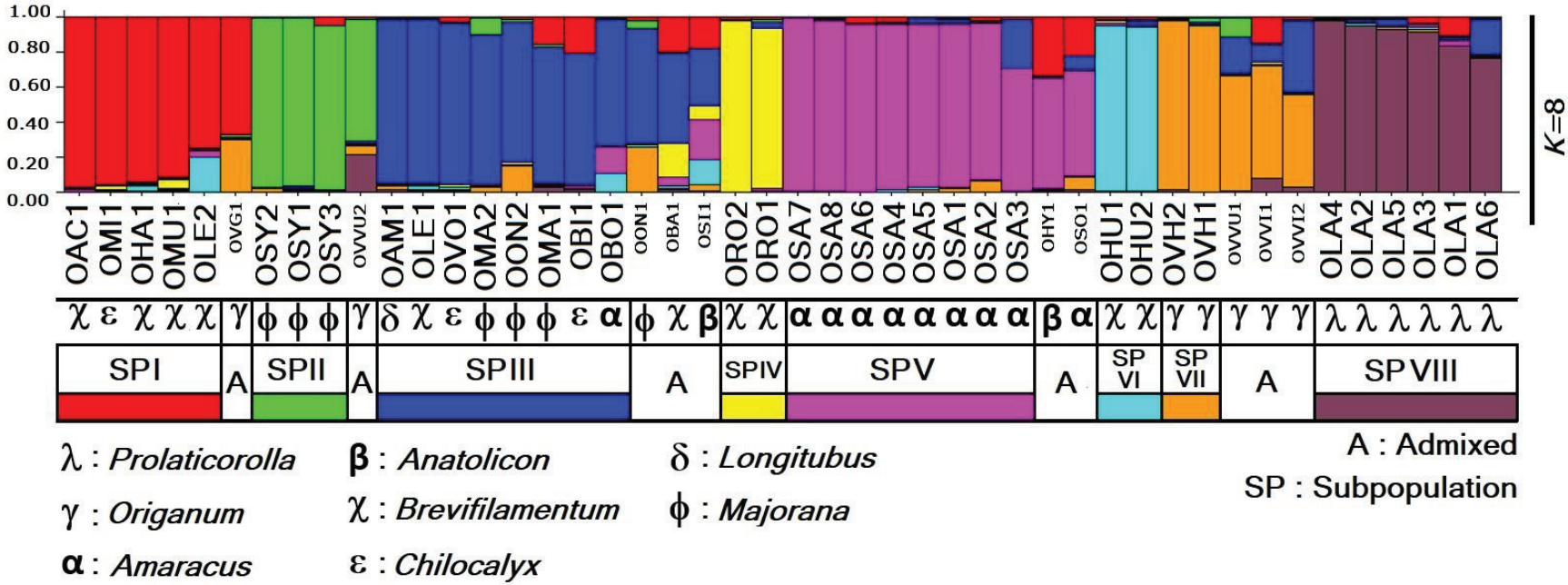


Figure 2.7.The structure of native Turkish oregano herbarium specimens for *K*=8. Each accession is coded and listed along the x-axis, the proportion of identity to a given subpopulation is indicated by the different colored bars in the y-axis.

Table 2.11. Subpopulations and admixed individuals as determined by population structure analysis at *K*=8 and *K*=3

***K*=8**				***K*=3**			
Individual	**Inferred Ancestry**	**Significance Value**	**Section**	**Individual**	**Inferred Ancestry**	**Significance Value**	**Section**
OSO1	Admixed	0.60	***Amaracus, Anatolicon, Brevifilamentum, Majorana,* and *Origanum***	OBO1	Admixed	0.52	***Amaracus, Anatolicon, Brevifilamentum, Chilocalyx Majorana,* and *Origanum***
OHY1	Admixed	0.62		*OSO1*	Admixed	0.60	
OSI1	Admixed	0.33		*OHY1*	Admixed	0.62	
OBA1	Admixed	0.51		*OSI1*	Admixed	0.43	
OON1	Admixed	0.66		OBA1	Admixed	0.57	
OVVU1	Admixed	0.66		OHU1	Admixed	0.59	
OVVU2	Admixed	0.69		OHU2	Admixed	0.58	
OVG1	Admixed	0.70		OBI1	Admixed	0.60	
OVVI1	Admixed	0.65		OVO1	Admixed	0.59	
OVVI2	Admixed	0.53		OMA1	Admixed	0.62	
OAC1	SP I	0.97	***Brevifilamentum* and *Chilocalyx***	OSY3	Admixed	0.54	
OHA1	SP I	0.94		*OVG1*	Admixed	0.63	
OLE2	SP I	0.75		OAC1	SP I	0.97	***Brevifilamentum* and *Chilocalyx***
OMU1	SP I	0.91		OHA1	SP I	0.92	
OMI1	SP I	0.96		OLE2	SP I	0.89	
OSY1	SP II	0.96	***Majorana***	OMU1	SP I	0.94	
OSY2	SP II	0.96		ORO1	SP I	0.87	
OSY3	SP II	0.94		ORO2	SP I	0.97	
OBO1	SP III	0.73	***Amaracus, Brevifilamentum, Longitubus, Chilocalyx,* and *Majorana***	OMI1	SP I	0.96	
OLE1	SP III	0.93		OSA1	SP II	0.91	***Amaracus***
OAM1	SP III	0.94		OSA2	SP II	0.87	
OBI1	SP III	0.75		OSA3	SP II	0.78	
OVO1	SP III	0.92		OSA4	SP II	0.92	

(Cont. on the next page)

Table 2.11. (cont.)

OMA1	SP III	0.78		OSA5	SP II	0.94	
OMA2	SP III	0.86		OSA6	SP II	0.90	
OON2	SP III	0.80		OSA7	SP II	0.99	
ORO1	SP IV	0.92	***Brevifilamentum***	OSA8	SP II	0.96	
ORO2	SP IV	0.98		OLE1	SP III	0.79	***Brevifilamentum,***
OSA1	SP V	0.93	***Amaracus***	OAM1	SP III	0.85	***Longitubus, Majorana,***
OSA2	SP V	0.90		OMA2	SP III	0.86	***Origanum,*** **and**
OSA3	SP V	0.70		OON1	SP III	0.84	***Prolaticorolla***
OSA4	SP V	0.95		OON2	SP III	0.80	
OSA5	SP V	0.94		OSY1	SP III	0.80	
OSA6	SP V	0.95		OSY2	SP III	0.92	
OSA7	SP V	0.99		OVVU1	SP III	0.98	
OSA8	SP V	0.97		OVVU2	SP III	0.92	
OHU1	SP VI	0.94	***Brevifilamentum***	OVH1	SP III	0.98	
OHU2	SP VI	0.94		OVH2	SP III	0.98	
OVH1	SP VII	0.95	***Origanum***	OVVI1	SP III	0.89	
OVH2	SP VII	0.96		OVVI2	SP III	0.96	
OLA1	SP VIII	0.84	***Prolaticorolla***	OLA1	SP III	0.75	
OLA2	SP VIII	0.95		OLA2	SP III	0.92	
OLA3	SP VIII	0.92		OLA3	SP III	0.78	
OLA4	SP VIII	0.98		OLA4	SP III	0.96	
OLA5	SP VIII	0.93		OLA5	SP III	0.97	
OLA6	SP VIII	0.77		OLA6	SP III	0.95	

Definitions are as follows: Individual codes with *italic* font indicate the observed admixed individuals for K=3 and K=8; SP = Sub population.

2.5. Discussion

In this chapter, herbarium material for 22 native oregano species including 24 taxa with 15 endemic species from eight sections of the genus *Origanum* (*Amaracus*, *Anatolicon*, *Brevifilamentum*, *Longitubus*, *Chilocalyx*, *Majorana*, *Origanum,* and *Prolaticorolla*) were screened with EST-SSR and SRAP markers. Only one endemic species, *O. brevidens* (Bornmüller) Dinsmore (in section *Brevifilamentum*), was not included due to an absence of specimens. (Ietswaart, 1980; Duman et al., 1995; Guzelmansur and Lise, 2013).

2.5.1. SRAP and EST-SSR markers in *Origanum*

The genetic diversity among *Origanum* species was investigated in 46 individuals from 24 taxa with SRAP and EST-SSR markers. The SRAP markers yielded 12.04 fragments per primer combination and were highly informative in oregano as is often expected from genetic markers that are based on random or non-sequence specific amplification. Similar results (an average of 13.30 markers) were obtained with RAPD markers in 27 Greek *O. vulgare* subsp. *hirtum* accessions (Katsiotis et al., 2009).

The EST-SSR markers were also highly polymorphic with an average of four fragments per primer pair. The markers were developed by Novak et al. (2008) who obtained an average of 0.87 fragments in 39 *O. vulgare* and *O. majorana* accessions. This discrepancy in polymorphism is probably due to the fact that the current study examined more accessions from a wider variety of taxa. Some of the same EST-SSR markers were also used to examine *O. vulgare* populations from Tunisia and found to be highly polymorphic with an average of five fragments per marker (Mechergui et al., 2017). Additional EST-SSR markers were developed by Ince et al. (2014). Thirty of these markers were tested on 65 samples from eight *Origanum* species (Ince et al., 2014) while 15 were surveyed on 670 *O. compactum* individuals (Aboukhalid et al., 2017). In both studies, the markers yielded a similar number of fragments (one to six) as obtained in our work.

2.5.2. Genetic diversity of Turkish *Origanum*

Overall genetic diversity of the Turkish *Origanum* accessions was high with a mean Jaccard dissimilarity index of 0.76. Ince et al. (2014) also used EST-SSR primers to examine genetic diversity of Turkish *Origanum*. They analyzed 65 individuals from eight species and obtained an average genetic dissimilarity of 0.46. Higher diversity was expected in our study given the broad range of material examined: 46 individuals representing 24 of the 25 *Origanum* taxa. Nearly all *Origanum* species have identical chromosome numbers (2n=30) (Ietswaart, 1980) and the genus can be either self or cross-pollinated with a reported lack of hybridization barriers between species and taxa (Kıtıkı et al., 1997). Cross-pollination and the resulting high diversity are also aided by the geographical proximity of different *Origanum* populations (Loveless and Hamrick, 1984). Because the samples were collected from contiguous regions in Türkiye, the transfer of pollen or seed from one population into another can result in enhanced gene flow (Loveless and Hamrick, 1984). Furthermore, rivers or streams can also spread seed or pollen of *Origanum* species as described by Van Looy et al. (2009).

2.5.2.1. *Section Amaracus*

Ten accessions from three species were sampled from section *Amaracus*. The average diversity of the section was 0.41 (*h*) with highest *NGI* to section *Origanum* (0.87). In the dendrogram and PCoA analyses, the section was most closely related to sections *Anatolicon* and *Brevifilamentum*. This agreed with the gene flow analysis which indicated that *Amaracus* had the highest flow and number of migrants with these two sections. In addition, *Amaracus* and *Brevifilamentum* species have similar habit and inflorescence structure (Ietstwaart, 1980).

NGI and gene flow between the *Amaracus* species could not be measured because two of the taxa (OBO and OSO) had only one sample. The OSA accessions formed their own cluster with OSO indicating their close genetic relationship which was expected given their membership in the same section as well as the geographical proximity of their collection locations in Burdur and Antalya. Unexpectedly, the OBO1 accession was clustered with a mixed group of individuals from various sections in the dendrogram (cluster A3) and population structure analyses. This discrepancy suggests

that this particular sample should be re-examined for its morphology and that additional samples of this species should be collected and molecularly characterized. Based on these results, it should be clear if the position of OBO1 is due to its genetic distinctness from the other *Amaracus* species or if it has been misidentified.

2.5.2.2. *Section Anatolicon*

Two accessions from two species were sampled from section *Anatolicon* with an average heterozygosity of 0.41. *Anatolicon* had highest genetic identity (0.86) with *Origanum* and *Brevifilamentum*. Both *Anatolicon* accessions were admixed according to the population structure analysis and were not closely related in the dendrogram. Instead, the accessions clustered with individuals from sects. *Brevifilamentum*, *Chilocalyx* and *Amaracus*. In agreement with this result, *Anatolicon* had high gene flow with these three sections. Indeed, the most migrants observed between sections in the entire study was seen between *Anatolicon* and *Brevifilamentum*. Moreover, natural hybrids between *Anatolicon* species OSI and *Brevifilamentum* (OBA) have been documented (Ietstwaart, 1980). Thus, high rates of cross-pollination could result in the admixed population structure seen in the OHY and OSI samples. Both OHY and OSI are widely distribution in Türkiye (Sadikoglu, 2012) which may also contribute to their admixed structure. *Anatolicon* has also been reported to hybridize with individuals from section *Majorana* and may hybridize with section *Origanum* (Ietstwaart, 1980). However, especially high gene flow was not observed between these sections in our study. More samples from these species should be collected and analyzed before any conclusions about their genetic structure and relationships with other taxa can be made.

Cross-hybridization is common in the genus *Origanum* and has played a significant role in speciation (Ietswaart, 1980). According to Ietswaart (1980), section *Anatolicon* arose from many years of hybridization between species in sects. *Origanum* and *Amaracus*, and genus *Thymus*. The admixed population structure of the *Anatolicon* accessions studied in this work may reflect the diverse origin of this section. This idea is also supported by the high gene flow observed between *Anatolicon* and *Amaracus* which could be a remnant of the section's origin or could arise from more recent hybridizations.

2.5.2.3. *Section Brevifilamentum*

Ten accessions from seven species were analyzed from section *Brevifilamentum*. The section had an average diversity of 0.40 (*h*) and highest genetic identity with section *Origanum* (0.85). Individual samples were widely spread in the dendrogram and population structure analysis. The section had the highest gene flow and *Nm* with *Anatolicon*. High gene flow were also observed with *Amaracus, Chilocalyx, Origanum* and *Majorana*. The dendrogram and gene flow results indicate the diverse genetic background of *Brevifilamentum*. These findings are in an agreement with Ietswaart's hypothesis (1980) that many of the species in this section arose through ancient hybridizations when *Origanum* populations were restricted to mountainous regions due to climate change and a more arid environment. Natural hybrids between *Brevifilamentum* and *Majorana* were identified by Ietstwaart (1980). The tested *Brevifilamentum* samples were collected from all regions included in our study which spanned from the Mediterranean to the Black Sea and Eastern Anatolia. Thus, the section is widely dispersed in Türkiye and has the opportunity for cross-pollination with many Origanum species.

Of the three *Brevifilamentum* species that had more than one sample, OHU had the highest *h* (0.35) followed by OLE (0.34) and ORO (0.32). *NGI* between these species ranged from 0.75 to 0.76. The two OHU samples clustered together in the dendrogram and formed their own subpopulation. These samples were collected in eastern Antalya and were most closely related to OLE2 which was from the adjacent province, Karaman. Interestingly, in the dendrogram, the other OLE accession was placed in the mixed group from various sections (cluster A3) as described previously. The PCoA agreed with this placement as OLE1 was found at the junction between Clusters II and III. Therefore, it would be useful to test other OLE individuals to determine where they are placed in the analyses. The two ORO accessions formed their own subcluster in the dendrogram and PCoA and their own subpopulation in structure analysis. These samples were most closely related to the OBA individual which is also from section *Brevifilamentum*. The single OAC, OMU and OHA samples clustered in the dendrogram and were in a subpopulation which contained mainly other *Brevifilamentum* accessions. These three samples (OAC, OMU, OHA) were collected from Tunceli and Erzincan which share a border in Eastern Anatolia. These results suggest cross-pollination between these

individuals. However, it is necessary to collect additional samples from these species to get an estimate of their gene flow.

According to Ietswaart (1980), section *Brevifilamentum* arose from hybridizations involving *Amaracus* and the genus Saturejeae. This hybrid origin may still be evident in the fact that the *Brevifilamentum* species were the most widely distributed in the dendrogram and population structure analyses. In addition, the results can be attributed to the high gene flow that is ongoing between *Brevifilamentum* and most of the other sections as well as between some of the *Brevifilamentum* species. Another key factor is the known endemism of most of these species. For example, OHU is endemic to only two locations (Sadikoglu, N; personal communication) and formed its own subpopulation in the structure analysis (K=8). Similarly, ORO which has unknown endemism but is considered to be local (Sadikoglu, N; personal communication), also formed its own subpopulation. Both OHU and ORO had very low gene flow with the other species as expected for populations with restricted geographical locations. In the study of Lukas et al. (2013), there was also low gene flow between pure isolated OON populations and OON accs. in putative hybrid zone with ODU, supporting the idea of geographical isolation effects habitat fragmentation and speciation of new species (Singh, 2022).

2.5.2.4. *Section Chilocalyx*

Three samples from the three species (OBI, OVO, OMI) in section *Chilocalyx* were examined and had an *h* of 0.42. *Chilocalyx* had the highest *NGI* with *Origanum* (0.87). Indeed, putative hybrids between these two sections have been recorded (Ietswaart, 1980). The *Chilocalyx* samples formed a distinct cluster in dendrogram analysis and their own subcluster in PCoA. They were most closely associated with the *Brevifilamentum* accessions, ORO and OBA; and overall, had high gene flow with this section. Significant gene flow was also observed between *Chilocalyx*, *Anatolicon* and *Majorana*. The samples from these sections were collected in the Mediterranean region, thus, facilitating cross-pollination.

Chilocalyx (*CHI*) arose by hybridizations involving sects. *Majorana*, *Origanum* and an unknown genus (Ietswaart, 1980). In our work, *Chilocalyx* had the highest genetic identity with section *Origanum* reflecting its origins. In addition, significant gene flow

was observed between *Chilocalyx* and its other progenitor, *Majorana*. All the *Chilocalyx* species are endemic, and our samples were collected from adjoining Mediterranean locations. Individuals belonging to this section grouped together in our analyses indicating a shared population, however, not enough accessions were studied to examine gene flow and genetic distance.

The accessions from sect. *CHI* were located in subcluster C1 within Cluster C of dendrogram together with sect. *ANA* and the subcluster that they constructed was together with another subcluster, C2, with full of individuals from sect. *BRE*. In the case *K*=3 in Chapter 2, section *AMA* had its own exclusive sub population in SPII, while sections *PRO, ORG, MAJ, LONGI*, and *BRE* was grouped together in SPIII. The involvement of five different sections in SPIII might reflect a hybridization pattern among those sections which were screened with native specimens. From section *CHI*, there were one accession fell in SPI, and the remaming two accs. were admixed. In the study of Tanhaş (2019), a population consisted of 21 specimens with 18 species from eight sections that are naturally grows in Türkiye was investigated with 11 EST-SSR and 16 SRAP combinations. A number of 91 alleles were gathered with a mean value of 0.28 genetic diversity value (Polymorphism Information Content – PIC) and a range of dissimilarities of 0.13 to 0.89 for NJ was observed. In their study, both NJ dendrogram and Structure graph was divided into three groups. In SPI, individuals from sections *CHI*, *BRE*, *MAJ*, *AMA*, and *LONGI* were structured. In SPII, accs. from *AMA*, *PRO*, *MAJ*, *BRE*, *ANA*, and *CHI* were grouped together. In SPIII, *ORG*, *MAJ*, *CHI*, and *BRE* was ensembled together. As a comparison, in their study, *CHI* specimens were fell into all subpopulations, while *ORG* specimen was grouped together with individuals from *MAJ*, *CHI*, and *BRE* sections. In our *K*=3, our *ORG* population (with higher number of specimens) had genetic clustering with *PRO*, *MAJ*, *LONGI*, and *BRE*; while *AMA* and *CHI* had their own sub populations and *ANA* accessions were in admixed structure. The admixed structure of two *CHI* accessions and the placement of *CHI* in subpopulations in both study might reflect the shared intermediate morphological features between section pairs *AMA – ORG* and *MAJ – ORG* with *CHI* (Dirmenci et al., 2020).

2.5.2.5. *Section Longitubus*

Only one sample was used from section *Longitubus* which is comprised of only one species, OAM. The accession clustered with the mixed group of accessions from different sections (Cluster A3) that contained OBO1 (see section *Amaracus*) and was found at the junction between Clusters II and III in the PCoA. Natural hybrids between species of *Longitubus* and *Prolaticorolla* have been described (Ietstwaart, 1980); however, due to a lack of samples, we could not measure gene flow between these sections. Thus, it is evident that additional samples from this species must be collected and analyzed to understand more about the genetic diversity and relationships of this species and section.

Longitubus (*LONGI*) is hypothesized to be originated from genus Saturejeae and section *Amaracus* from genus Origanum (Ietswaart, 1980). According to the same source, *Longitubus* has unique corolla and stamen morphology which distinguish it from the other Origanum sections. In our molecular genetic study, only one individual was included from the section, and it was found to cluster with a variety of species from other sections. Thus, it is apparent that morphological differences are not necessarily reflected at the molecular level. Of course, additional *Longitubus* individuals must be examined to learn more about the genetic relationships of this section with the other oregano species.

2.5.2.6. *Section Majorana*

Seven accessions from the three species in section *Majorana* were analyzed and had an average genetic diversity of 0.41 (*h*). *Majorana* had the highest *NGI* with *Origanum* (0.88). All of the samples were found in the same cluster of the dendrogram. Moreover, OSY and OON accessions formed their own distinct subclusters. The *Majorana* samples were most closely related to individuals from section *Origanum* as seen in the dendrogram and PCoA. These results were confirmed by a high gene flow and *Nm* between these two sections. Relatively high gene flow was also observed between *Majorana*, *Brevifilamentum* and *Chilocalyx*. Natural hybridization was previously observed between *Majorana* and sects. *Anatolicon*, *Brevifilamentum*,

Origanum and *Prolaticorolla* (Ietswaart, 1980) which indicates that *Majorana* alleles may migrate even more than observed in our study.

Within *Majorana*, OSY (0.35) had slightly higher heterozygosity than OMA (0.34) and OON (0.33). High genetic diversity was also identified in the ITS (internal transcribed spacer) region of OSY (Lukas et al., 2013). Genetic identity among the *Majorana* species ranged from 0.74 to 0.78. As in the dendrogram analysis, population structure analysis indicated that OSY accessions formed their own subpopulation. Interestingly, these samples had high *NGI* (0.78) with and were most closely related to one of the two OVVU samples. This relationship was also reflected in a high gene flow between OSY and OVVU. As previously mentioned, the OON samples formed their own dendrogram subcluster. These results are consistent with those of Lukas et al. (2013) who examined the ITS sequences of species in *Majorana* and found that OON individuals formed a distinct group that did not include OSY or OMA. OON is also unusual in the genus *Origanum* because its morphology is relatively homogenous and can be easily distinguished from other species (Lukas et al., 2013). Highest gene flow for OON was with OMA and OVVI.

The two OMA samples did not cluster most closely with each other but were both found in the mixed cluster A3 and in a subpopulation with multiple sections according to population structure analysis. This situation is mirrored at the morphological level in that OMA has characters which vary in natural populations and are not distinct from OSY (Ietswaart, 1980). OMA is an interesting species because, depending on the expert/genebank, it may or may not include individuals from *O. dubium,* a morphologically similar taxon (Lukas et al., 2013). Ietswaart (1980) classified *O. dubium* as a synonym of OMA. However, others who have examined samples at the molecular level argue that *O. dubium* arose by hybridization between OSY and OON (Lukas et al., 2013). Therefore, it is possible that one of our OMA samples could be *O. dubium*, thereby explaining why they did not cluster closely in the dendrogram. OMA had relatively high gene flow with the OLE in *Brevifilamentum* and OVVI in *Origanum*. The close relationships and high gene flow between OSY, OON and the *O. vulgare* subspecies may be a result of their geographical proximity as many of the samples were collected in Mersin, Antalya, and their surrounding regions.

More recently, some researchers have suggested a departure from Ietswaart's classification and have proposed that section *Majorana* be categorized as its own genus (Kaufmann & Wink, 1994). According to this research which examined *rbcL* sequence,

the genetic distance between *O. majorana* (syn. *Majorana hortensis*) and *O. vulgare* (subsp. not given) was 1.4%, a value which is typical for inter-genera comparisons. However, our results did not support this hypothesis as the *Majorana* accessions did not form an outgroup. Ince et al. (2014) obtained similar results with a different set of SSR markers. Moreover, high gene flow (this study) and natural hybridization (Ietswaart, 1980) were observed between section *Majorana* and other *Origanum* sections. Thus, our work suggests that *Majorana* should remain within the genus.

2.5.2.7. *Section Origanum*

Seven accessions from four *Origanum* subspecies were analyzed and found to have an average genetic diversity of 0.42 (*h*). In the dendrogram, all but one of the accessions, OVG1 grouped in cluster A1. However, OVG1 did cluster with all the *Origanum* samples in the PCoA. This disparity could be due to exceptional situation of OVG1 as being the only *Origanum* accession not collected from the Mediterranean. Instead OVG1 was sampled from Tunceli, a remote region in Eastern Anatolia. This region does not contain natural populations of any of the other *O. vulgare* subspecies. Thus, OVG was isolated from the rest. The *Origanum* accessions were most closely related to each other and to *Majorana* accessions. This close relationship with *Majorana* was also reflected by a high level of gene flow and migrants between the two sections and a high value for *NGI* (0.88). In addition, both natural and artificial hybrids have been reported for sections *Origanum* and *Majorana* (Ietswaart, 1980). *Origanum* also had high gene flow with *Brevifilamentum*.

The three subspecies with more than one individual (OVVU, OVH, and OVVI), had similar levels of genetic diversity. The OVH individuals formed their own subpopulation and their own subcluster in dendrogram analysis. OVH also clustered separately from OVVU in the work of Mechergui et al. (2016), which, in common with our work, used the EST-SSRs developed by Novak et al. (2008). Moreover, separate clustering of OVH and OVVU was reported by Katsiotis et al. (2009) using sequence from the ITS1-5.8S-ITS2 region. The remaining *Origanum* individuals were admixed in terms of population structure when the number of subpopulations was assumed to be eight, in agreement with Ietswaart's classification (1980). Gene flow was high between OVH, OVVU and OVVI as expected given that they are subspecies and not distinct

species. Genetic identity between the subspecies was moderate (~0.74) with some "non-Origanum" taxa more closely related to *O. vulgare* subspecies. For example, OVVU had more genetic identity with OHU and OSY (~0.78) than with OVH and OVVI. These results are in an agreement with the gene flow analysis which indicated high interchange with both *Majorana* and *Brevifilamentum* species.

2.5.2.8. *Section Prolaticorolla*

Section *Prolaticorolla* contains only one species, *O. laevigatum* (OLA), and six samples from this species were analyzed in the study. The section had *h* of 0.41 and highest *NGI* with *Origanum*. The section did not have high gene flow with any other section. Average genetic diversity of OLA was 0.33 and this species had relatively high gene flow with only OVVU. All the OLA samples formed a distinct subcluster in the dendrogram and PCoA. This species was most closely related to both *Origanum* and *Majorana* accessions. Natural hybrids between *Prolaticorolla* and *Majorana* have been described as well as between *Prolaticorolla* and *Longitubus* (Ietswaart, 1980). The reason for the seeming genetic distinctness of OLA is unknown given the fact that interspecific hybrids occur, and the species is not geographically isolated.

Prolaticorolla (PRO) originated from hybridizations involving section *Origanum* and the genus Saturejeae (Ietswaart, 1980). Indeed, the section had the highest *NGI* with section *Origanum (ORG)* as may be expected from its origin. The section contains only one species which is endemic to the eastern Mediterranean region of Türkiye (Sadikoglu, 2012). Perhaps as a result of this endemism, gene flow between section *PRO* and the other sections: *AMA, ANA, BRE, CHI, MAJ,* and *ORG*; was the lowest overall and OLA formed its own subpopulation and very distinct clusters in the other analyses. As Tauzet (2012) mentioned about the impact of genetic drift on population size in the process of rare allele fixation, the probability of isolated populations might involve female only plants should be considered for endemic and genetically pure plant species such as OLA collected from Osmaniye, Hatay, and UNK provinces from section *PRO* as they built isolated clusters of endemic OLA all together, which will make them open for hybridization cross-sectional, with other species but their rare alleles for sex determination might allow its fixation and creation of OLA species unless there would be another rare allele related to gender determiners from another taxa.

2.5.3. Population structure of the genus *Origanum*

The population structure analysis of the eight *Origanum* L. sections based on molecular genetic data did not have complete concordance with Ietswaart's classification based on morphology (1980). When the results for eight subpopulations are examined, most of the *Amaracus* (80%, all of the OSA acc.), *Prolaticorolla* (100%) and *Origanum* (71%) individuals fell into distinct subpopulations as expected based on their section assignments. *Anatolicon* individuals (2 acc.) were admixed and did not fall into any subpopulation which was also true of some *Amaracus* (1 acc.), *Origanum* (5 accs.), *Brevifilamentum* (1 acc.) and *Majorana* (1 acc.) accessions. The remaining individuals in *Brevifilamentum* fell into four subpopulations. Two of the subpopulations were exclusive containing only the individuals from one *Brevifilamentum* species, ORO (2 accs.) and OHU (2 accs.). The other two subpopulations also included species from other sections. These mixed subpopulations contained individuals from *Longitubus* and *Chilocalyx*. The members of section *Majorana* were equally split between two subpopulations, one of which was exclusively OSY (3 acc.) from *Majorana*.

Thus, the main difference between Ietswaart's classification and our subpopulations assignments lies in the occurrence of mixed subpopulations. These were most notable for sects. *Brevifilamentum* and *Majorana*. This difference may be simply the result of examining the material at two different levels—morphological versus DNA. Although morphological differences require changes at the DNA level, many more mutations occur in the genome than are apparent from morphology. In addition, the results suggest the possibility that cross-hybridization in these two sections might lead to divergence and speciation. This hypothesis agrees with Ietswaart's work (1980) which indicated that hybridization is the main driver of speciation in the genus. A much weaker factor in speciation is geographical isolation (Ietswaart, 1980). This hypothesis is also supported by our finding that endemic species such OHU formed their own subpopulations.

2.5.4. Hybridization pattern of oregano

Homoploid hybridization is the most common factor in oregano that effects generation of new oregano species and inbred species generation over evolutionary time.

In fact, the oregano species are accumulation lots of allelic variation after each hybridization via recombination frequency. The reported conservation of chromosome numbers among oregano species and findings about morphological conservation of some characters in the literature, molecular observations of high genetic identity values between different *Origanum* L. taxa and the suggests that there is an ongoing homoploid hybridization within and among natural populations. Consequently, the ancestral species are continuing to evolve while new hybrids are being arisen.

Native oregano populations without genetic barriers have the cross-hybridization ability to drive speciation. In some cases, this natural speciation has led to divergence of species at the molecular and morphological levels while; in other cases, species have become admixed or more similar because of transfer of alleles between *Origanum* L. species. Also, the genetic barriers between some oregano species due to their sterile forms (male sterility) with only carrying female organs may affect possible hybridizations negatively as species located near to each other may have mating problem if they are both carrying female organs and it can make them local endemics. This behavior can have different effects on adaptation of the genus to its local environment and result in highly endemic species such as OMU which is only found in one location in Tunceli and mostly found in Cyprus (Lukas et al., 2013). It can also result in broadly distributed species like OON. As it is known, some oregano species are known as ancestors of other oregano species via hybridization within sections such as OMA (ODU in Türkiye) being descended from OSYBE species in nature, and also ODU has a gene flow towards OON. In addition, the assembled contribution of *ANA* to to gene flow was observed as the highest for native Turkish *Origanum* L. herbarium material from eight sections (*ANA – BRE*: 6.08).

The origin of the different *Origanum* sections was proposed by Ietswaart in 1980. According to his hypothesis which assumes 10 sections, sections *Amaracus, Majorana,* and *Origanum* were directly descended from species in the ancestral genus Saturejeae (Ietswaart, 1980). Thus, taxa in these sections are the oldest *Origanum* species. According to Ietswaart (1980), hybridization not only happens between species in the same section, but also occurs cross sectionally (Figure 2.8). The matrix in Figure 2.8 shows the species in sections *Anatolicon, Brevifilamentum, Longitubus, Chilocalyx, Majorana, Origanum* and *Prolaticorolla* that tend to have natural hybrids. This may explain why OON (*MAJ*) and OVVU (*ORG*) are the most numerous and widely distributed oregano species in Türkiye, respectively (Sadikoglu, 2012). This is also

evident in the large numbers of migrants that were observed between sections *Majorana* and *Origanum* in our study as well as the natural hybrids identified previously (Ietswaart, 1980; Dirmenci et al., 2021). Interestingly, despite its ancient origins *Amaracus* did not exhibit high gene flow with the other two original sections in our work. This may be attributed to the very limited distribution of *Amaracus* species in Türkiye (Sadikoglu, N; personal communication). In contrast, artificial hybridization and hybridization with one cultivated parent are more common within section *Amaracus* and between sections *Amaracus, Anatolicon* and *Longitubus*.

Worldwide, 20 hybrids in the genus *Origanum* have been reported and 13 of them naturally occur in Türkiye (Arabacı et al., 2021; Dirmenci et al., 2021a). The hybrid species mostly share phenotypic similarities in calyx, leaf, corolla size and number of spikes (Davis, 1949; Dirmenci et al., 2019). However, it is reported that intermediate hybrid species mostly conserve a common morphology with one of the parents (Arabacı et al., 2021). There are both natural and artificial hybrids reported in the literature and some of them are: *O. x dolichosiphon* (OAM x OLA), *O. x intermedium* (OSI x OON), *O. x haradjanii* (OSYBE x OLA), *O. intercedens* (OVH – OON), *O. x adanense* (OBA – OLA), *O. x adae* (*O. aylineae* – OSI) , *O. x aytacii* (OSI – OVH), *O. malyeri* (OBO x OVH) (Taş, 2010). In addition, a molecular study supported that *O. munzurense* is no longer a species in section *BRE*, it is a hybrid species that must be accepted as *O. x munzurense* (OAC x OVG) (Dirmenci et al., 2019).

An update of the hybridization scheme among oregano sections suggested by Ietswaart (1980) has been prepared by Dirmenci et al. (2021) (Figure 2.9; Figure 2.10). In comparison, Dirmenci reports hybridizations between sections *Amaracus – Brevifilamentum*, *Amaracus – Majorana*, *Amaracus – Origanum*, *Brevifilamentum – Anatolicon*, *Brevifilamentum – Origanum*, *Brevifilamentum – Prolaticorolla*, *Majorana – Amaracus*, *Origanum – Amaracus*, *Origanum – Brevifilamentum*, and *Prolaticorolla – Brevifilamentum* that were not reported in Ietswaart's earlier hybridization scheme. On the contrary, hybridization between sections *Longitubus – Amaracus* was not included in the updated work when compared to Ietswaart's (1980) report.

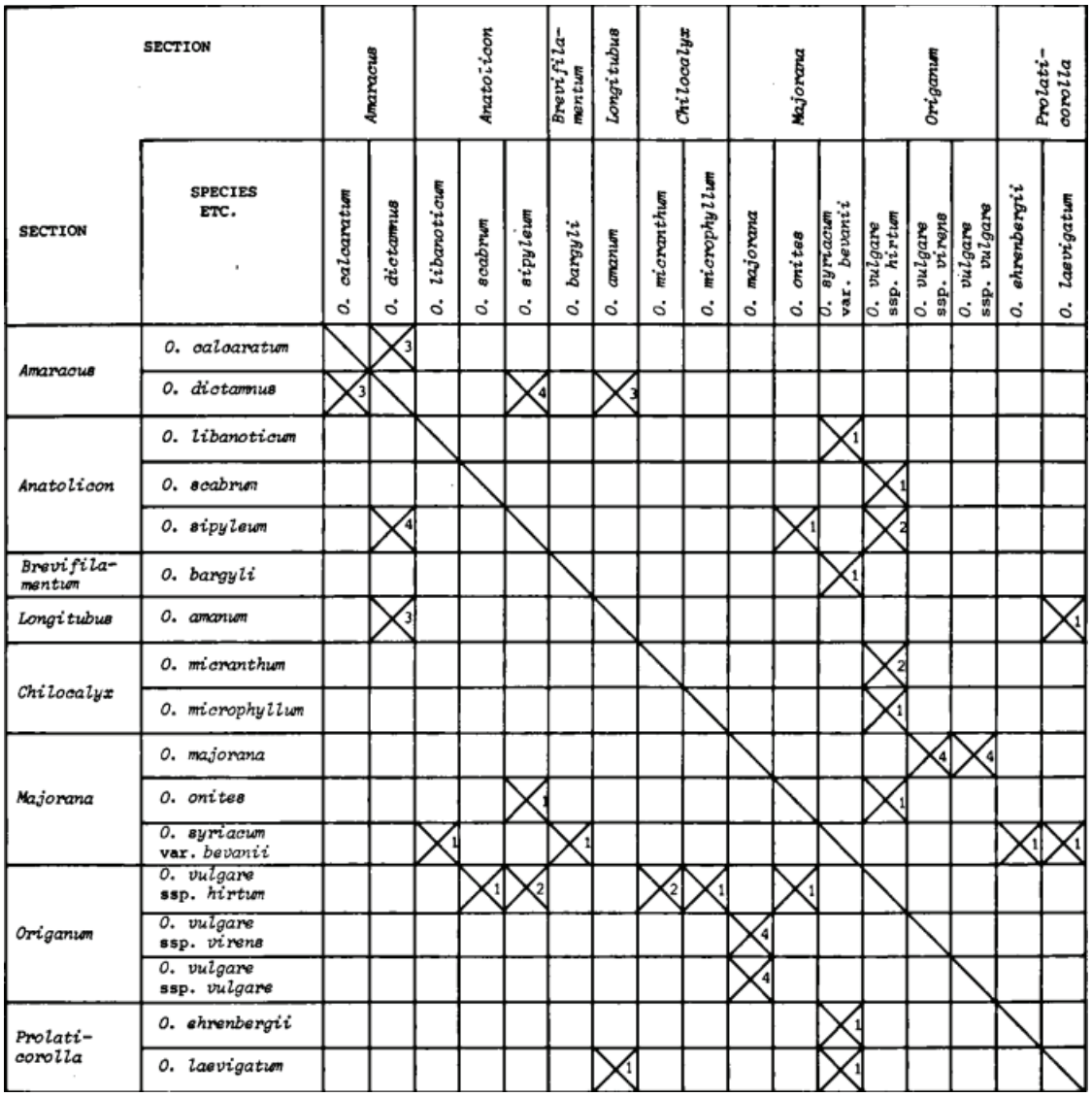


Figure 2.8. Hybrid matrix according to Ietswaart (1980). The four main types of hybridization events are categorized as 1) hybrid formation between parents in natural habitats, 2) putative hybrids occurring at natural sites, 3) successfully made artificial hybridization between cultivated parents, and 4) hybrids from at least one cultivated parent.

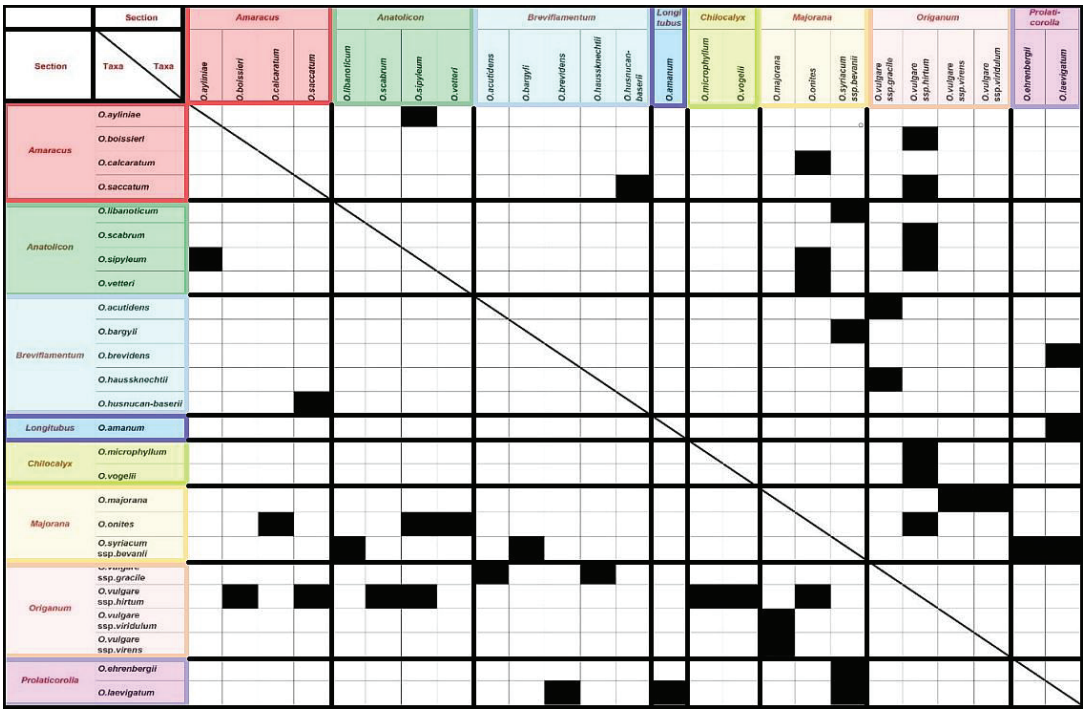


Figure 2.9.Update in cross-sectional hybridization matrix of Ietswaart's (1980) by Dirmenci et al. (2021). Black boxes indicate hybridizations.

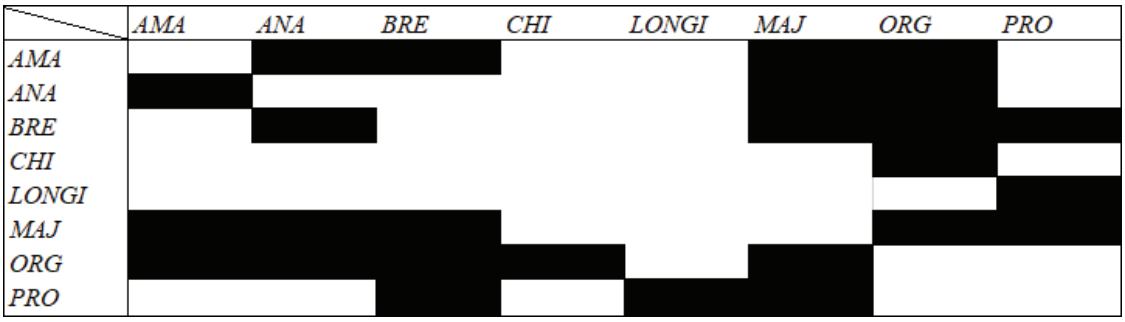


Figure 2.10. Representation of cross-sectional hybridizations for natural oregano hybrids according to knowledge given by Dirmenci et al. (2021). Black boxes indicate hybridizations.

The recent scheme for cross-sectional hybridizations of Dirmenci et al. (2021) indicates that *Chilocalyx*, *Longitubus*, *Amaracus*, *Anatolicon* and *Prolaticorolla* sections have low tendency to cross pollinate with most of the sections. Instead, they prefer to hybridize with certain sections in nature. In contrast, sections *Origanum*, *Brevifilamentum* and *Majorana* had the highest likelihood to cross hybridize with several sections. The findings of our work support this hyphothesis by the observation of the highest gene flow between section *Anatolicon* and *Brevifilamentum* and a by the

observation of the highest genetic similarity between sections *Majorana* and *Origanum*, respectively.

Given the complex relationships among oregano accessions and species, we studied genetic diversity and relationships using molecular markers.

2.5.5. Conclusion

In our study, population structure analysis at *K*=8 suggested that some of the species from sections such as *Brevifilamentum* and *Majorana* may eventually form their own sections. *K*=8 was selected because it matches Ietswaart's taxonomy and gave the highest likelihood value. However, *K*=3 gave the highest delta *K* value indicating that the data also fit the hypothesis of three subpopulations. According to this hypothesis, 11 of the accessions were admixed, *Amaracus* formed its own subpopulation, most of the *Brevifilamentum* samples formed another subpopulation and the remaining sections were mixed in the third subpopulation. Thus, our results stress that the hybridization behavior of *Origanum* has complicated its taxonomy and that both morphological and molecular data should be considered when proposing revisions to the genus. In addition, it is evident that more samples from each species must be examined to understand diversity and genetic relationships at the species and section levels in more depth. At the same time such work will aid in conservation efforts by providing methods for species identification. Thus, seeds of known specimens can be collected from nature for tissue culture, greenhouse, field and breeding applications. Such conservation will allow researchers to examine more samples and to devise a robust classification which prevents misidentification and mislabeling of seeds, plants, and culinary and medicinal products. Combination of all knowledge in the scientific reports and molecular data gained in this chapter for the genus *Origanum* L. strongly suggests that some of the oregano taxa are still under natural selection and this leads to a high genetic diversity among sections and within species. For a global coverage of speciation dynamics of oregano species, the fundamentals of hybridization behaviors must be investigated in further studies to discriminate possible reverse or forward speciation in recent events and the evolutionary predominancy of any ancestral species must be assured.

CHAPTER 3

MOLECULAR CHARACTERIZATION OF *Origanum* L. SPECIMENS FROM ARRI GENE BANK NATIONAL COLLECTION

3.1. Introduction

Türkiye has three topographical regions resulting in a diverse biogeography and rich flora containing many economically important, endemic, and widely distributed plant species. Conservation of wild and cultivated plant species is under governmental control with authorized Institutions. In Türkiye, there are two main Gene Banks: in İzmir and Ankara. There are also 57 herbaria in 36 provinces (Index Herbariorum online webserver, 2021). Worldwide there are ten *in situ* plant conservation fields for crop wild relatives and eight of them are in Türkiye (Zair et al., 2021). This is due to the geographic location of Türkiye as a center of origin for many plants. Crop wild relatives are also conserved "*ex situ*", however this preservation only covers 70% of species in gene banks meaning that the rest of the wild relatives are not conserved in gene banks. Thus, there is an urgent need to add under-represented species to gene banks (Zair et al., 2021). The responsibilities of gene banks are considerable—they must manage the collected plants, register them, cultivate and propagate them. In addition, the specimens should be defined not only for their morphological features but also should be investigated at the molecular level. The more genetic resources that are conserved, the more genetic richness can be achieved in both nature and cultivated plants.

In the last decades, molecular markers are frequently utilized to discriminate species by combining molecular evidence with known morphological characters. The morphological characterization of oregano species by Ietswaart (1980) is an invaluable review of the taxonomy of the genus *Origanum* L. As Ietswaart found, natural hybridization is the main source of variation in the genus and it is important to investigate the sources of this variation by means of determining which specific hybridizations have occured. The high frequency of cross hybridization within the genus *Origanum* makes it

hard to estimate the speciation dynamics of oreganos. Moreover, hybridization behavior can change over time. For example, endemic oregano species and their intermediate hybrids generate adaptive behaviors to altering climate and land conditions. Chemotype variation may arise from "lineage specific gene expression" of enzymes such as terpene synthase which occupies a central role for monoterpene synthesis in the Lamiaceae (Lichman et al., 2020). Such microevolutionary changes in oregano chemical diversity can contribute to macroevolutionary pathways by affecting plant-pollinator interactions therby resulting in hybridization of oregano species.

A few previous studies have examined oregano species with molecular markers. The genetic diversity of 14 native *O. onites* clones collected from three provinces (Antalya, Muğla and İzmir) were investigated by Tonk et al. (2010) with 26 Random amplified polymorphic DNA (RAPD) markers. Their analyses resulted in 75.3% marker polymorphism with a total of 412 alleles. According to Jaccard analysis, their clone population clustered into three subgroups and individuals clustered together according to their geographical locations. The low In previous chapter (Chapter 2), molecular genetic diversity and population structure of 46 oregano genotypes from 22 species were investigated with 25 SRAP combinations (Taşcıoğlu et al., 2018). As a result, a total of 325 polymorphic alleles were observed with a mean of 12.04 alleles. Jaccard analysis and Principle Coordinate Analysis (PCoA) resulted in three main clusters while the best number of populations within germplasm ware assumed as K=3 and 8 with the Bayesian approach. As a result, individuals from section *Prolaticorolla* and *Amaracus* formed their exclusive subpopulations for K=8. In 2017, Mechergui et al. developed 13 new SSR markers and investigated genetic diversity of *O. glandulosum* (OGL) specimens from Tunisia districts and samples from the German Gene Bank [*O. vulgare* subsp. *hirtum* (USA) and *O. vulgare* subps. *vulgare* (Italy)] and observed high gene flow between OGL accessions but low gene flow was observed in samples from the gene bank (Mechergui et al., 2017). They hypothesized that populations with self-pollination are more strongly differentiated from each other than populations of cross-pollinated species. Recently, Alekseeva and colleagues (2021) screened a Bulgarian OVH population consisting of 239 individuals (eight populations) with 11 SSRs and eight SRAPs and found ten genetic clusters within the OVH population and concluded that SRAP markers are more informative than SSR markers. Their work resulted in F_{ST} values between 0.0047 and 0.11, reflecting a low and moderate gene flow among OVH taxa.

In the current work, accessions from the Aegean Agricultural Research Institute (AARI) in Izmir representing the Turkish oregano germplasm with 130 specimens belonging to eight taxa (11 species: ODU, OHA, OHY, OLE, OON, OSI, OSYBE, OVG, OVH, OVVI, and OVVU) from the genus *Origanum* were examined with 10 SRAP markers to gain knowledge about their genetic diversity and population structure. The determination of gene flow among these taxa will contribute to revision of the taxonomy of the sections *Anatolicon*, *Brevifilamentum*, *Majorana*, and *Origanum* within the genus *Origanum* which is currently based on morphological variation.

3.2. Plant Material

Accessions belonging to the *Origanum* collection at AARI (Table 3.1) consisted of 130 specimens that were collected from six region of Türkiye (Central Anatolia, Marmara, Mediterranean, East Anatolia, Black Sea and Aegean regions) between the years 1989 and 2015 (Figure 3.3). Seeds of these accessions were planted in the field at AARI in 2019 (Figure 3.1) with multiple replicates. Specimens were collected from mature leaves as bulks from multiple plants at the flower bud stage.

Figure 3.1. Plant material in the field at AARI.

Soil and other contaminants were discarded by washing the samples and specimens kept at -80℃ after homogenization with liquid nitrogen (Figure 3.2).

Figure 3.2. An image displaying the cleaning process of specimens collected from AARI field.

Figure 3.3. Collection locations of accessions used in this study that are conserved at AARI. Numbers in parentheses: The Darwin codes for multiple samples of a certain taxa given in Table 3.1 that were used in this study from the same province.

Table 3.1. Plant material belonging to oregano species that are conserved at Aegean Agricultural Research Institute (AARI) and were used in the genetic diversity analyses in Chapter 3. ENo: code number for experimental; AARI No: code number of *Origanum* species for each specimen conserved at the AARI gene bank; Darwin Code: the code as an abbreviation of taxa names in dissimilarity analyses; S: Section; *MAJ*: *Majorana* (each section is followed by the symbol used in the Structure graph; *ANA*: *Anatolicon*; *BRE*: *Brevifilamentum*; *ORG*: *Origanum*; GR: geographic region; MT: Mediterranean region; BS: Black Sea region; A: Aegean region; M: Marmara region; CA: Central Anatolia region; EA: Eastern Anatolia region; E: Endemic species (indicated with plus; +); CY-GBRY: Collection Year – Gene Bank Registration Year; TR: Türkiye; minus (-): unknown/ no information.

ENo	AARI No	RNo	Species Name	Darwin Code	Section	E	Type	Discrete Location	Sampling Location	GR	CY-GBRY
1	1	TR 53149	*Origanum dubium*	ODU1	*MAJ* – ϕ	-	Wild	Gazipaşa	Antalya, TR	MT	1990
2	2	TR 53153	*Origanum dubium*	ODU2	*MAJ* – ϕ	-	Wild	Alanya	Antalya, TR	MT	1990
3	3	TR 53209	*Origanum dubium*	ODU3	*MAJ* – ϕ	-	Wild	Araç	Kastamonu, TR	BS	1990
4	4	TR 53194	*Origanum onites*	OON1	*MAJ* – ϕ	-	Wild	Perge	Antalya, TR	MT	1990
5	5	TR 53162	*Origanum dubium*	ODU4	*MAJ* – ϕ	-	Wild	Manavgat	Antalya, TR	MT	1990
6	6	TR 53169	*Origanum dubium*	ODU5	*MAJ* – ϕ	-	Wild	Akseki	Antalya, TR	MT	1990
7	7	TR 53244	*Origanum dubium*	ODU6	*MAJ* – ϕ	-	Wild	Küre	Kastamonu, TR	BS	1990
8	8	TR 54467	*Origanum onites*	OON2	*MAJ* – ϕ	-	Wild	Düden	Antalya, TR	MT	1990
9	9	TR 54471	*Origanum onites*	OON3	*MAJ* – ϕ	-	Wild	Milas	Muğla, TR	A	1991
10	10	TR 54473	*Origanum onites*	OON4	*MAJ* – ϕ	-	Wild	Muğla	Muğla, TR	A	1991
11	11	TR 54480	*Origanum onites*	OON5	*MAJ* – ϕ	-	Wild	Köyceğiz	Muğla, TR	A	1991
12	12	TR 54483	*Origanum onites*	OON6	*MAJ* – ϕ	-	Wild	Dalaman	Muğla, TR	A	1991
13	13	TR 54484	*Origanum onites*	OON7	*MAJ* – ϕ	-	Wild	Göcek	Muğla, TR	A	1991
14	14	TR 54477	*Origanum onites*	OON8	*MAJ* – ϕ	-	Wild	Marmaris	Muğla, TR	A	1991
15	15	TR 54485	*Origanum onites*	OON9	*MAJ* – ϕ	-	Wild	Göcek	Muğla, TR	A	1991

(Cont. on the next page)

Table 3.1. (cont.)

16	16	TR 54490	*Origanum onites*	OON10	*MAJ* – ϕ	-	Wild	Fethiye	Muğla, TR	A	1991
17	17	TR 54502	*Origanum onites*	OON11	*MAJ* – ϕ	-	Wild	Göknük	Antalya, TR	MT	1991
18	18	TR 54493	*Origanum onites*	OON12	*MAJ* – ϕ	-	Wild	Kekova	Antalya, TR	MT	1991
19	19	TR 54512	*Origanum onites*	OON13	*MAJ* – ϕ	-	Wild	Tire	İzmir, TR	A	1991
20	20	TR 54513	*Origanum onites*	OON14	*MAJ* – ϕ	-	Wild	Tire	İzmir, TR	A	1991
21	21	TR 54514	*Origanum onites*	OON15	*MAJ* – ϕ	-	Wild	Tire	İzmir, TR	A	1991
22	22	TR 54516	*Origanum onites*	OON16	*MAJ* – ϕ	-	Wild	Kiraz	İzmir, TR	A	1991
23	23	TR 54517	*Origanum onites*	OON17	*MAJ* – ϕ	-	Wild	Köşk	Aydın, TR	A	1991
24	24	TR 54522	*Origanum onites*	OON18	*MAJ* – ϕ	-	Wild	Nazilli	Aydın, TR	A	1991
25	25	TR 54525	*Origanum onites*	OON19	*MAJ* – ϕ	-	Wild	Bozdoğan	Aydın, TR	A	1991
26	26	TR 54530	*Origanum onites*	OON20	*MAJ* – ϕ	-	Wild	Denizli	Denizli, TR	A	1991
27	27	TR 54531	*Origanum onites*	OON21	*MAJ* – ϕ	-	Wild	Denizli	Denizli, TR	A	1991
28	28	TR 54536	*Origanum onites*	OON22	*MAJ* – ϕ	-	Wild	Demirci	Manisa, TR	A	1991
29	29	TR 54544	*Origanum onites*	OON23	*MAJ* – ϕ	-	Wild	Akhisar	Manisa, TR	A	1991
30	30	TR 54545	*Origanum onites*	OON24	*MAJ* – ϕ	-	Wild	Ayvacık	Çanakkale, TR	M	1991
31	31	TR 54547	*Origanum onites*	OON25	*MAJ* – ϕ	-	Wild	Ayvacık	Çanakkale, TR	M	1991
32	32	TR 54548	*Origanum onites*	OON26	*MAJ* – ϕ	-	Wild	Ayvacık	Çanakkale, TR	M	1991
33	33	TR 54561	*Origanum onites*	OON27	*MAJ* – ϕ	-	Wild	Bergama	İzmir, TR	A	1991
34	34	-	-	UNK1	-	-	-	-	-	-	-
35	35	TR 54442	*Origanum onites*	OON28	*MAJ* – ϕ	-	Wild	Köyceğiz	Muğla, TR	A	1989
36	36	TR 54435	*Origanum dubium*	ODU7	*MAJ* – ϕ	-	Wild	Yatağan	Muğla, TR	A	1989
37	37	TR 54497	*Origanum onites*	OON29	*MAJ* – ϕ	-	Wild	Demre	Antalya, TR	MT	1991
38	38	TR 54520	*Origanum onites*	OON30	*MAJ* – ϕ	-	Wild	Nazilli	Aydın, TR	A	1991
39	39	TR 54563	*Origanum onites*	OON31	*MAJ* – ϕ	-	Wild	Menemen	İzmir, TR	A	1991
40	40	TR 54476	*Origanum onites*	OON32	*MAJ* – ϕ	-	Wild	Yenice	Muğla, TR	A	1991
41	42	TR 57124	*Origanum sipyleum*	OSI1	*ANA* – β	+	Wild	Manisa	Manisa, TR	A	1993

(Cont. on the next page)

Table 3.1. (cont.)

42	43	TR 54445	*Origanum onites*	OON33	*MAJ* – ϕ	-	Wild	Köyceğiz	Muğla, TR	A	1989
43	44	TR 54596	*Origanum onites*	OON34	*MAJ* – ϕ	-	Wild	Köşk	Aydın, TR	A	1991
44	45	TR 54549	*Origanum onites*	OON35	*MAJ* – ϕ	-	Wild	Ayvacık	Çanakkale, TR	M	1991
45	46	TR 54552	*Origanum onites*	OON36	*MAJ* – ϕ	-	Wild	Havran	Balıkesir, TR	M	1991
46	47	TR 54554	*Origanum onites*	OON37	*MAJ* – ϕ	-	Wild	Burhaniye	Balıkesir, TR	M	1991
47	48	TR 54557	*Origanum onites*	OON38	*MAJ* – ϕ	-	Wild	Ayvalık	Balıkesir, TR	M	1991
48	49	TR 54562	*Origanum onites*	OON39	*MAJ* – ϕ	-	Wild	Söke	Aydın, TR	A	1991
49	50	TR 54595	*Origanum onites*	OON40	*MAJ* – ϕ	-	Wild	Sütçüler	Isparta, TR	MT	1991
50	51	TR 54594	*Origanum onites*	OON41	*MAJ* – ϕ	-	Wild	Bucak	Burdur, TR	MT	1991
51	52	TR 54579	*Origanum onites*	OON42	*MAJ* – ϕ	-	Wild	-	-	-	-
52	53	TR 54441	*Origanum vulgare*	OVVU1	*ORG* – γ	-	Wild	Köyceğiz	Muğla, TR	A	1989
53	54	TR 58558	*Origanum dubium*	ODU8	*MAJ* – ϕ	+	Wild	Kumluca	Antalya, TR	MT	1993-1995
54	56	TR 60214	*Origanum leptocladum*	OLE1	*BRE* – χ	+	Wild	Ermenek	Karaman, TR	CA	1994-1995
55	57	TR 60424	*Origanum syriacum* subsp. *bevanii*	OSYBE1	*MAJ* – ϕ	-	Wild	Savastepe	Balıkesir, TR	M	1994-1995
56	58	TR 60427	*Origanum sipyleum*	OSI2	*ANA* – β	+	Wild	Savastepe	Balıkesir, TR	M	1994-1995
57	59	TR 60516	*Origanum sipyleum*	OSI3	*ANA* – β	+	Wild	İskilip	Çorum, TR	BS	1994-1995
58	60	TR 60561	*Origanum haussknechtii*	OHA1	*BRE* – χ	+	Wild	Divriği	Sivas, TR	CA	1994-1995
59	61	TR 61058	*Origanum dubium*	ODU9	*MAJ* – ϕ	-	Wild	Taşeli	Antalya, TR	MT	1993-1995
60	62	TR 61104	*Origanum hypericifolium*	OHY1	*ANA* – β	+	Wild	Beyağaç	Denizli, TR	A	1994-1995

(Cont. on the next page)

Table 3.1. (cont.)

61	63	TR 69502	*Origanum onites*	OON43	*MAJ – ϕ*	-	Wild	Anamur	İçel, TR	MT	1996-2000
62	64	TR 73007	*Origanum dubium*	ODU10	*MAJ – ϕ*	-	Wild	Gündoğmuş	Antalya, TR	MT	2002-2005
63	65	TR 73014	*Origanum vulgare* subsp. *viride*	OVVI1	*ORG – γ*	-	Wild	Alanya	Antalya, TR	MT	2002-2005
64	66	TR 73548	*Origanum vulgare*	OVVU2	*ORG – γ*	-	Wild	Bozüyük	Bilecik, TR	M	2005
65	67	TR 73563	*Origanum sipyleum*	OSI4	*ANA – β*	+	Wild	Eskigediz	Kütahya, TR	A	2005
66	68	TR 76974	*Origanum syriacum* subsp. *bevanii*	OSYBE2	*MAJ – ϕ*	-	Wild	Karaisalı	Adana, TR	MT	2007
67	69	TR 76979	*Origanum syriacum* subsp. *bevanii*	OSYBE3	*MAJ – ϕ*	-	Wild	Topçalı	Kahramanmaraş, TR	MT	2007
68	70	TR 76983	*Origanum sipyleum*	OSI5	*ANA – β*	+	Wild	Kırkağaç	Manisa, TR	A	2007
69	71	TR 76984	*Origanum onites*	OON44	*MAJ – ϕ*	-	Wild	Kırkağaç	Manisa, TR	A	2007
70	72	TR 76985	*Origanum sipyleum*	OSI6	*ANA – β*	+	Wild	Spil	Manisa, TR	A	2007
71	73	TR 76988	*Origanum onites*	OON45	*MAJ – ϕ*	-	Wild	Elmalı	Antalya, TR	MT	2002-2008
72	74	TR 76990	*Origanum syriacum* subsp. *bevanii*	OSYBE4	*MAJ – ϕ*	-	Wild	Elmalı	Antalya, TR	MT	2002-2008
73	75	-	-	UNK2	-	-	-	-	-	-	-
74	76	-	-	UNK3	-	-	-	-	-	-	-
75	77	TR 76997	*Origanum vulgare*	OVVU3	*ORG – γ*	-	Wild	Alanya	Antalya, TR	MT	2002-2008
76	78	TR 76999	*Origanum vulgare* subsp. *hirtum*	OVH1	*ORG – γ*	-	Wild	Gazipaşa	Antalya, TR	MT	2002-2008
77	79	-	-	UNK4	-	-	-	-	-	-	-

(Cont. on the next page)

Table 3.1. (cont.)

78	81		-	UNK5	-	-			-		
79	82	TR 77673	*Origanum vulgare* subsp. *hirtum*	OVH2	*ORG – γ*	-	Wild	Göksun	Kahramanmaraş, TR	MT	2008-2009
80	83	TR 77675	*Origanum vulgare* subsp. *hirtum*	OVH3	*ORG – γ*	-	Wild	Kahramanmaraş	Kahramanmaraş, TR	MT	2008-2009
81	84	TR 77676	*Origanum vulgare* subsp. *hirtum*	OVH4	*ORG – γ*	-	Wild	Kahramanmaraş	Kahramanmaraş, TR	MT	2008-2009
82	85	TR 82335	*Origanum vulgare* subsp. *gracile*	OVG1	*ORG – γ*	-	Wild	Erzincan	Erzincan, TR	EA	2011-2014
83	86	TR 82855	*Origanum sipyleum*	OSI7	*ANA – β*	+	Wild	Acıpayam	Denizli, TR	A	2014-2015
84	87	TR 80181	*Origanum dubium*	ODU11	*MAJ – ϕ*	-	Wild	Menemen	İzmir, TR	A	2010-2011
85	88	TR 80182	*Origanum vulgare*	OVVU4	*ORG – γ*	-	Wild	Tire	İzmir, TR	A	2005-2011
86	90	-	-	UNK6	-	-	-	-	-	-	-
87	95	-	-	UNK7	-	-	-	-	-	-	-
88	118	-	-	UNK8	-	-	-	-	-	-	-
89	120	-	-	UNK9	-	-	-	-	-	-	-
90	144	-	-	UNK10	-	-	-	-	-	-	-
91	148	TR 77668	*Origanum onites*	OON46	*MAJ – ϕ*	-	Wild	Erdemli	İçel, TR	MT	2008-2009
92	149	TR 77669	*Origanum onites*	OON47	*MAJ – ϕ*	-	Wild	Erdemli	İçel, TR	MT	2008-2009
93	150	TR 77672	*Origanum vulgare* subsp. *hirtum*	OVH5	*ORG – γ*	-	Wild	Göksun	Kahramanmaraş, TR	MT	2008-2009
94	151	TR 77674	*Origanum syriacum* subsp. *bevanii*	OSYBE5	*MAJ – ϕ*	-	Wild	Kahramanmaraş	Kahramanmaraş, TR	MT	2008-2009
95	154	TR 54475	*Origanum onites*	OON48	*MAJ – ϕ*	-	Wild	Yeniceköy	Muğla, TR	A	1991

(Cont. on the next page)

Table 3.1. (cont.)

96	155	TR 54488	*Origanum onites*	OON49	*MAJ – ϕ*	-	Wild	Fethiye	Muğla, TR	A	1991
97	156	-	-	UNK11	-		-		-		
98	157	TR 54491	*Origanum onites*	OON50	*MAJ – ϕ*	-	Wild	Kalkan	Antalya, TR	MT	1991
99	158	TR 54499	*Origanum onites*	OON51	*MAJ – ϕ*	-	Wild	Finike	Antalya, TR	MT	1991
100	160	-	-	UNK12	-	-	-	-	-	-	-
101	161	TR 54556	*Origanum onites*	OON52	*MAJ – ϕ*	-	Wild	Burhaniye	Balıkesir, TR	M	1991
102	162	TR 63126	*Origanum dubium*	ODU12	*MAJ – ϕ*	-	Wild	Ağlasun	Burdur, TR	MT	1995
103	163	-	-	UNK13	-	-	-	-	-	-	-
104	165	-	-	UNK14	-	-	-	-	-	-	-
105	166	-	-	UNK15	-	-	-	-	-	-	-
106	172	-	-	UNK16	-	-	-	-	-	-	-
107	175	-	-	UNK17	-	-	-	-	-	-	-
108	176	-	-	UNK18	-	-	-	-	-	-	-
109	178	-	-	UNK19	-	-	-	-	-	-	-
110	180	-	-	UNK20	-	-	-	-	-	-	-
111	181	-	-	UNK21	-	-	-	-	-	-	-
112	190	-	-	UNK22	-	-	-	-	-	-	-
113	193	-	-	UNK23	-	-	-	-	-	-	-
114	198	-	-	UNK24	-	-	-	-	-	-	-
115	199	-	-	UNK25	-	-	-	-	-	-	-
116	200	TR 71538	*Origanum sipyleum*	OSI8	*ANA – β*	+	Wild	Eskigediz	Kütahya, TR	A	2003
117	201	TR 76973	*Origanum syriacum* subsp. *bevanii*	OSYBE6	*MAJ – ϕ*	-	Wild	Kahramanmaraş	Kahramanmaraş, TR	MT	2007
118	202	TR 76975	*Origanum vulgare* subsp. *hirtum*	OVH6	*ORG – γ*	-	Wild	Göksun	Kahramanmaraş, TR	MT	2007

(Cont. on the next page)

Table 3.1. (cont.)

119	203	TR 76976	*Origanum vulgare* subsp. *hirtum*	OVH7	*ORG – γ*	-	Wild	Kahramanmaraş	Kahramanmaraş, TR	MT	2007
120	204	TR 76977	*Origanum vulgare* subsp. *hirtum*	OVH8	*ORG – γ*	-	Wild	Bulanık	Kahramanmaraş, TR	MT	2007
121	205	TR 76978	*Origanum syriacum* subsp. *bevanii*	OSYBE7	*MAJ – ϕ*	-	Wild	Topçalı	Kahramanmaraş, TR	MT	2007
122	206	TR 64495	*Origanum dubium*	ODU13	*MAJ – ϕ*	-	Wild	İçel	İçel, TR	MT	1996
123	207	-	-	UNK26	-	-	-	-	-	-	-
124	208	TR 64641	*Origanum dubium*	ODU14	*MAJ – ϕ*	-	Wild	Tarsus	İçel, TR	MT	1996
125	211	-	-	UNK27	-	-	-	-	-	-	-
126	212	-	-	UNK28	-	-	-	-	-	-	-
127	213	-	-	UNK29	-	-	-	-	-	-	-
128	215	-	-	UNK30	-	-	-	-	-	-	-
129	216	TR 76824	*Origanum sipyleum*	OSI9	*ANA – β*	+	Wild	Gediz	Kütahya, TR	A	2007
130	217	TR 69501	*Origanum onites*	OON53	*MAJ – ϕ*	-	Wild	Silifke	İçel, TR	MT	1996-2000

3.3. Methods

3.3.1. DNA extraction

Mature leaves of oregano specimens were used for genomic DNA isolation. The DNA extraction was conducted according the CTAB protocol with some modifications for additional purification steps (Doyle and Doyle, 1987; Costa and Roberts, 2014). DNA concentration and quality were measured by SkanIt software for Multiscan Go 3.2 spectrophotometer (Thermo Scientific). Isolated DNA stocks were diluted (50 ng/µl) and loaded on 0.8 % agarose gel (1 X TAE Buffer), ethidium bromide was used to stain DNA and samples were visualized under UV light.

3.3.2. SRAP analyses

A total of 10 SRAP primer combinations (Li and Quiros, 2001) with the highest GD from Chapter 2 were selected to screen the AARI collection (Table 3.2).

Table 3.2. SRAP primer pairs used in this study

Pair Code	Forward	Sequence of Forward (5' to 3')	Reverse	Sequence of Reverse (5' to 3')
1	**me2**	TGAGTCCAAACCGGAGC	**em3**	GACTGCGTACGAATTGAC
2			**em8**	GACTGCGTACGAATTAGC
3			**em11**	GACTGCGTACGAATTTCG
4			**em15**	GACTGCGTACGAATTCGT
5	**me3**	TGAGTCCAAACCGGAAT	**em2**	GACTGCGTACGAATTTGC
6			**em3**	GACTGCGTACGAATTGAC
7			**em4**	GACTGCGTACGAATTTGA
8			**em5**	GACTGCGTACGAATTAAC
9	**me4**	TGAGTCCAAACCGGACC	**em1**	GACTGCGTACGAATTAAT
10			**em8**	GACTGCGTACGAATTAGC

SRAP markers amplified PCR fragments in 25 µl final volume with 1 X Reaction Buffer (with BSA), 3 mM $MgCl_2$, 0.125 mM deoxyribonucleotide triphosphates (dNTPs), 1 U *Taq* DNA polymerase, “me” (forward) and “em” (reverse) primers were combined and added as 2 pmol and template DNA was 50 ng. For PCR reactions, an initial denaturation was set at 94 ^oC for 5 min, then amplification steps were applied: a) 5 cycles of [94 ^oC – 1 minute, 35 ^oC – 1 minute, 72 ^oC – 1 minute] followed by b) 35

cycles of [94 ºC – 1 minute, 50ºC – 1 minute, 72 ºC – 1 minute) with final elongation at 72 ºC – 10 minutes. Reaction products were loaded on 2% agarose gel (1 X TAE Buffer), stained with ethidium bromide and run at 110 V for 2.5 hours and visualized under UV light with the BioRad (Universal Hood II) visualization instrument.

3.3.3. Diversity and population structure analyses

The agarose gel images were used to assess the genetic diversity of the AARI population according to the presence "1" or absence "0" of each band for each SRAP marker. Thus, markers were scored dominantly. Genetic Diversity (*GD*) was calculated with the online software, GDdom (Abuzayed et al., 2016).

The missing data were coded as "9", and OON20, OSI1, OON35, OSI3, OON44, OON45, OVH1, OVH3, ODU12, UNK17, and UNK28 samples with high missing data were discarded from Clustering Anaysis and Principle Coordinate Analysis (PCoA). A Neighbor Joining (NJ) Dendrogram was constructed for the accessions using data for the 10 SRAP markers with DARwin 6.0.8 software (Perrier and Jacquemoud, 2006) with Dice Coefficient. Dendrograms were colored according to taxon names.

A PCoA factorial analysis was performed in DARwin 6.0.8 software to create five axes to plot the relationship among accessions in a Euclidian spatial organization (Perrier and Jacquemound, 2006).

The predominant taxon, OON, was used to create a separate dendrogram and PCoA for Darwin software. Units OON1, OON20 and OON44 were not included in this clustering analysis due to deficient data.

GenAlEx 6.5 plugin was used to create binary and random binary matrices for the oregano accessions at both the taxon and section levels (Peakall and Smouse, 2006, 2012). The number of effective alleles (*Ne*), Shannon's Index (*I*), the mean diversity (*h*) and genetic divergence within and between the six taxa and unknown accessions having more than one individual (*O. dubium*, ODU; *O. onites*, OON; *O. sipyleum*, OSI; *O. syriacum* subsp. *bevanii*, OSYBE; *O. vulgare* subsp. *hirtum*, OVH; *O. vulgare* subsp. *vulgare*, OVVU) were investigated with *PhiPT* analysis (analogous to F_{ST} analysis). *PhiPT* < 0.15 indicated significant gene flow between sections and taxon (Frankham et al., 2002). "9999" pairwise permutations were used and comparisons with *P* values below 0.001 were assumed to be statistically significant. AMOVA was estimated to find the

genetic diversity among sections/taxa for those accessions with more than one individual in GenAlEx 6.5.

Nei's genetic distances (*NGD*) and identities (*NGI*) were determined according to pairwise correlation in GenAlEx (Peakall and Smouse, 2006). Gene flow was measured by calculation of the number of migrants per generation (*Nm*) using the formula given in Chapter 2, section 2.3.4 (Wood and Gardner, 2007).

Population structure was evaluated with Bayesian inference with STRUCTURE 2.3.4 software (Pritchard et al., 2000). The subpopulations (*K*) were tested with *K* from 1 to 10 groups. Each iteration was made 10 times for each *K*. The initial burn in replications (50,000) were followed by 300,000 Markov Chain Monte Carlo (MCMC) replications. The most suitable number of *K* was determined using Structure Harvester (Earl and vonHoldt, 2012). Identity thresholds, 0.6 and 0.7, were set for understanding the effect of threshold value on admixed individuals (<0.6 and <0.7) to determine the best representative of the population (Scutari and Denis, 2014).

The AARI population was analyzed with various numbers of subpopulations (*K*): i) *K*=2 for which the highest likelihood value was observed, ii) *K*=3 for the number of clusters indicated by the NJ dendrogram, iii) *K*=4 because the population consisted of accessions from four sections (*Anatolicon*, *Brevifilamentum*, *Majorana* and *Origanum*), iv) *K*=5 to represent the four main taxa of sections in the germplasm (*ANA, BRE, MAJ, ORG*) and considering unknown (UNK) accessions separately, and v) *K*=8 because there are unknown individuals that might represent any one of the eight sections that naturally grow in Türkiye.

To investigate the relationship between genetic diversity and the collection locations, i) specimens from the same region and ii) specimens from the same province were assumed to be populations. With this aim, gene flow (*PhiPT*) and number of migrants (*Nm*) were calculated for the five regions and 13 provinces with more than one individual via GenAlEx 6.51 plugin (Smouse et al., 2017). Specimens with a high number of missing values and admixed individuals (according to *K*=4) were excluded from the analyses to avoid any conflict. Results for populations with at least four individuals were discussed. The highest number of individuals per populations were 39 for Aegean region and 37 for Mediterranean region, followed by eleven for Marmara region. Among populations at the province level, the highest number of individuals was observed for Antalya province followed by Muğla.

3.4. Results

3.4.1. Constitution of AARI germplasm collection

The *Origanum* species germplasm at AARI includes accessions of unknown species from unknown collection locations and accessions of known species from the sections *Anatolicon*, *Brevifilamentum*, *Majorana*, *Origanum.* These samples were collected from 20 locations in six geographic regions of Türkiye. Among regions, Aegean and Mediterranean regions were the specimen-rich collection areas, whereas Eastern Anatolian region had only one province representing the region's oregano diversity.

Section *Majorana* was represented by the most material from with 74 accessions (57% of the total) from 14 provinces. The next largest group of accessions (30) were of unknown species (27%). The remaining material included 14, 10, and 2 individuals belonging to sections *Origanum* (11%) from six provinces, *Anatolicon* (8%) from five provinces and *Brevifilamentum* (1.5%) from two provinces, respectively. Within section *Majorana*, ODU, OON, and OSYBE accounted for 19, 72, and 9% of the total *Majorana* accessions. In section *Anatolicon*, species OHY and OSI represented 10 and 90% of the accessions. Two species, OHY and OLE, equally contributed to section *Brevifilamentum* (50%). In section *Origanum,* OVG, OVH, OVVU, and OVVI represented 7, 57, 29, and 7% of the accessions, respectively.

The distribution of AARI registered oregano taxa by province is shown in Table 3.3. Eleven province were represented by a single taxon including OSYBE – Adana/MT, OVVU – Bilecik/M, OSI – Çorum/BS, OVG – Erzincan/EA, OON – Çanakkale/M, OLE – Karaman/CA, OHA, and ODU in the AARI gene bank. Antalya was the richest province in terms of taxa with ODU, OON, OSYBE, OVH, OVVI, and OVVU. The number of collection locations per taxon was most diverse for OON with eleven provinces (Table 3.4). In contrast, the species OHY, OVG, OLE, OHA, and OVVI were registered from only one province.

Table 3.3. The distribution of taxa in each province. R: region, A: Aegean, BS: Black Sea, CA: Central Anatolia, EA: Eastern Anatolia, M: Marmara, MT: Mediterranean, PerCov: Coverage Percent (%) of the species in that province.

R	Province	Taxa	PerCov (%)	R	Province	Taxa	PerCov (%)
A	Aydın	OON	100	EA	Erzincan	OVG	100
	Denizli	OHY	25	M	Balıkesir	OON	66
		OON	50			OSI	17
		OSI	25			OSYBE	17
	İzmir	ODU	12.5		Bilecik	OVVU	100
		OON	75		Çanakkale	OON	100
		OVVU	12.5	MT	Adana	OSYBE	100
	Kütahya	OSI	100		Antalya	ODU	37
	Manisa	OON	50			OON	42
		OSI	50			OSYBE	5.3
	Muğla	ODU	6.6			OVH	5.3
		OON	86.7			OVVI	5.3
		OVVU	6.6			OVVU	5.3
BS	Çorum	OSI	100		Burdur	ODU	50
	Kastamonu	ODU	100			OON	50
CA	Karaman	OLE	100		Isparta	OON	100
	Sivas	OHA	100		İçel	ODU	33
						OON	67
					Kahramanmaraş	OSYBE	36.3
						OVH	63.7

Among twenty locations, thirteen of the provinces (Antalya, Aydın, Balıkesir, Burdur, Çanakkale, Denizli, İçel, İzmir, Kahramanmaraş, Kastamonu, Kütahya, Manisa, and Muğla) were represented by more than one accession which made them suitable for their use as a population for AMOVA analyses. Antalya was the richest province in terms of number of taxa (ODU, OON, OSYBE, OVH, OVVI, OVVU) and number of individuals (18) from two sections, *Majorana* and *Origanum* (Table 3.3, Table 3.4). Aydın was represented by six OON accessions from section *Majorana*. Balıkesir had six specimens from section *Anatolicon* (OSI taxon) and section *Majorana* (OON and OSYBE taxa). Çanakkale only had four specimens from OON from section *Majorana*. İçel was represented by six individuals of ODU and OON from section *Majorana*. İzmir had eight individuals from two sections, *Majorana* (ODU and OON) and *Origanum* (OVVU). Kahramanmaraş had nine accessions from section *Majorana* (OSYBE) and *Origanum* (OVH). Manisa had four specimens collected from sections *Anatolicon* (OSI)

and *Majorana* (OON). Muğla province had 15 accessions representing sections *Majorana* (ODU and OON) and *Origanum* (OVVU). Denizli and Kütahya provinces had only three accessions from sections *Anatolicon* and *Majorana*; while Burdur and Kastamonu provinces had only two individuals from section *Majorana*. Provinces having fewer then four accessions are not discussed in the results.

Table 3.4. The distribution of each taxon over provinces. Ns: Number of specimens, R: region, PerLoc: distribution of species per location in percentage (%) [SD: ±5 in total per Species]. A: Aegean, BS: Black Sea, CA: Central Anatolia, EA: Eastern Anatolia, M: Marmara, MT: Mediterranean, and UNK: Unknown.

Taxa	Ns	R	Province	PerLoc (%)	Taxa	Ns	R	Province	PerLoc (%)
ODU	1	A	İzmir	7	OSI	3	A	Kütahya	33
	1	A	Muğla	7		1	A	Denizli	11
	2	BS	Kastamonu	14		3	A	Manisa	33
	1	MT	Burdur	7		1	BS	Çorum	11
	2	MT	İçel	14		1	M	Balıkesir	11
	7	MT	Antalya	50	OSYBE	1	M	Balıkesir	14
OHA	1	CA	Sivas	100		1	MT	Adana	14
OHY	1	A	Denizli	100		1	MT	Antalya	14
OLE	1	CA	Karaman	100		4	MT	Kahramanmaraş	57
OON	2	A	Denizli	4	OVG	1	EA	Erzincan	100
	6	A	Aydın	11	OVH	1	MT	Antalya	12
	3	A	Manisa	6		7	MT	Kahramanmaraş	88
	13	A	Muğla	24	OVVI	1	MT	Antalya	100
					OVVU	1	M	Bilecik	25
						1	MT	Antalya	25
						1	A	İzmir	25
						1	A	Muğla	25
	6	A	İzmir	11	UNK	30	UNK	Unknown	100
	1	MT	Isparta	2					
	1	MT	Burdur	2					
	4	M	Çanakkale	8					
	4	M	Balıkesir	8					
	4	MT	İçel	8					
	8	MT	Antalya	15					
	1	UNK	Unknown	2					
								∑ N_s:	130

3.4.2. SRAP analyses

The oregano germplasm used in this research consisted of 130 genotypes with 10, 2, 74 and 14 individuals from four sections; *Anatolicon*, *Brevifilamentum*, *Majorana* and

Origanum, respectively. There were also an additional 30 unknown accessions. Screening of this material with ten SRAP marker pairs resulted in 148 alleles. The number of polymorphic alleles per marker ranged from 3 to 35 with a mean of 14.8. Allele frequencies ranged from 0.32 to 0.86 with a mean of 0.64. The highest polymorphism was observed for the me3-em2 primer pair with a GD of 0.42 (Figure 3.4), while the least polymorphic marker pair was me4-em1 with a GD of 0.22 (Table 3.5).

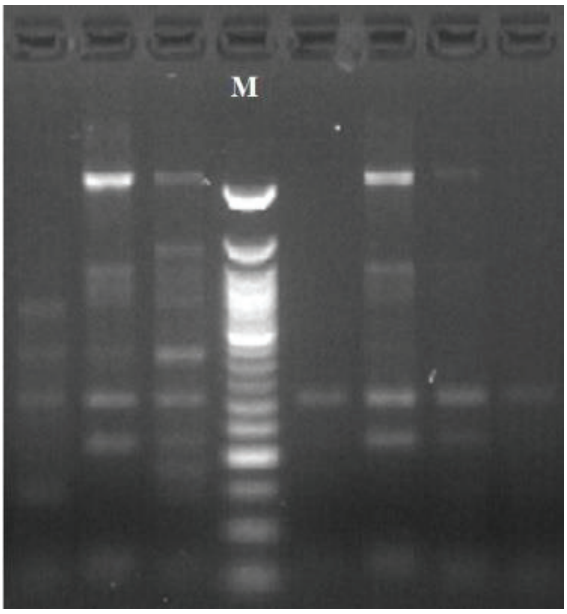


Figure 3.4. Allelic pattern of the most polymorphic SRAP marker pair (me3-em2) for AARI *Origanum* L. material. M: Marker/ 50 bp ladder.

Table 3.5. Allele frequencies and genetic diversity values of AARI oregano germplasm for SRAP markers. Number of alleles (*Na*), allele frequency (af), gene diversity (GD) value with standard deviation (SD) per SRAP marker, and average GD value.

SRAP Marker	***Na***	**af**	**Mean GD ± SD**	**Average GD**
me3-em2	11	0.63	0.42 ± 0.09	0.33
me3-em5	20	0.32	0.40 ± 0.08	
me2-em15	9	0.79	0.36 ± 0.11	
me2-em3	35	0.69	0.34 ± 0.19	
me3-em4	3	0.67	0.34 ± 0.13	
me2-em8	18	0.44	0.32 ± 0.15	
me3-em3	16	0.67	0.31 ± 0.13	
me4-em8	9	0.62	0.30 ± 0.12	
me2-em11	17	0.86	0.28 ± 0.11	
me4-em1	10	0.75	0.22 ± 0.15	

In AMOVA analysis, within section diversity was high (96%) and the remaining diversity (4%) was between sections (Table 3.6). At the taxon level, diversity within oregano taxa was 95% in AMOVA analysis (Figure 3.5, Table 3.7). Taxon and section level AMOVA results are given in Table 3.6.

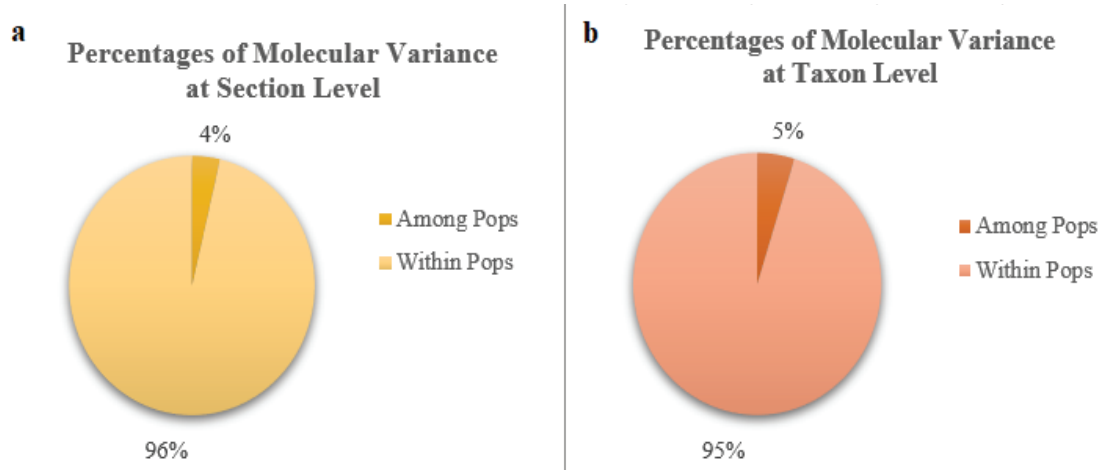


Figure 3.5. AMOVA results showing among and within population diversity at section and taxon levels

Table 3.6. AMOVA results for Turkish *Origanum* collection at AARI at section level.

Section Level	df	SS	MS	Est. Var.	%
Among Pops	4	191.609	47.902	1.046	4%
Within Pops	125	3416.383	27.331	27.331	96%
Total	129	3607.992		28.377	100%

Definitions are as follows: df=degrees of freedom, SS=sum of squares, MS=mean of squares, Est.Var.=estimated variance, %=percentage of variation.

Table 3.7. AMOVA results for Turkish *Origanum* collection at AARI at taxon level.

Source	df	SS	MS	Est. Var.	%
Among Pops	5	225.850	45.170	1.418	5%
Within Pops	89	2475.960	27.820	27.820	95%
Total	94	2701.811		29.238	100%

Definitions are as follows: df=degrees of freedom, SS=sum of squares, MS=mean of squares, Est.Var.=estimated variance, %=percentage of variation.

At the section level, genetic diversity (*h*) was approximately 0.48 for each section. Number of effective alleles ranged between 1.92 and 1.94 (*Ne*). The Shannon Index (*I*) values were all 0.67 for the five subpopulations of the sections (data not shown). *NGI*

varied between 0.95 and 0.96 among the sections indicating that the sections were genetically close to each other at the molecular level (Table 3.8).

Table 3.8. Section level *Nei*'s genetic distance (*NGD*) values shown below the diagonal vs. *Nei*'s genetic identity (*NGI*) values given above the diagonal.

***Nei's* Genetic Distance vs. *Nei's* Genetic Identity**	***Anatolicon***	***Brevifilamentum***	***Majorana***	***Origanum***	**Unknown**
Anatolicon	-	0.96	0.96	0.96	0.96
Brevifilamentum	0.04	-	0.96	0.96	0.96
Majorana	0.04	0.04	-	0.96	0.95
Origanum	0.04	0.04	0.04	-	0.96
Unknown	0.05	0.04	0.05	0.04	-

The highest *PhiPT* value (F_{ST} analogue) was 0.05 for section pairs: *Origanum* – *Majorana* and Unknown species – section *Origanum*. The lowest *PhiPT* value was zero between section pairs: *Brevifilamentum* – *Anatolicon*, *Majorana* – *Brevifilamentum*, and *Origanum* – *Brevifilamentum*, indicating high genetic similarity between these sections. The gene flow (*Nm*) between sections ranged from 4.75 to 24.75 with the highest observed between *Brevifilamentum* and the unknown accessions (Table 3.9). The lowest gene flow between sections was 4.75 between: a) *Majorana* and *Origanum* and b) *Majorana* and the unknown accessions.

Table 3.9. Section level *PhiPT* values below diagonal and *Nm* values above diagonal.

***PhiPT* values vs. *Nm* Values at Section Level**	***Anatolicon***	***Brevifilamentum***	***Majorana***	***Origanum***	**Unknown**
Anatolicon	-	0.00	8.07	24.75	6.00
Brevifilamentum	0.00	-	0.00	0.00	24.75
Majorana	0.03	0.00	-	4.75	4.75
Origanum	0.01	0.00	0.05	-	12.25
Unknown	0.04	0.01	0.05	0.02	-

At the taxon level, mean diversity (*h*) was 47% and the number of effective alleles (*Ne*) ranged between 1.87 and 1.99 with a mean value of 1.89. Shannon Index (*I*) values ranged between 0.65 and 0.67 with a mean of 0.66 for taxon pairs (data not shown). The *NGI* values were between 0.93 and 0.95 indicating high genetic similarity between all taxa. The highest *NGI* was observed between taxon pairs: ODU – OSYBE, OSI – OVVU, and OSYBE – OVVU (Table 3.10).

Table 3.10.*Nei's* genetic distances (*NGD*) (below diagonal) and *Nei's* genetic identities (*NGI*) between taxon pairs.

***Nei's* Genetic Distance vs. *Nei's* Genetic Identity**	**ODU**	**OON**	**OSI**	**OSYBE**	**OVH**	**OVVU**
ODU	-	0.93	0.93	0.95	0.94	0.93
OON	0.08	-	0.93	0.94	0.94	0.94
OSI	0.07	0.08	-	0.94	0.93	0.95
OSYBE	0.06	0.06	0.06	-	0.94	0.95
OVH	0.07	0.06	0.07	0.06	-	0.94
OVVU	0.07	0.07	0.06	0.05	0.06	-

The highest *PhiPT* value at the taxon level was 0.10 between taxa OVH and OON (0.10). The lowest *PhiPT* value was calculated as zero between taxon pairs: OVVU – ODU, OVVU – OSI, and OVVU – OVH. The highest *Nm* value was 24.75 for taxon pairs: ODU – OSI, ODU – OSYBE, OSI – OVH, and OSYBE – OVVU within the germplasm. The lowest gene flow was zero between taxon pairs: ODU – OVVU and OSI – OVVU, respectively (Table 3.11).

Table 3.11. *PhiPT* (below diagonal) and *Nm* (above diagonal) values among taxa.

***PhiPT* values vs. *Nm* Values at Taxon Level**	**ODU**	**OON**	**OSI**	**OSYBE**	**OVH**	**OVVU**
ODU	-	6.00	24.75	24.75	6.00	0.00
OON	0.04	-	3.92	3.92	2.25	6.00
OSI	0.01	0.06	-	12.25	24.75	0.00
OSYBE	0.01	0.06	0.02	-	3.92	24.75
OVH	0.04	0.10	0.01	0.06	-	0.00
OVVU	0.00	0.04	0.00	0.01	0.00	-

At the regional level, the within population diversity among the five regions of Türkiye (Aegean, Black Sea, Central Anatolia, Marmara, and Mediterranean) was 98% (Figure 3.6). Genetic diversity (*h*) was approximately 0.47 for each region. Number of effective alleles ranged between 1.89 and 1.92 (*Ne*). The Shannon Index (*I*) values were 0.67 for almost all regional populations (data not shown). *NGI* varied between 0.94 and 0.96 among the regions indicating that the populations within regional restricted areas were genetically close to each other at the molecular level (Table 3.12).

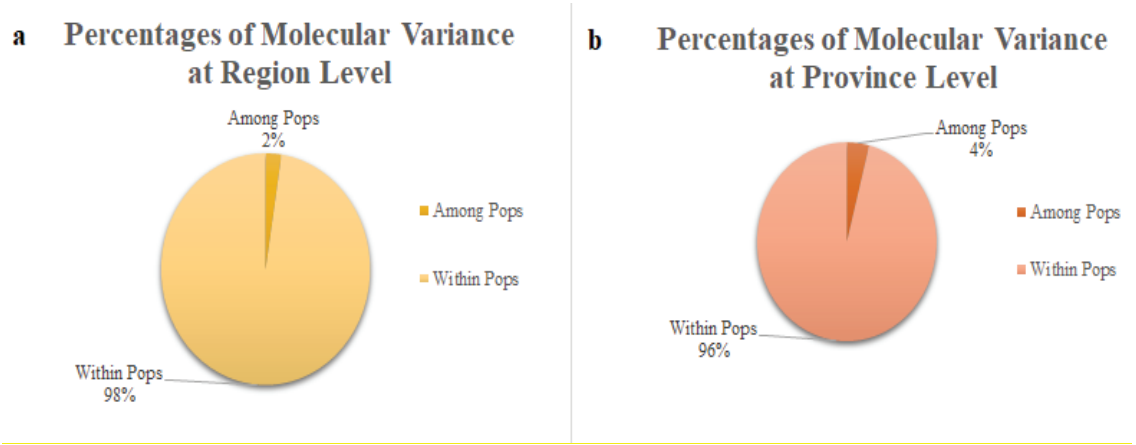


Figure 3.6. AMOVA results showing among and within population diversity for geographical regions for AARI collection at a) region and b) province level

Table 3.12.*Nei's* genetic distances (*NGD*) (below diagonal) and *Nei's* genetic identities (*NGI*) between regional population pairs.

***Nei's* Genetic Distance vs. *Nei's* Genetic Identity**	**Aegean**	**Black Sea**	**Central Anatolia**	**Marmara**	**Mediterranean**
Aegean	-	0.94	0.94	0.95	0.94
Black Sea	0.06	-	0.95	0.95	0.96
Central Anatolia	0.06	0.05	-	0.94	0.95
Marmara	0.05	0.05	0.06	-	0.95
Mediterranean	0.06	0.05	0.05	0.06	-

Although the highest and lowest *PhiPT* values were for pairs including the Black Sea and Central Anatolia regions, conclusions about their gene flow cannot be made due to the low number of individuals (2) in these populations. For the remaining regions, the highest *PhiPT* was between the Mediterranean and Aegean regions reflecting low gene flow due to their geographical separation (Table 3.13). The lowest *PhiPT* value was between the Marmara and Aegean regions which are geographically adjacent to each other allowing higher gene flow. In addition, the highest *Nm* value was between Marmara and Mediterranean regions (10.99). This relatively high number of migrants was followed by the Aegean – Mediterranean comparison (7.70). The lowest number of migrants was observed between the Aegean and Marmara regions which reflects their geographical separation.

Table 3.13. *PhiPT* (below diagonal) and *Nm* (above diagonal) values among regions.

***PhiPT* values vs. *Nm* values at regional level**	**Aegean**	**Black Sea**	**Central Anatolia**	**Marmara**	**Mediterranean**
Aegean	-	2.22	0.00	0.00	7.70
Black Sea	0.10	-	0.00	2.36	6.35
Central Anatolia	0.00	0.00	-	0.00	0.00
Marmara	0.00	0.10	0.00	-	10.99
Mediterranean	0.03	0.04	0.00	0.02	-

At the province level, AMOVA indicated that 96% of the molecular variance was within provinces while only 4% was among provinces (Figure 3.6). Genetic diversity (*h*) was between 0.40 and 0.43 for each province. Number of effective alleles ranged between 1.74 and 1.78 (*Ne*). The Shannon Index (*I*) values were between 0.58 and 0.62 for all populations (data not shown). *NGI* varied between 0.82 and 0.89 among the provinces. Among these, the highest *NGI* was observed between five pairs of provinces: Aydın – Çanakkale, Balıkesir – Çanakkale, İçel – İzmir, İçel – Kahramanmaraş, and Muğla – Çanakkale. Thus populations within the same regional areas were genetically close to each other at the molecular level (Table 3.14). There were also some geographically distance provinces which had high *NGI*. These high genetic identity values might be due to the predominant number of OON accessions that were collected from different provinces. For instance, Çanakkale had four OON accessions and Aydın had six OON accessions and shared an *NGI* of 0.88. The most distant populations were observed for Balıkesir and Antalya from the Marmara and Mediterranean regions with a great geographical distance. In addition, specimens from Kahramanmaraş – Balıkesir, Manisa – Antalya, and Manisa – Çanakkale was also found to be distant to each other agreeing with the location of these provinces in different geographical regions of Türkiye. The highest *PhiPT* value was 0.18 for province pairs: Kahramanmaraş – Balıkesir and Kahramanmaraş – Muğla. The lowest *PhiPT* values (zero) indicating high gene flow were observed between Balıkesir – Aydın, Çanakkale – Aydın, İzmir – Aydın, İzmir – Çanakkale, Manisa – Çanakkale, Muğla – Çanakkale, İçel – Kahramanmaraş, Manisa – İzmir, and Muğla – İzmir (Table 3.15). The highest *Nm* value was observed between Manisa – Aydın provinces (1201.75). The lowest number of migrants (*Nm*) was observed mostly between provinces from different geographic regions.

Table 3.14. *Nei's* genetic distances (*NGD*) (below diagonal) and *Nei's* genetic identities (*NGI*) between province pairs.

***Nei's* Genetic Distance vs. *Nei's* Genetic Identity**	**Antalya**	**Aydın**	**Balıkesir**	**Burdur**	**Çanakkale**	**Denizli**	**İçel**	**İzmir**	**Kahramanmaraş**	**Kastamonu**	**Kütahya**	**Manisa**	**Muğla**
Antalya	-	0.86	0.84	0.82	0.85	0.87	0.87	0.85	0.86	0.86	0.87	0.84	0.86
Aydın	0.15	-	0.87	0.82	0.88	0.87	0.85	0.86	0.85	0.87	0.87	0.84	0.85
Balıkesir	0.18	0.14	-	0.82	0.88	0.85	0.87	0.85	0.83	0.88	0.89	0.84	0.86
Burdur	0.20	0.20	0.20	-	0.86	0.82	0.87	0.84	0.86	0.86	0.85	0.85	0.85
Çanakkale	0.17	0.13	0.13	0.16	-	0.87	0.87	0.86	0.87	0.85	0.86	0.84	0.88
Denizli	0.14	0.14	0.17	0.20	0.14	-	0.89	0.86	0.85	0.89	0.85	0.85	0.86
İçel	0.14	0.17	0.14	0.14	0.13	0.11	-	0.88	0.88	0.85	0.88	0.86	0.87
İzmir	0.17	0.15	0.16	0.17	0.16	0.15	0.13	-	0.86	0.84	0.85	0.85	0.86
Kahramanmaraş	0.15	0.16	0.18	0.15	0.14	0.16	0.12	0.15	-	0.85	0.86	0.85	0.86
Kastamonu	0.15	0.14	0.13	0.15	0.16	0.12	0.16	0.17	0.16	-	0.87	0.87	0.87
Kütahya	0.14	0.14	0.12	0.16	0.15	0.16	0.13	0.16	0.15	0.14	-	0.85	0.87
Manisa	0.18	0.18	0.17	0.16	0.18	0.16	0.15	0.16	0.16	0.14	0.16	-	0.85
Muğla	0.15	0.17	0.15	0.16	0.13	0.15	0.14	0.15	0.15	0.14	0.14	0.16	-

Table 3.15. *PhiPT* (below diagonal) and *Nm* (above diagonal) values for provinces.

***PhiPT* values vs. *Nm* values at province level**	**Antalya**	**Aydın**	**Balıkesir**	**Burdur**	**Çanakkale**	**Denizli**	**İçel**	**İzmir**	**Kahramanmaraş**	**Kastamonu**	**Kütahya**	**Manisa**	**Muğla**
Antalya	-	43.27	9.18	106.37	37.18	0.00	5.87	20.48	3.28	2.94	9.42	14.51	7.32
Aydın	0.01	-	0.00	0.00	0.00	13.75	3.18	0.00	1.28	2.42	4.16	1201.75	38.52
Balıkesir	0.03	0.00	-	0.00	11.90	4.36	3.13	14.70	1.14	1.51	1.78	7.57	3.71
Burdur	0.00	0.00	0.00	-	0.00	5.05	0.00	14.77	1.74	0.00	0.00	0.00	1.95
Çanakkale	0.01	0.00	0.02	0.00	-	0.00	6.04	0.00	1.66	5.14	4.86	0.00	0.00
Denizli	0.00	0.02	0.05	0.05	0.00	-	28.16	27.68	2.48	4.40	3.08	4.66	24.64
İçel	0.04	0.07	0.07	0.00	0.04	0.01	-	3.08	0.00	0.00	0.00	17.63	2.09
İzmir	0.01	0.00	0.02	0.02	0.00	0.01	0.08	-	1.83	3.04	3.95	0.00	0.00
Kahramanmaraş	0.07	0.16	0.18	0.13	0.13	0.09	0.00	0.12	-	2.41	56.45	1.86	1.17
Kastamonu	0.08	0.09	0.14	0.00	0.05	0.05	0.00	0.08	0.09	-	0.00	0.00	1.26
Kütahya	0.03	0.06	0.12	0.00	0.05	0.08	0.00	0.06	0.00	0.00	-	0.00	2.11
Manisa	0.02	0.00	0.03	0.00	0.00	0.05	0.01	0.00	0.12	0.00	0.00	-	7.07
Muğla	0.03	0.01	0.06	0.11	0.00	0.01	0.11	0.00	0.18	0.17	0.11	0.03	-

3.4.3. Dissimilarity analyses

3.4.3.1. Dendrogram analysis

Neighbor Joining (NJ) dendrogram dissimilarities were calculated with Dice coefficient and resulted in a mean of 52% diversity in the germplasm (Figure 3.7). The dissimilarity values ranged between a minimum and maximum of 0.11 and 0.96 with a 94% cophenetic r value indicating that the dendrogram was a very good fit to the distance matrix data.

The NJ dendrogram had three main clusters: A, B and C (Figures 3.8, 3.9, and 3.10). Accessions from *O. onites* species (OON), which is the predominant taxon of the collection, were spread throughout all clusters. In Figure 3.6, the predominancy of specimens from the Mediterranean region was observed in cluster A (72%); while the placement of most accessions from the Aegean region was observed in cluster B (45%). The third, smallest cluster contained mostly accessions from the Aegean (15%) together with single accessions from the Mediterranean (3%) and Marmara regions (7%).

Cluster A was the most accession rich group in the dendrogram with 63 accessions and distribution per taxon as follows: OON – 22 individuals, ODU – 10, OSYBE – 4, OVH – 4, OHY – 1, OLE – 1, OVVI – 1, and OVVU – 1, including 19 unknown specimens (Figure 3.7). All four sections (*ANA*, *BRE*, *MAJ*, and *ORG*) and 14 provinces in five regions in Türkiye were included in cluster A.

Cluster B had a total number of 42 accessions, distributed as follows: OON – 19, OSI – 4, OSYBE – 3, OVVU – 3, ODU – 2, OVH – 2, OHA – 1, and OVG – 1, including seven unknown specimens (Figure 3.8). These indicated that Cluster B was composed of individuals from unknown species and all four sections; *ANA, BRE*, *MAJ* and *ORG*; from 15 provinces in six regions of Türkiye.

Cluster C contained nine accessions (ODU – 1; OON – 7; in addition to one unknown specimen) in section *MAJ* from five province in three regions: A, M, and MT (Figure 3.9).

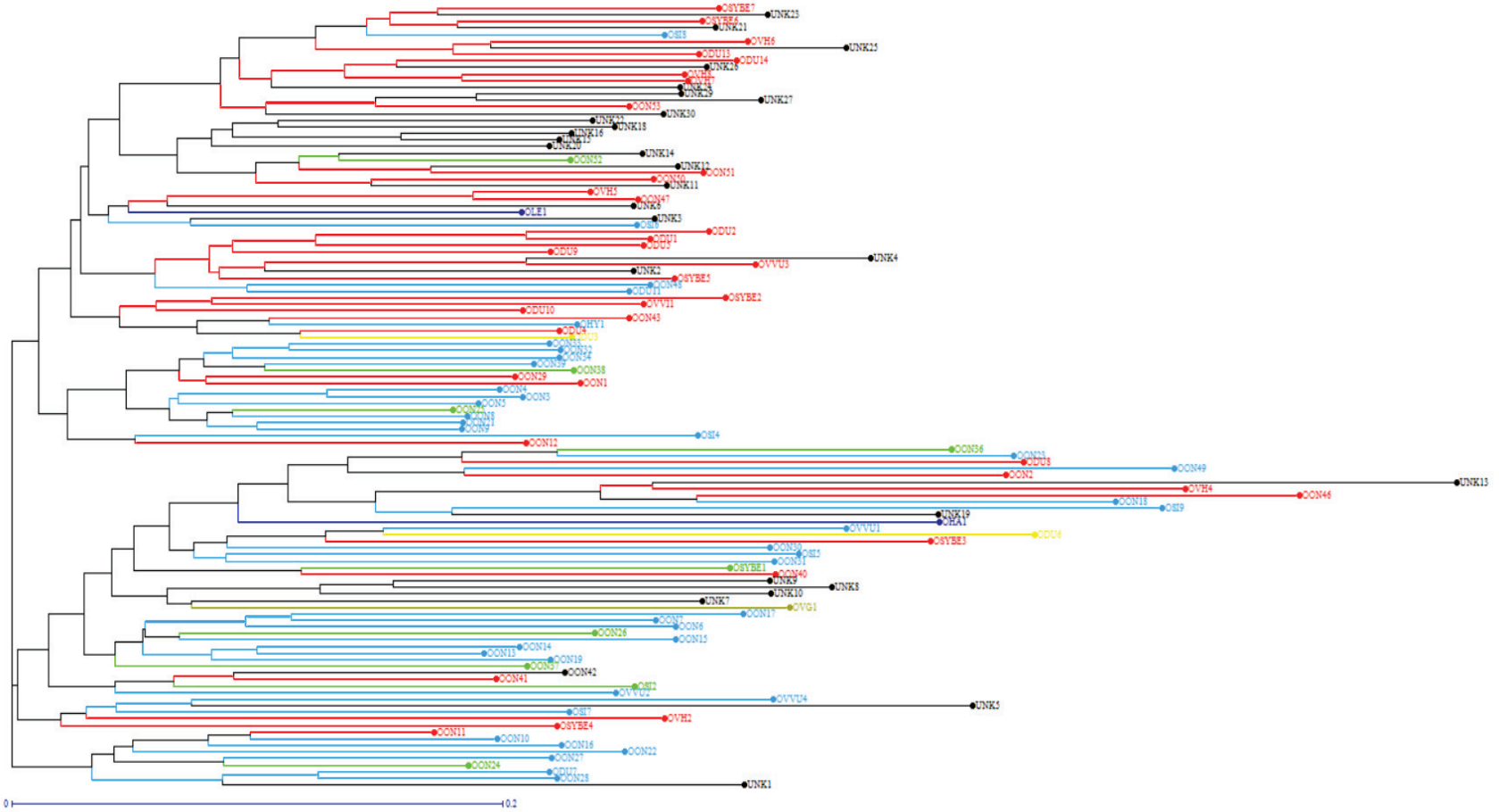


Figure 3.7. The neighbor joining (NJ) dendrogram colored according to geographic regions for AARI collection from sections *Anatolicon, Brevifilamentum*, *Majorana*, and *Origanum* conserved at AARI Institute. Samples are color coded according to regional status: red, Mediterranean region; blue, Aegean region; green, Marmara region, yellow, Black Sea region; dark blue: Central Anatolia region, and khaki, Eastern Anatolia region. Samples with unkown origin are in black.

3.4.3.1.1. Clustering by province/region

There was some clustering according to province/region in the dendrogram. Cluster A.1.1.1.1 contained almost exclusively Mediterranean specimens with only two exceptions and accessions from unknown locations. (Figure 3.7). In addition, accessions from Kahramanmaraş and Antalya tended to group together in cluster A despite the fact that they represented different sections and species. Accessions from Muğla also clustered together and were all OON accessions. In cluster B.1.2.1 there was a grouping of Aegean samples from OON. Cluster C was almost exclusively limited to Aegean specimens of OON with two exceptions.

3.4.3.1.2. Clustering by section

Clusters A and B consisted of accessions from all four sections: *ANA*, *BRE*, *MAJ* and *ORG*. (Figures 3.7, 3.8, and 3.9). Of all of these sections, only *MAJ* specimens showed exlusive grouping within the larger clusters. For example, in cluster A, group A.2 contained mainly *MAJ* accessions with one *ANA* specimen. *MAJ* also clustered together in group B.1.1.2 in cluster B. In addition, cluster C had only individuals from section *MAJ*. *ANA* and *ORG* were always found together with *MAJ* and never formed exclusive groups. Interestingly the two *BRE* accessions from the same region (CA) were found in two different clusters (A and B).

3.4.3.1.3. Clustering by taxon

The germplasm collection contained specimens from 11 taxa. Some taxa formed clusters in the dendrogram. OSI from section *ANA* was mostly found in cluster B (57% of the samples); while the remaining accessions were located in cluster A. In both clusters, OSI individuals were scattered throughout the clusters. Of the seven OSI accessions in the dendrogram, three of them (43%) were most closely related to OON accessions from section *MAJ*. OHY from section *ANA* was only represented by one accession which was found in Cluster A. OLE from section *BRE* had only one sample as did OHA from the same section. Interestingly OHA was most closely related with a cluster that was almost exclusively OON from *MAJ*.

In section *MAJ*, most of the ODU accessions were found in subcluster A.1 with an exclusive group of four accessions in A.1.1.2.1. Four of the ODU accessions were most closely related to OON accessions which is also in section *MAJ*. OSYBE accessions were most closely related to OVVU and OSI from sections *ORG* and *ANA*, respectively. OON was the predominant taxon of the germplasm collection. As a result, OON specimens were found to be dispersed in all three clusters. In addition, OON tended to form subclusters in the dendrogram. For example, subgroup A.2 was composed of 15 OON accessions with the addition of one OSU sample. Another OON subcluster fell in subgroup B.1.1.1.1.1.1 with six OON samples and OSI, OVH and ODU individuals as well as two unknown samples. Subcluster B1.1.2 was exclusively OON with nine accessions. Moreover, cluster C was almost exclusively OON samples with one ODU and one unknown accession.

In section *ORG*, there was only one OVG sample which clustered with unknown accessions in cluster A. OVH accessions fell into both clusters A and B. Although in one case a OVH sample was most closely related to another OVH sample, this species also formed close clusters with OSI and OON. OVVI was represented by only one sample in cluster A that was most closely related to OSYB. Three of the four OVVU accessions were found in cluster B. In two instances, these accessions were most similar to OSI samples from section *ANA*.

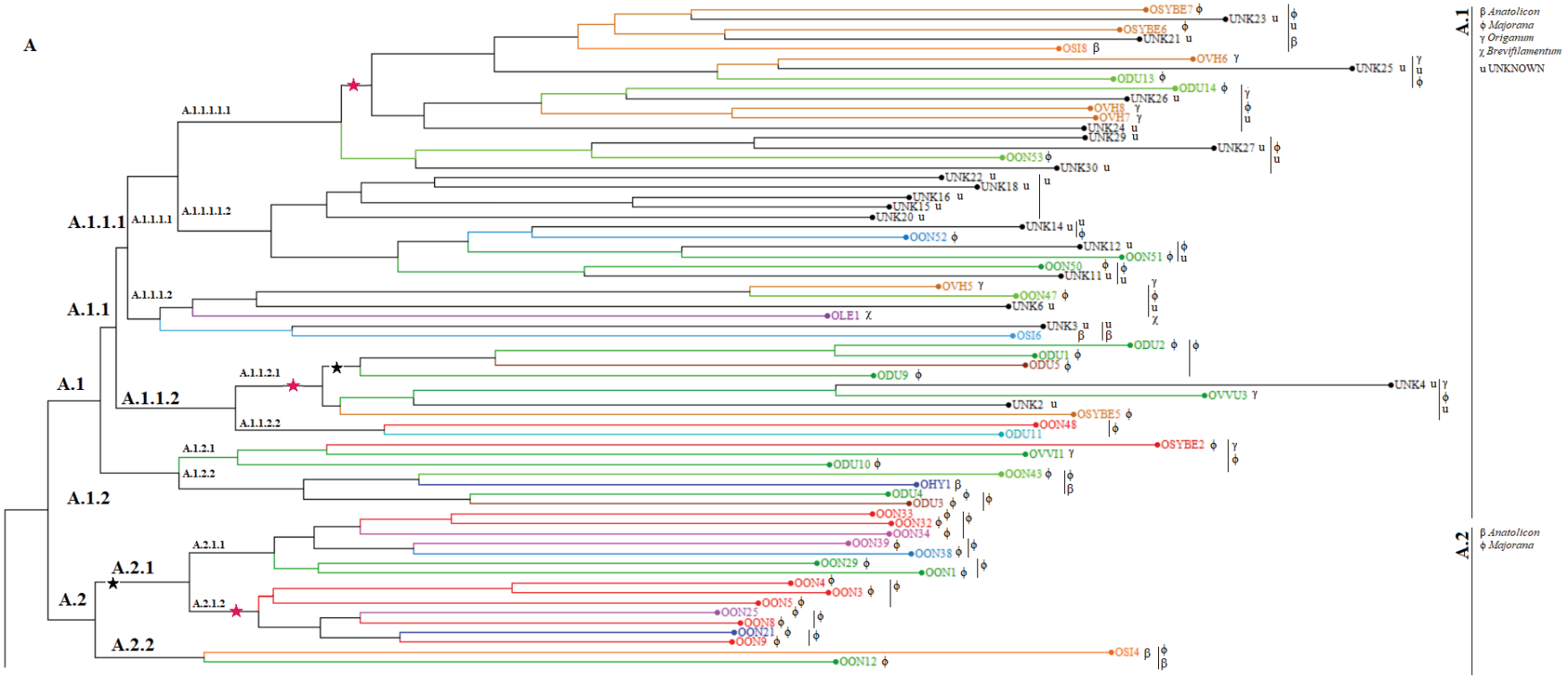


Figure 3.8. The Neighbor Joining (NJ) dendrogram for oregano genotypes conserved at AARI Instiute. Cluster A. A.1 and A.2 are the major subclusters of cluster A in the dendrogram. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas. Sections are represented by symbols; β: *Anatolicon*, χ: *Brevifilamentum*, ϕ: *Majorana* and γ: *Origanum*. Black stars indicate clustering by taxon, while maroon stars indicate clustering by region

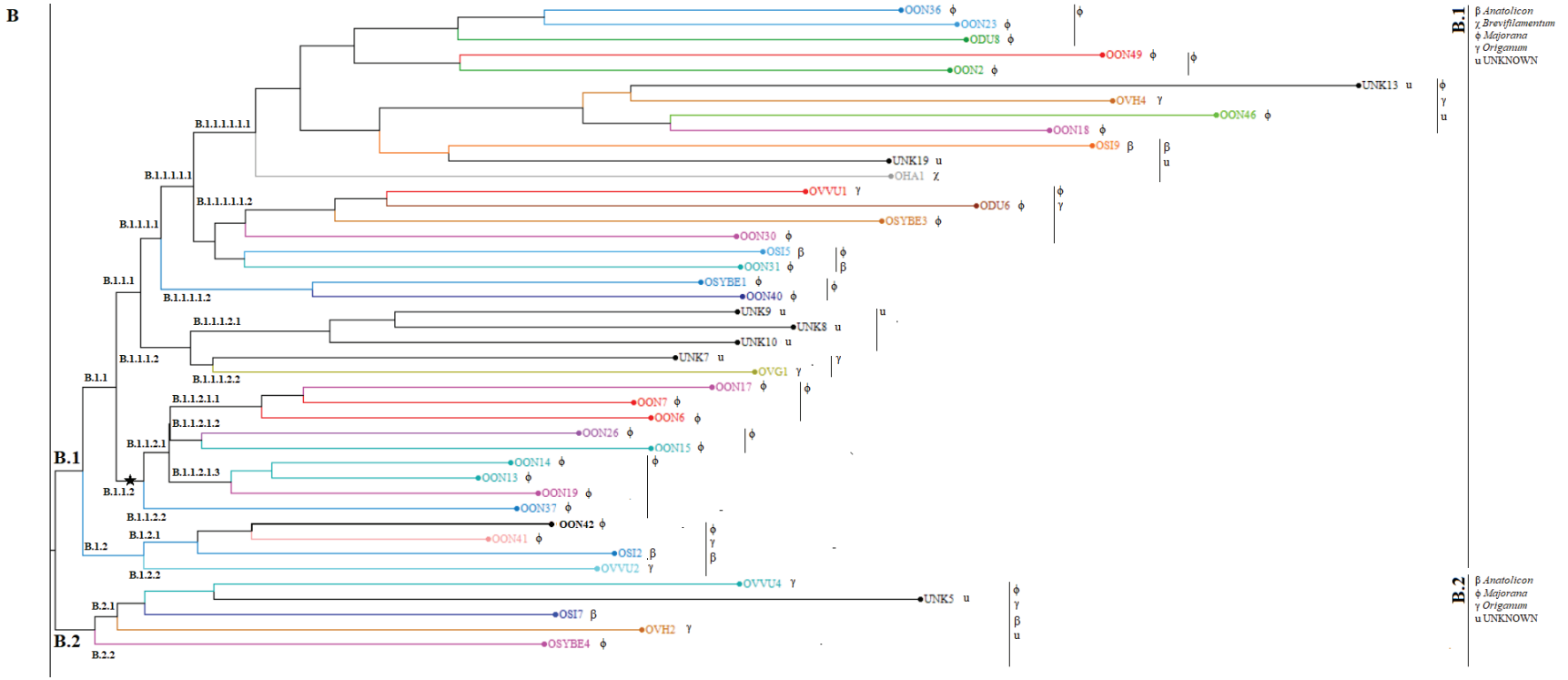


Figure 3.9. The Neighbor Joining (NJ) dendrogram for oregano genotypes conserved at AARI Instiute. Cluster B. B.1 and B.2 represents subclusters of cluster B in dengrogram. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, soft medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas. Star indicates monophyletic grouping of individuals from the same taxa.

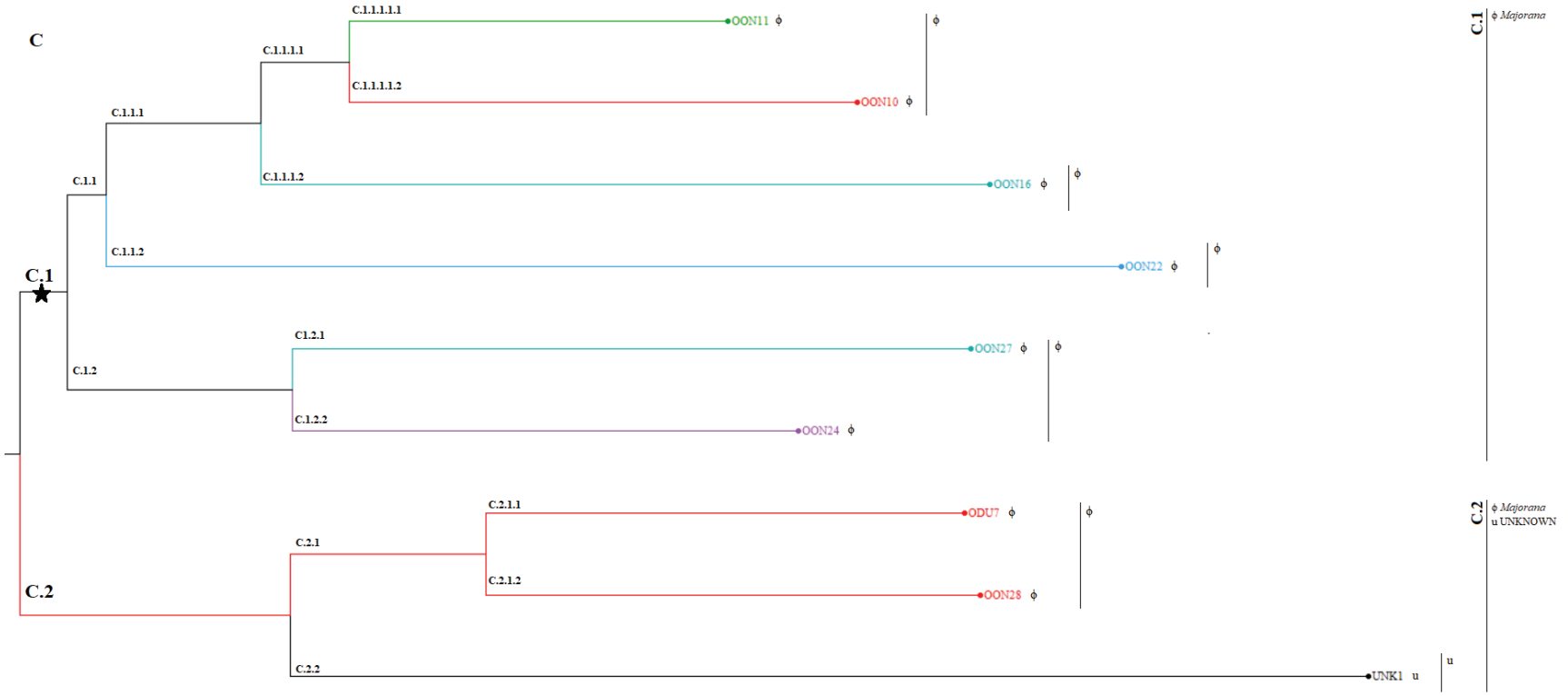


Figure 3.10. The Neighbor Joining (NJ) dendrogram for oregano genotypes conserved at AARI Instiute. Cluster C. C.1 and C.2 represents subclusters of cluster C in dendrogram. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, soft medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas. Sections were represented by signs β: *Anatolicon*, χ: *Brevifilamentum*, ϕ: *Majorana* aand γ: *Origanum*. Star indicates monophyletic grouping of individuals from the same taxa.

3.4.3.1.4. Dendrogram analysis of OON accessions

Because OON was the most common taxon in the germplasm collection, a separate dendrogram was drawn to examine the relationships among these accessions (Figure 11). In only a few cases (marked with stars in the figure), OON accessions from the same province clustered together. In the most notable case, five OON accessions from Muğla formed a nearly exclusive cluster with a Çanakkale accession. OON13 and OON14 from Tire, İzmir also grouped together with another Aegean specimen (from Aydin). Two accessions from İçel were also most closely related to each other in the dendrogram. The map in Figure 3.12 shows the proximity and locations of the aforementioned accessions. Despite these cases of regional clustering, in general, position in the dendrogram was not related to province or region for OON.

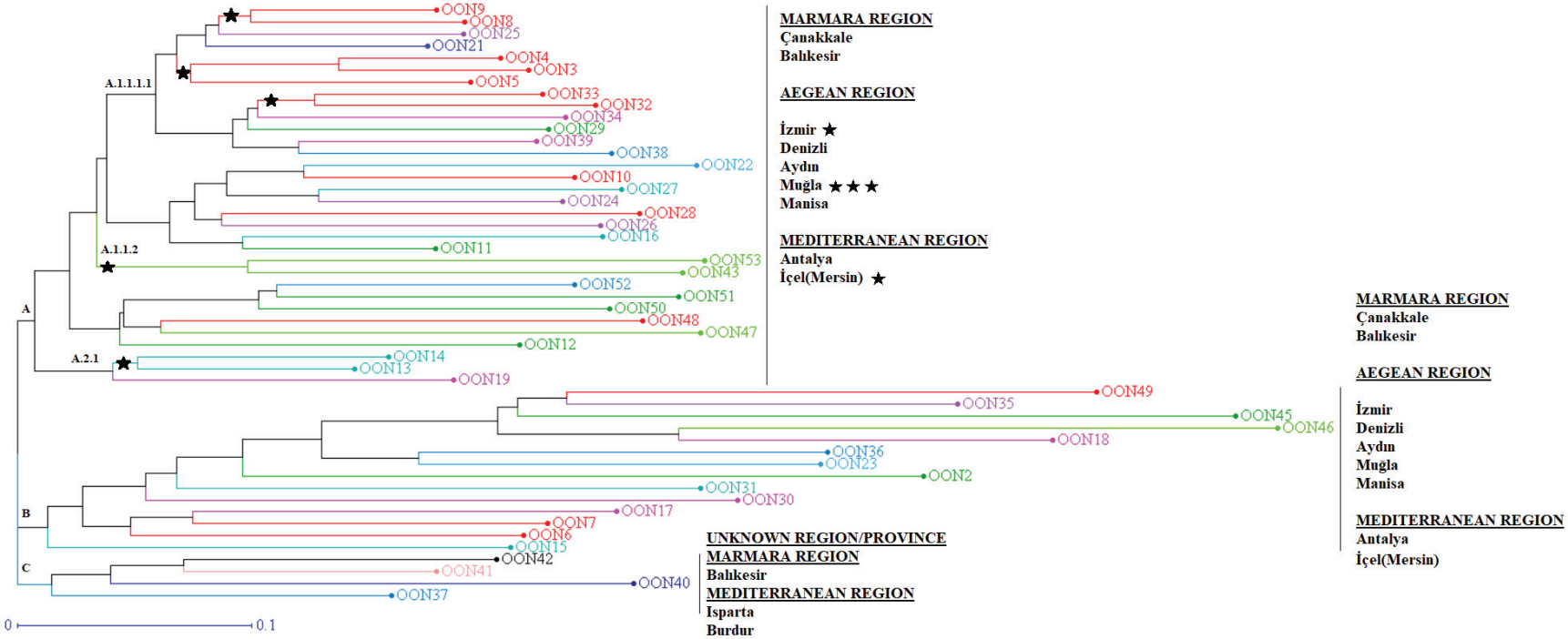


Figure 3.11. The Neighbor Joining (NJ) dendrogram for the 50 *Origanum onites* L. genotypes conserved at AARI Instiute. A, B and C represents the subclusters in dendrogram. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, soft medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas. Stars indicate monophyletic grouping of individuals from the same taxa. The number of stars next to provinces indicates the number of subclusters with certain taxa; e.g. Muğla has three subclusters having same subspecies.

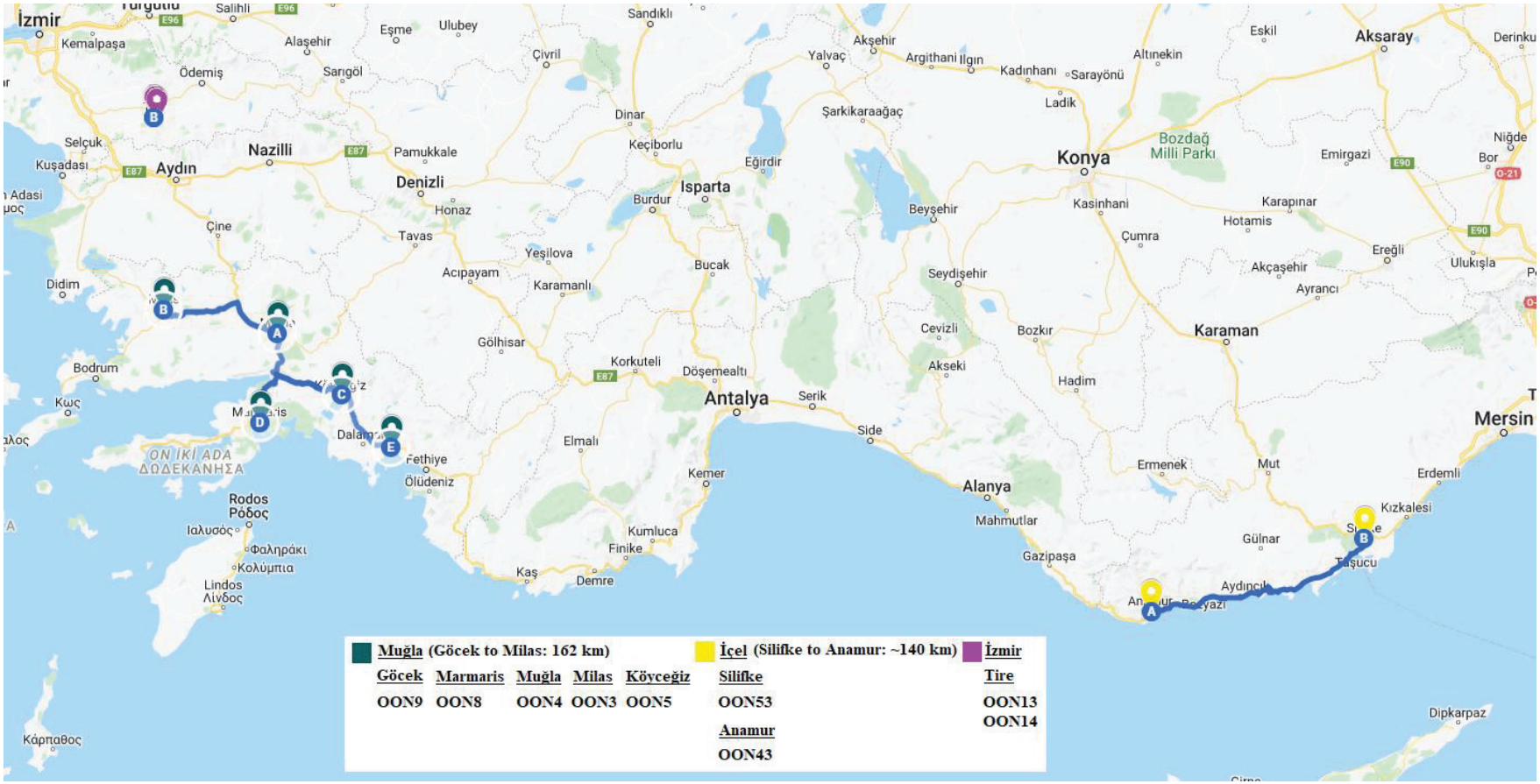


Figure 3.12. Representative map of OON accessions that were clustered exclusively from same provinces: Muğla, İçel and İzmir. (I can do the same for main dendrogram – same geo ori clusters and same taxa clusters from different regions per cluster A, B and C).

3.4.3.2. PCoA analysis

Principal Coordinate Analysis (PCoA) was performed for the 119 accession units using the SRAP marker genotypic data (Figure 3.13). The eigen values for the PCoA were 11.12, 7.40 and 5.61% of variance for the first three axes, respectively. In section *Anatolicon*, the eight OSI individuals were dispersed over all four quadrants while OHY1 was placed closer to the OSI individuals located in the (X, -Y) section of the graph (Figure 3.13). For section *Brevifilamentum*, there were only two individuals: OHA1 and OLE1 which were not closely placed in the graph. Section *Majorana* was respresented by the most specimens which were located throughout the graph. Within this section, the OON specimens were mostly concentrated in (X, -Y) spatial organization in PCoA plot. ODU species was also spread over the PCoA graph but mostly placed in the (-Y) section of the graph. Seven OSYBE individuals from the same section were distributed in all areas of the PCoA. Samples from section *Origanum* were distributed in three of the four quadrants of the graph. OVVU1, OVVU2 and OVVU4 created their own subcluster in the (-X, Y) quadrant. OVH accessions OVH6, OVH7 and OVH8 group in the (X, Y) region of the PCoA plot; while OVH2, OVH4 and OVH5 individuals were spread in the plot.

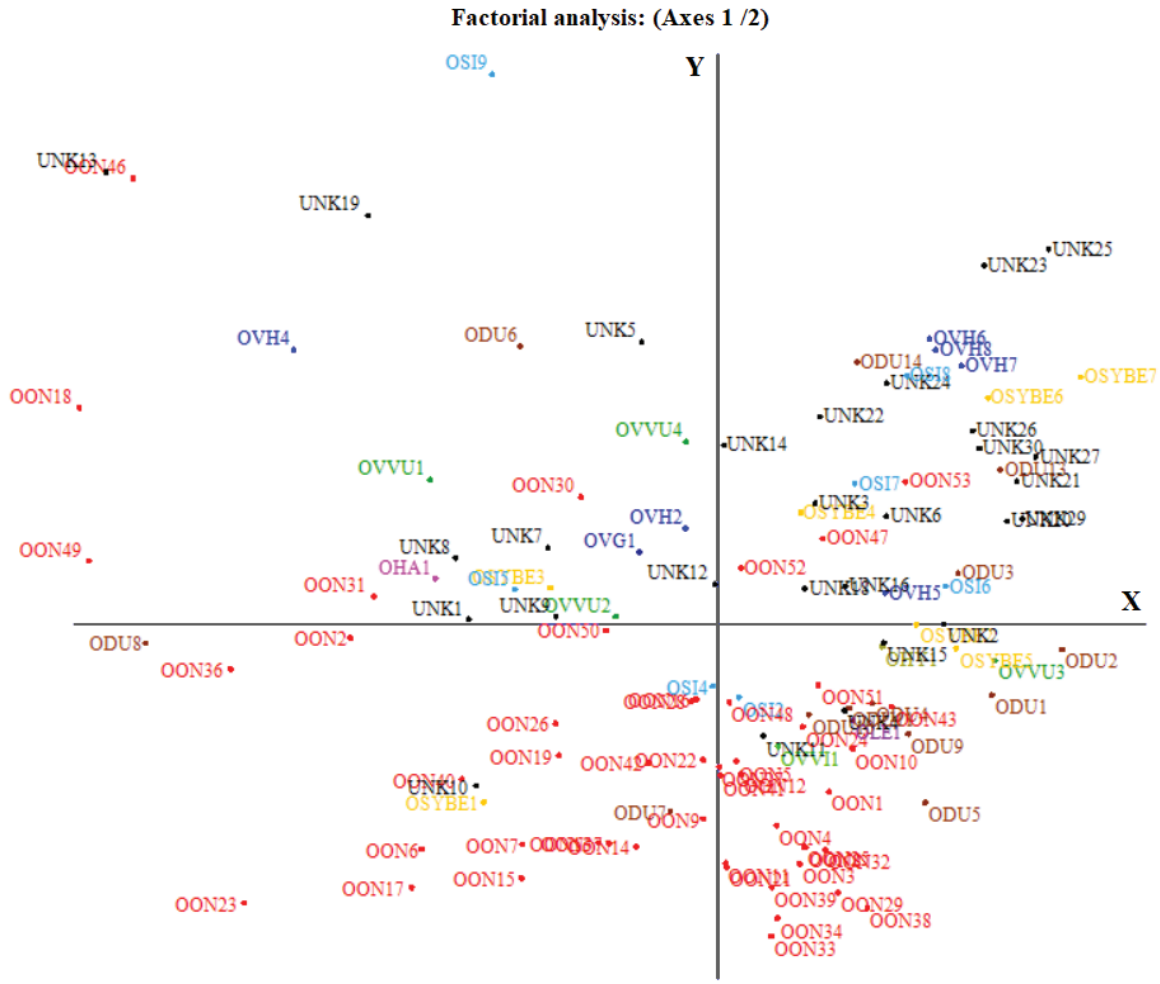


Figure 3.13. Principal Coordinate Analysis (PCoA) graph colored according to species names among AARI oregano collection. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, soft medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas.

A PCoA graph was also plotted for the predominant taxon of the germplasm, OON. The eigenvalues for the first three axes were 21.87, 8.12 and 7.07 %, respectively (Figure 3.14). Most OON specimens were concentrated in two quadrants of the PCoA graph (-X, Y) and (-X, -Y). In contrast, OON31 (İzmir), OON2 (Antalya), OON23 (Manisa), OON36 (Balıkesir), OON18 (İçel), OON35 (Çamakkale), OON49 (Muğla), OON45 (Antalya), and OON46 (İçel) specimens were distinctly spread over (X, Y) and (X, -Y) sections of the graph. Accessions OON45 (Antalya), OON46 (İçel), OON49 (Muğla), OON18 (Aydın), and OON35 (Çanakkale) were the most distinct specimens in the PCoA plot. According to the PCoA plot, OONs from provinces Muğla and Antalya were dispersed over all coordinates with some concentration in the (-X, -Y) and (-X, Y) quadrants. Five of the OON specimens (OON3, OON5, OON8, OON9, and OON28) collected from Muğla province were found in close proximity in the (-X, -Y) section of the plot. Antalya, Çanakkale and İzmir had samples over all four quadrants. Aydın and Muğla specimens were located in three quadrants. OONs from Balıkesir were located in quadrants (-X, Y) and (X, -Y). Manisa specimens were located in (-X, -Y) and (X, -Y) sections of the quadrants. Specimens from Burdur, Denizli and Isparta were found in singular quadrants as follows: (-X, Y), (-X, -Y), and (X, Y). OON42 from unknown geographic origin was located in the (-X, Y) section ofthe plot.

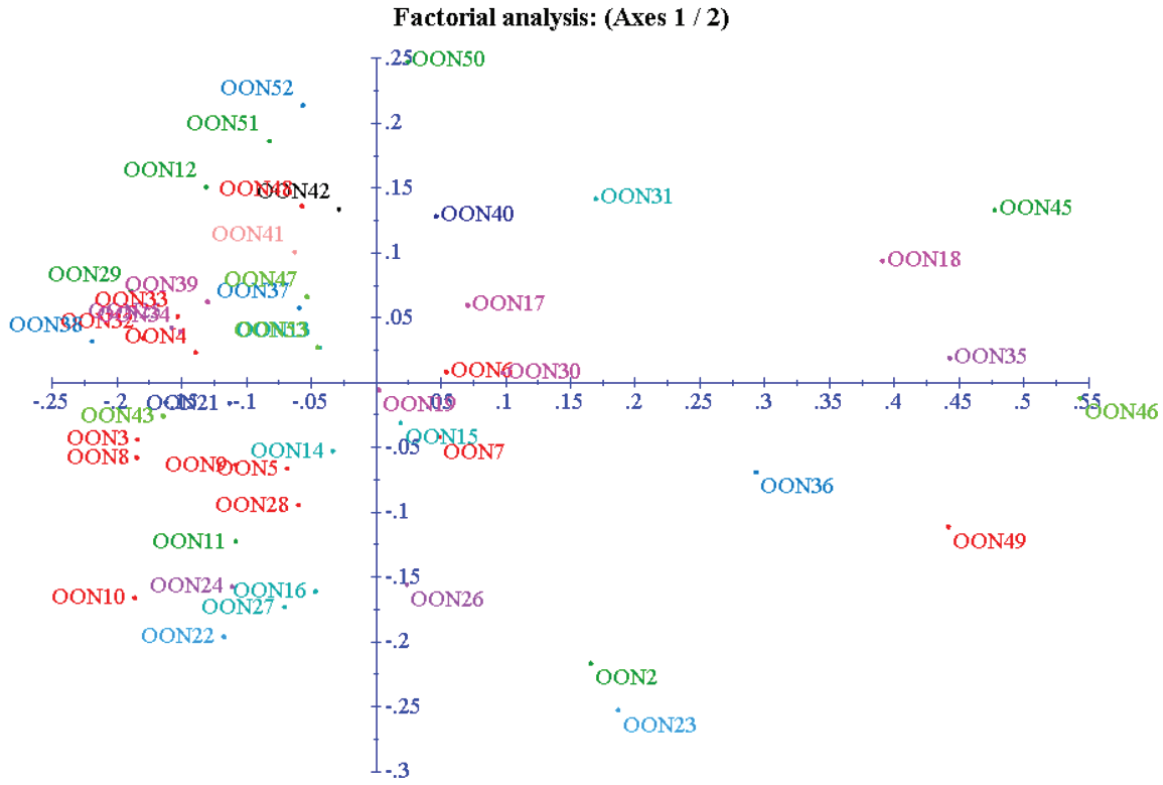


Figure 3.14. PCoA plot built for the geographical display of OON taxa predominant in the AARI germplasm. Colors represent different collection origins: dark red: Adana, dark green: Antalya, fuchsia: Aydın, soft medium blue: Balıkesir, light cyan blue: Bilecik, salmon: Burdur, light purple: Çanakkale, cobalt blue: Denizli, khaki: Erzincan, navy dark blue: Isparta, lime green: İçel(Mersin), soft teal green-blue: İzmir, light brown: Kahramanmaraş, dark purple: Karaman, dark brown: Kastamonu, orange: Kütahya, sky blue: Manisa, red: Muğla, and grey: Sivas.

3.4.3.3. Population structure

The population structure of the AARI collection which is composed of 130 individuals from four sections (*Anatolicon – ANA*, *Brevifilamentum – BRE*, *Majorana – MAJ* and *Origanum – ORG*) was determined for four different optimal *K*s and two genetic identity thresholds (0.60, 0.70). *K*=2 was selected because it gave the most significant (Δ*K*) value (Figure 3.15; Figure 3.16). *K*=4 was used because it represents the number of sections sampled. *K*=5 was tested because some of the unknown samples could belong to other sections in the genus. *K*=8 was examined because there are a total of eight sections of *Origanum* found in Türkiye and the unknown specimens could potentially fall into any of these sections. In addition, *K*=8 gave a high log likelihood value (Figure 3.17). According to the comparison of different threshold values, 0.70 was chosen because it provided a more stringent separation of the subpopulations.

In overall consideration for each tested number of subpopulations, individuals from *ANA, BRE, MAJ,* and *ORG* sections fell into the same sub populations (Table 3.18). However, as the number of *K* increased, the UNK samples were increasingly classified as admixed and then into new subpopulations. This may reflect the hybrid constitution of the unknown specimens or that the unknown samples belong to other species in the eight sections.

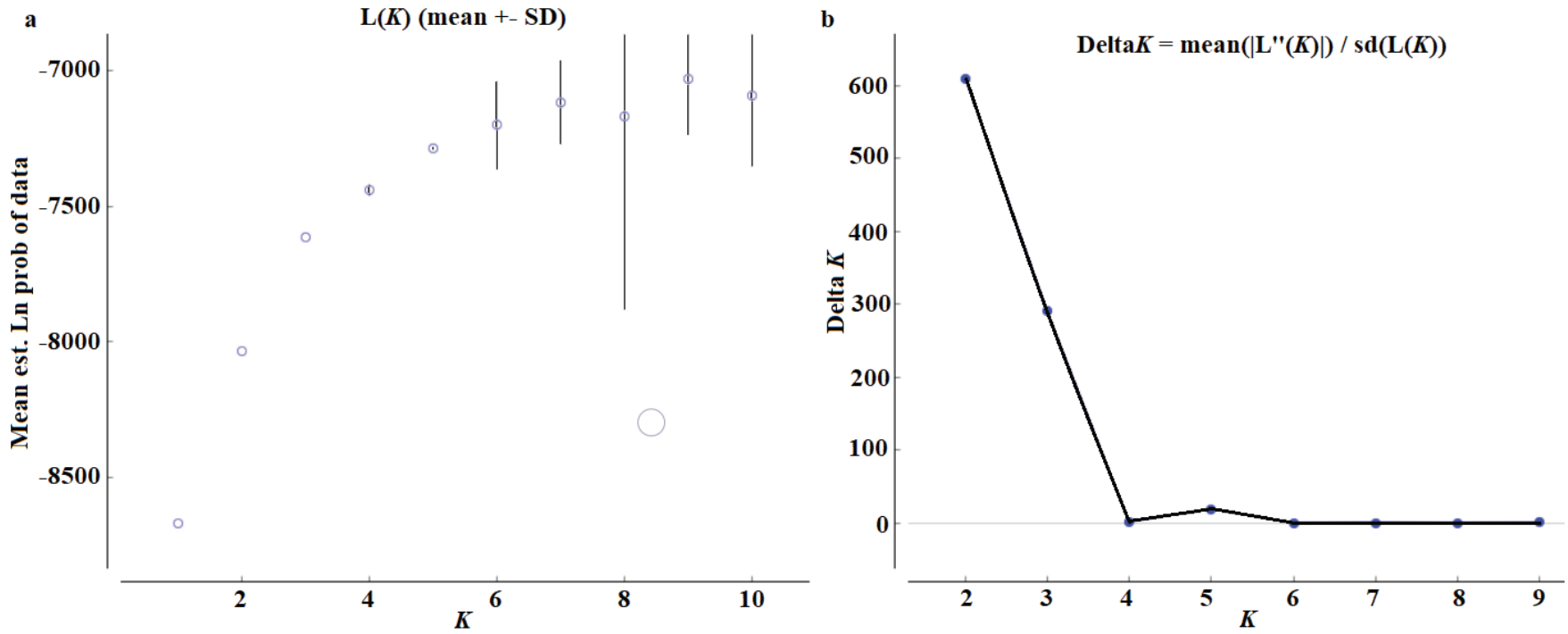


Figure 3.15. Likelihood and Delta*K* (Δ*K*) values gathered from Structure Harvester software. a) Likelihood values plotted on online Structure Harvester analysis, b) Delta*K* (Δ*K*) values were fitting best to *K*=5 and especially, *K*=2.

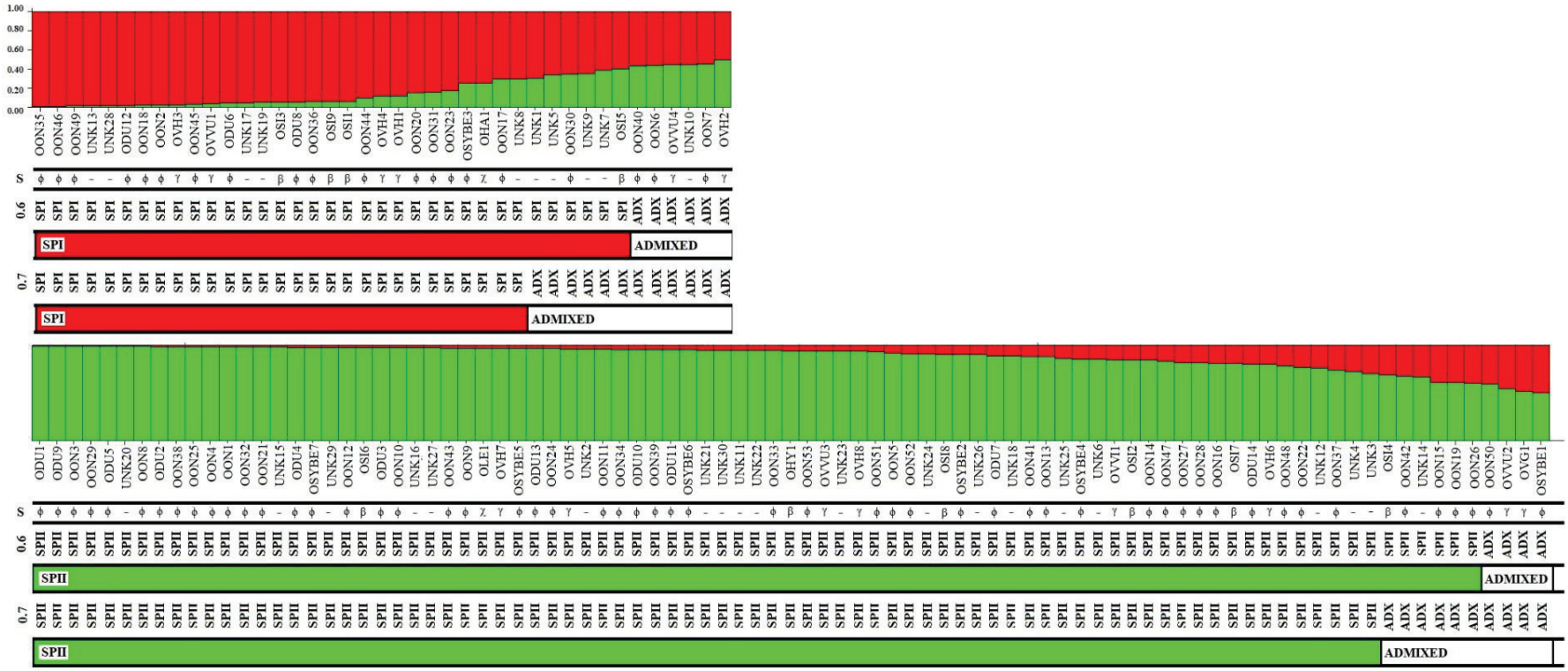


Figure 3.16. Population structure graph for optimal number of *K* determined with Structure Harvester (*K*=2). SPI: subpopulation I, SPII: subpopulation II, SPIII: subpopulation III.

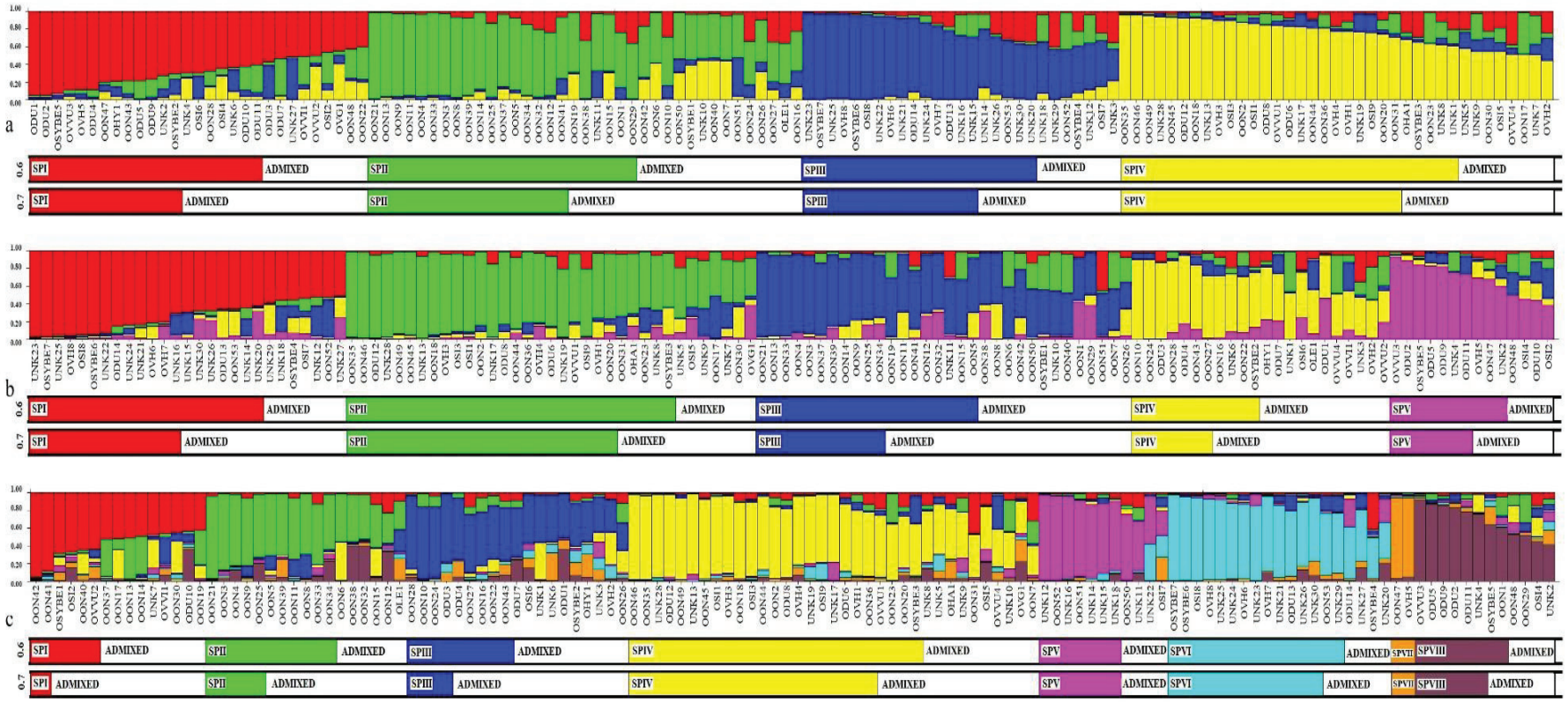


Figure 3.17. Population structure graphs for *K*=4, 5, and 8: Structure results for *K*= a) 4, b) 5, and c) 8. SPI: subpopulation I, SPII: subpopulation II, SPIII: subpopulation III, SPIV: subpopulation IV, SPV: subpopulation V, SPVI: subpopulation VI, SPVII: subpopulation VII, and SPVIII: subpopulation VIII. 0.6 and 0.7 indicates accepted threshold value for grouping individuals into subpopulations.

Table 3.16.Percentages of the number of individuals per section belong to certainsubpopulations . The total number of accessions was 130 in 16 different taxa. ∑#: total number, %: percentage, *ANA*: *Anatolicon*, *BRE*: *Brevifilamentum*, *MAJ*: *Majorana*, *ORG*: *Origanum*, UNK: unknown, *Nt*: number of taxa, *Ni*: number of individuals, ADX: Admixed, SPI: subpopulation I, SPII: subpopulation II, SPIII: subpopulation III, SPIV: subpopulation IV, SPV: subpopulation V, SPVI: subpopulation VI, SPII: subpopulation VII, and SPVIII: subpopulation VIII.

***K*=2**			**SPI**		**SPII**		**ADX**	
Section	*Ni*	Taxon	Ni	%	Ni	%	Ni	%
ANA	9	OSI	3	33	4	45	2	22
	1	OHY	-	-	-	-	1	100
∑ of *ANA*	10	*ANA*	3	30	4	40	3	30
BRE	1	OHA	1	100	-	-	-	-
	1	OLE	-	-	1	100	-	-
∑ of *BRE*	2	*BRE*	1	50	1	50	-	-
MAJ	14	ODU	3	22	11	78	-	-
	53	OON	12	23	32	60	9	17
	7	OSYBE	1	14	5	72	1	14
∑ of *MAJ*	74	*MAJ*	16	22	48	65	10	13
ORG	1	OVG	-	-	-	-	1	100
	8	OVH	3	38	4	50	1	12
	1	OVVI	-	-	1	100	-	-
	4	OVVU	1	25	1	25	2	50
∑ of *ORG*	14	*ORG*	4	29	6	42	4	29
No section info: UNK	30	UNK	5	17	19	63	6	20
∑ of *UNK*	30	UNK	5	17	19	63	6	20

(Cont. on the next page)

Table 3.16. (cont.)

K=4			SPI		SPII		SPIII		SPIV		ADX	
Section	*Ni*	Taxon	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%
ANA	9	OSI	-	-	-	-	1	11	3	33	5	56
	1	OHY	1	100	-	-	-	-	-	-	-	-
∑ of *ANA*		*ANA*	1	10	-	-	1	10	3	30	5	50
BRE	1	OHA	-	-	-	-	-	-	-	-	1	100
	1	OLE	-	-	-	-	-	-	-	-	1	100
∑ of *BRE*		*BRE*	-	-	-	-	-	-	-	-	2	100
MAJ	14	ODU	5	36	-	-	2	14	3	21	4	29
	53	OON	2	4	16	30	-	-	10	19	25	47
	7	OSYBE	2	29	-	-	2	29	-	-	3	42
∑ of *MAJ*		*MAJ*	9	12	16	22	4	5	13	18	32	43
ORG	1	OVG	-	-	-	-	-	-	-	-	1	100
	8	OVH	1	13	-	-	3	37	3	37	1	13
	1	OVVI	-	-	-	-	-	-	-	-	1	100
	4	OVVU	1	25	-	-	-	-	1	25	2	50
∑ of *ORG*		*ORG*	2	14	-	-	3	21	4	36	5	29
No section info: UNK	30	UNK	2	7	-	-	7	23	4	13	17	57
∑ of *UNK*		UNK	2	7	-	-	7	23	4	13	17	57

(Cont. on the next page)

Table 3.16. (cont.)

***K*=5**			**SPI**		**SPII**		**SPIII**		**SPIV**		**SPV**		**ADX**	
Section	*Ni*	Taxon	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%
ANA	9	OSI	1	11	3	33	-	-	-	-	-	-	5	56
	1	OHY	-	-	-	-	-	-	-	-	-	-	1	100
∑ of *ANA*		*ANA*	1	10	3	30	-	-	-	-	-	-	6	60
BRE	1	OHA	-	-	-	-	-	-	-	-	-	-	1	100
	1	OLE	-	-	-	-	-	-	-	-	-	-	1	100
∑ of *BRE*		*BRE*	-	-	-	-	-	-	-	-	-	-	2	100
MAJ	14	ODU	1	7	3	21	-	-	2	14	4	27	3	29
	53	OON	-	-	9	17	11	21	5	9	-	-	28	53
	7	OSYBE	2	29	-	-	-	-	-	-	1	14	4	57
∑ of *MAJ*		*MAJ*	3	4	12	16	11	15	7	9	5	7	34	46
ORG	1	OVG	-	-	-	-	-	-	-	-	-	-	1	100
	8	OVH	3	37	3	38	-	-	-	-	-	-	2	25
	1	OVVI	-	-	-	-	-	-	-	-	-	-	1	100
	4	OVVU	1	25	-	-	-	-	1	25	-	-	2	50
∑ of *ORG*		*ORG*	4	29	3	21	-	-	2	14	-	-	6	43
No section info: UNK	30	UNK	6	20	4	13	-	-	-	-	1	3	19	63
∑ of *UNK*		UNK	6	20	4	13	-	-	-	-	1	3	19	63

(Cont. on the next page)

Table 3.16. (cont.)

***K*=8**			**SPI**		**SPII**		**SPIII**		**SPIV**		**SPV**		**SPVI**		**SPVII**		**SPVIII**		**ADX**	
Section	*Ni*	Taxon	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%	Ni	%
ANA	9	OSI	-	-	-	-	-	-	3	33	-	-	1	11	-	-	-	-	5	56
	1	OHY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100
∑ of *ANA*	10	*ANA*	-	-	-	-	-	-	3	30	-	-	1	10	-	-	-	-	6	60
BRE	1	OHA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100
	1	OLE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100
∑ of *BRE*	2	*BRE*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	100
MAJ	14	ODU	-	-	-	-	1	7	3	21	-	-	1	7	-	-	4	29	5	36
	53	OON	2	4	5	9	3	6	8	15	3	6	-	-	1	2	-	-	31	58
	7	OSYBE	-	-	-	-	-	-	-	-	-	-	2	29	-	-	-	-	5	71
∑ of *MAJ*		*MAJ*	2	3	5	7	4	5	11	13	3	4	3	4	1	1	4	5	41	55
ORG	1	OVG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100
	8	OVH	-	-	-	-	-	-	3	37	-	-	3	38	1	13	-	-	1	12
	1	OVVI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100
	4	OVVU	-	-	-	-	1	25	-	-	-	-	-	-	-	-	-	-	3	75
∑ of *ORG*		*ORG*	-	-	1	7	1	7	3	21	-	-	3	21	1	7	-	-	6	43
No section info: UNK	30	UNK	-	-	-	-	1	3	4	13	5	17	6	20	-	-	-	-	14	47
∑ of *UNK*		UNK	-	-	-	-	1	3	4	13	5	17	6	20	-	-	-	-	14	47
∑*Ni*=130		∑*Nt*=16																		

3.4.3.3.1. *K*=2

When the accessions were examined according to *K*=2, both subpopulations contained accessions from every section as well as unknown samples with the majority (61%) in subpopulation II (SPII) (Figure 3.15, Table 3.16). In addition, 17% of the accessions were identified as admixed meaning that their genetic ancestry did not fit into either subpopulation at the 70% threshold chosen for all analyses. For K=2, both *He* and F_{ST} values were calculated to estimate the expected heterozygosity and gene flow for each subpopulation, respectively (Table 3.18). SPI had 10% lower *He* and less gene flow among subpopulation members than SPII. This could be due to the fact that SPII had more than twice as many samples as SPI.

Within section *ANA*, 40% of the accessions fell into SPII with 30% in subpopulation I (SPI) and 30% as admixed (Table 3.17). The two accessions from *BRE* were found in different subpopulations which is not surprising given that they were from different taxa: OHA and OLE. Similar to *ANA*, accessions from *MAJ* mainly fell into SPII (65%) followed by SPI (22%) and the admixed category (13%) (Table 3.11, Table 3.17). *ORG* accessions also fell into three groups with 42% in SPII and 29% each in SPI and the admixed category. The accessions of unknown taxon had a similar pattern of population structure with 63% in SPII, 17% in SPI and 20% in the admixed group. The data were examined to see if all of the samples from a given taxon fell into the same subpopulation. At *K*=2, there was no such clustering observed (Table 3.18).

3.4.3.3.2. *K*=4

When the accessions were examined according to *K*=4, the majority of individuals were found to be categorized as admixed (47%) with the four subpopulations in nearly equal proportions (11 to 18%) (Table 3.16). Specimens from the four sections were found together in each subpopulations with the exception of SPII which contained only individuals of section *MAJ*. The highest gene flow was found in SPI with an F_{ST} value of 18%. This subpopulation also had the highest *He* (30%) as expected for individuals with high gene flow (Table 3.18). On the contrary, the highest F_{ST} was found as 55% for SPIII, the group with the lowest expected heterozygosity.

Within section *ANA*, 50% of the individuals had admixed genetic structure. In section *BRE,* the single individuals of OHA and OLE were both admixed. Specimens from section *MAJ* were found to be dispersed over all SPs and the admixed group of individuals as might be expected due to the predominance of samples from this section in the study. The majority of individuals from *MAJ* was admixed (43%). SPII contained 22% of the *MAJ* accessions which were distinct from all other sections in this subpopulation. Specimens of section *ORG* were mostly found in SPIV (36%) and 29% of the specimens were categorized as admixed. Unknown accessions mainly belonged to the admixed category (57%) and to SPIII (23%).

At the taxon level, OSI and OHY from *ANA* were placed in completely different subpopulations. Within section *MAJ*, OON was the most highly represented taxon and had 47% of specimens as admixed. Some of the OON accessions formed their own subpopulation in SPII (30%). Moreover, none of the OON specimens fell into SPIII. Accessions from ODU taxon were mostly concentrated on SPI (36%) and found to be in all subpopulations except SPII. In *ORG*, half of the specimens of taxon OVVU were admixed and the rest contributed equally to SPI and SPIV. Specimens of taxon OVH were mainly grouped together in SPIII and SPIV in equal proportions (37%). Single OVG and OVVI specimens had admixed structure.

3.4.3.3.3. *K*=5

K=5 was investigated as the unknown specimens could have been members of a single taxon of the eight sections. The specimens were mostly admixed (53%) for *K*=5 (Table 3.16). Two of the five subpopulations were exlusively *MAJ* while the remaining three had a mixture of specimens from *MAJ*, *ANA* and *ORG* as well as UNK samples. SPIII which consisted of only *MAJ* accession from the OON taxon and 8% of the total number of samples had the lowest F_{ST} value (28%) and the highest *He* (29%) reflecting the high gene flow among the different OON accessions.

Specimens from *ANA* were found only in SPI, SPII and the admixed group. The two BRE samples were admixed. *MAJ* was the only section with individuals spread over all subpopulations. In fact, two of the subpopulations (SPIII and SPIV) were exclusively *MAJ*. ORG specimens fell into three subpopulations and also in the admixed group. UNK accessions had a similar pattern as the ORG specimens.

Within section *ANA*, the OHY accession was admixed (100%), while the OSI samples fell into two subpopulations, SPI and SPII (Table 3.10). The remaining *ANA* accessions were admixed (56%). Both single *BRE* specimens, OHA and OLE, had admixed structure. For section *MAJ*, the highest proportion (53%) of OON samples were admixed. The rest of the OON samples fell into three subpopulations with 21% in the OON-exclusive SPIII. No OON samples were found in SPI and SPV. ODU and OON specimens were grouped together in SPII and SPIV, while ODU and OSYBE individuals were grouped together in SPI and SPV. Moreover, ODU specimens were mostly located in SPV (27%), while OSYBE specimens were mostly concentrated in SPI (29%). The individuals belonging to section *ORG* were 43% admixed. The rest of the samples fell into mainly SPI with both OVH and OVVU individuals in this subpopulation. The unknown specimens were found to be 63% admixed followed by SPI (20%) and SPII (13%).

3.4.3.3.4. *K*=8

For *K*=8, the number of admixed individuals was the same as it was for *K*=5 (53%)(Table 3.16). Of the eight subpopulations, SPIV was the largest with 16% of the specimens followed by SPVI with 10%. The remaining SPs had 6% or fewer of the total number of accessions indicating that they were minor groups. One reason for testing K=8 was to see if the UNK samples grouped exclusively in one or more subpopulations. This did not occur, instead, the UNK samples remained grouped with specimens from known sections. Interestingly, the use of K=8 caused 19 of the *MAJ* accessions to become divided into four separate exclusive subpopulations. The lowest gene flow and highest expected hertozygosity were observed in SPVIII which contained individuals from *MAJ*, *ORG* and UNK taxa. The highest F_{ST} value was observed as 64% for SPV which was exclusively MAJ (Table 3.18).

Specimens from section *ANA* were mostly admixed (60%) and the remaining accessions (from OSI) were located in SPIV (33%) and SPVI (10%) (Table 3.16). The taxa belonging to section *BRE* (OHA and OLE) was both admixed. A total of 55% of individuals in section *MAJ* were categorized as admixed, while the remaining individuals were spread over all subpopulations. Specimens of section *ORG* were divided into four

main subpopulations (SPIII, SPIV, SPVI, and SPVII) in addition 43% admixed accessions. Approximately half of UNK specimens (47%) had admixed structure.

Within section MAJ, OON accessions were isolated from other taxa of the section in SPI, SPII, SPV, and SPVII. The highest proportion of OON individuals were in SPIV with 15% membership. In SPIII and SPIV, ODU and OON taxa were clustered together while ODU and OSYBE accessions were grouped together in SPVI. In ORG, taxa OVG and OVVI were defined as admixed. Specimens from OVH and OVVU were not clustered and fell into different subpopulations. Specimens from taxon OVH were divided into three subpopulations. In contrast, OVVU accessions only fell into SPIII (25%). The unknown accessions which were not admixed, mainly accumulated in SPVI.

Table 3.17.Summary of subpopulation and dendrogram cluster membership for all studied accessions. Inferred subpopulations for *K*=2, 4, 5 and 8 are given for thresholds 0.6 and 0.7. Cluster location from dissimilarity analysis (Cluster A**, B, and C) is given in the last column. "-" refers to samples that were excluded in dissimilarity analyses due to their high number of missing data for the analyses (more than 50%). "ADX" indicates: "admixed". Accessions are grouped according to taxon followed by their species and sorted according to sampling location. GR: Geographical region of Türkiye. %MV: missing value. A*/A in GR: Aegean region, BS: Black Sea region, CA: Central Anatolia region, EA: Eastern Anatolia region, M: Marmara region, MT: Mediterranean region. A**: Cluster A.

Section	Species Name	Darwin Code	Sampling Location	GR	*K*=2		*K*=4		*K*=5		*K*=8		Darwin (Cluster)
					0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.7	
ANA	*Origanum hypericifolium* O. Schwarz et P.H. Davis	OHY1	Denizli	A*	SPII	SPII	SPI	SPI	ADX	ADX	ADX	ADX	A**
	Origanum sipyleum L.	OSI7	Denizli		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	B
		OSI4	Kütahya		SPII	ADX	SPI	ADX	ADX	ADX	ADX	ADX	A
		OSI8	Kütahya		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		OSI9	Kütahya		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OSI5	Manisa		SPI	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OSI6	Manisa		SPII	SPII	SPI	ADX	ADX	ADX	ADX	ADX	A
		OSI1	Manisa		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OSI3	Çorum	BS	SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OSI2	Balıkesir	M	SPII	SPII	ADX	ADX	ADX	ADX	SPI	ADX	B
BRE	*Origanum haussknechtii* Boiss.	OHA1	Sivas	CA	SPI	SPI	SPIV	ADX	SPII	ADX	ADX	ADX	B
	Origanum leptocladum Boiss.	OLE1	Karaman		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	A
MAJ	*Origanum dubium* L.	ODU11	İzmir	A	SPII	SPII	SPI	ADX	SPV	SPV	SPVIII	SPVIII	A
		ODU7	Muğla		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	C
		ODU3	Kastamonu	BS	SPII	SPII	ADX	ADX	SPIV	SPIV	SPIII	SPIII	A
		ODU6	Kastamonu		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B

(Cont. on the next page)

Table 3.17. (cont.)

MAJ	*Origanum dubium* L.	ODU1	Antalya	MT	SPII	SPII	SPI	SPI	ADX	ADX	ADX	ADX	A
		ODU10	Antalya		SPII	SPII	SPI	ADX	ADX	ADX	ADX	ADX	A
		ODU2	Antalya		SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	SPVIII	A
		ODU4	Antalya		SPII	SPII	SPI	SPI	SPIV	SPIV	SPIII	ADX	A
		ODU5	Antalya		SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	SPVIII	A
		ODU8	Antalya		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		ODU9	Antalya		SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	SPVIII	A
		ODU12	Burdur		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		ODU13	İçel		SPII	SPII	SPIII	SPIII	SPI	ADX	SPVI	SPVI	A
		ODU14	İçel		SPII	SPII	SPIII	SPIII	SPI	SPI	ADX	ADX	A
	Origanum onites L.	OON17	Aydın	A	SPI	SPI	ADX	ADX	ADX	ADX	ADX	ADX	B
		OON18	Aydın		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OON19	Aydın		SPII	ADX	SPII	ADX	SPIII	ADX	ADX	ADX	B
		OON30	Aydın		SPI	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OON34	Aydın		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	ADX	A
		OON39	Aydın		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	ADX	A
		OON20	Denizli		SPI	SPI	SPIV	SPIV	SPII	SPII	ADX	ADX	-
		OON21	Denizli		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	SPII	A
		OON13	İzmir		SPII	SPII	SPII	SPII	SPIII	SPIII	ADX	ADX	B
		OON14	İzmir		SPII	SPII	SPII	SPII	SPIII	SPIII	ADX	ADX	B
		OON15	İzmir		SPII	ADX	SPII	ADX	SPIII	ADX	ADX	ADX	B
		OON16	İzmir		SPII	SPII	ADX	ADX	SPIV	ADX	SPIII	ADX	C
		OON27	İzmir		SPII	SPII	ADX	ADX	SPIV	SPIV	SPIII	ADX	C
		OON31	İzmir		SPI	SPI	SPIV	SPIV	SPII	ADX	ADX	ADX	B
		OON22	Manisa		SPII	SPII	ADX	ADX	SPIV	ADX	SPIII	ADX	C
		OON23	Manisa		SPI	SPI	SPIV	ADX	SPII	ADX	SPIV	ADX	B
		OON44	Manisa		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OON10	Muğla		SPII	SPII	ADX	ADX	SPIV	SPIV	SPIII	SPIII	C
		OON28	Muğla		SPII	SPII	SPI	ADX	SPIV	SPIV	SPIII	SPIII	C
		OON3	Muğla		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	SPII	A
		OON32	Muğla		SPII	SPII	SPII	SPII	SPIII	ADX	ADX	ADX	A

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Table 3.17. (cont.)

MAJ	*Origanum onites* L.	OON33	Muğla		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	ADX	A
		OON4	Muğla		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	SPII	A
		OON48	Muğla		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	A
		OON49	Muğla		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OON5	Muğla		SPII	SPII	SPII	SPII	SPIII	ADX	SPII	ADX	A
		OON6	Muğla		ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OON7	Muğla		ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OON8	Muğla		SPII	SPII	SPII	SPII	ADX	ADX	SPII	ADX	A
		OON9	Muğla		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	SPII	A
		OON36	Balıkesir	M	SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OON37	Balıkesir		SPII	SPII	SPII	SPII	SPIII	SPIII	ADX	ADX	B
		OON38	Balıkesir		SPII	SPII	SPII	ADX	ADX	ADX	ADX	ADX	A
		OON52	Balıkesir		SPII	SPII	ADX	ADX	ADX	ADX	SPV	SPV	A
		OON24	Çanakkale		SPII	SPII	ADX	ADX	SPIV	SPIV	SPIII	SPIII	C
		OON25	Çanakkale		SPII	SPII	SPII	SPII	SPIII	SPIII	SPII	SPII	A
		OON26	Çanakkale		SPII	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OON35	Çanakkale		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OON1	Antalya	MT	SPII	SPII	SPII	ADX	ADX	ADX	SPVIII	ADX	A
		OON11	Antalya		SPII	SPII	SPII	SPII	SPIII	ADX	SPII	ADX	C
		OON12	Antalya		SPII	SPII	SPII	SPII	SPIII	ADX	ADX	ADX	A
		OON2	Antalya		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OON29	Antalya		SPII	SPII	SPII	ADX	ADX	ADX	ADX	ADX	A
		OON45	Antalya		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OON50	Antalya		ADX	ADX	ADX	ADX	ADX	ADX	SPV	SPV	A
		OON51	Antalya		SPII	SPII	ADX	ADX	ADX	ADX	SPV	SPV	A
		OON41	Burdur		SPII	SPII	SPII	ADX	SPIII	ADX	SPI	SPI	B
		OON40	Isparta		ADX	ADX	ADX	ADX	ADX	ADX	SPI	ADX	B
		OON43	İçel		SPII	SPII	SPI	SPI	SPIV	SPIV	SPIII	ADX	A
		OON46	İçel		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OON47	İçel		SPII	SPII	SPI	SPI	SPV	ADX	SPVII	SPVII	A
		OON53	İçel		SPII	SPII	SPIII	ADX	SPI	ADX	SPVI	ADX	A

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Table 3.17. (cont.)

MAJ	*Origanum onites* L.	OON42	-	-	SPII	ADX	ADX	ADX	ADX	ADX	SPI	SPI	B
	Origanum syriacum L. subsp. *bevanii* (Holmes) Ietsw.	OSYBE1	Balıkesir	M	ADX	ADX	ADX	ADX	ADX	ADX	SPI	ADX	B
		OSYBE2	Adana	MT	SPII	SPII	SPI	SPI	SPIV	ADX	ADX	ADX	A
		OSYBE4	Antalya		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	B
		OSYBE3	Kahramanmaraş		SPI	SPI	SPIV	ADX	SPII	ADX	ADX	ADX	B
		OSYBE5	Kahramanmaraş		SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	ADX	A
		OSYBE6	Kahramanmaraş		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		OSYBE7	Kahramanmaraş		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
ORG	*Origanum vulgare* L. subsp. *gracile*	OVG1	Erzincan	EA	ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
	Origanum vulgare L. subsp. *hirtum*	OVH1	Antalya	MT	SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OVH2	Kahramanmaraş		ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OVH3	Kahramanmaraş		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		OVH4	Kahramanmaraş		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		OVH5	Kahramanmaraş		SPII	SPII	SPI	SPI	SPV	ADX	SPVII	SPVII	A
		OVH6	Kahramanmaraş		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		OVH7	Kahramanmaraş		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		OVH8	Kahramanmaraş		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
	Origanum vulgare L. subsp. *viride*	OVVI1	Antalya	MT	SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	A
	Origanum vulgare L. subsp. *vulgare*	OVVU4	İzmir	A	ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		OVVU1	Muğla		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	ADX	B
		OVVU2	Bilecik	M	ADX	ADX	ADX	ADX	ADX	ADX	SPI	ADX	B
		OVVU3	Antalya	MT	SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	SPVIII	A
UNK	UNK	UNK1	-		SPI	ADX	SPIV	ADX	ADX	ADX	ADX	ADX	C
		UNK2	-		SPII	SPII	SPI	SPI	SPIV	ADX	ADX	ADX	A
		UNK3	-		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	A
		UNK4	-		SPII	SPII	SPI	SPI	SPV	SPV	SPVIII	SPVIII	A

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Table 3.17. (cont.)

UNK	UNK	UNK5	-		SPI	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		UNK6	-		SPII	SPII	SPI	ADX	SPIV	ADX	ADX	ADX	A
		UNK7	-		SPI	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		UNK8	-		SPI	SPI	SPIV	ADX	SPII	ADX	ADX	ADX	B
		UNK9	-		SPI	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		UNK10	-		ADX	ADX	ADX	ADX	ADX	ADX	ADX	ADX	B
		UNK11	-		SPII	SPII	SPII	ADX	SPIII	ADX	ADX	ADX	A
		UNK12	-		SPII	SPII	ADX	ADX	ADX	ADX	SPV	SPV	A
		UNK13	-		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		UNK14	-		B	ADX	SPIII	ADX	SPI	ADX	SPV	SPV	A
		UNK15	-		SPII	SPII	SPIII	SPIII	SPI	ADX	SPV	SPV	A
		UNK16	-		SPII	SPII	SPIII	SPIII	SPI	SPI	SPV	SPV	A
		UNK17	-		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		UNK18	-		SPII	SPII	ADX	ADX	ADX	ADX	SPV	SPV	A
		UNK19	-		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	B
		UNK20	-		SPII	SPII	SPIII	ADX	SPI	ADX	ADX	ADX	A
		UNK21	-		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		UNK22	-		SPII	SPII	SPIII	SPIII	SPI	SPI	ADX	ADX	A
		UNK23	-		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		UNK24	-		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		UNK25	-		SPII	SPII	SPIII	SPIII	SPI	SPI	SPVI	SPVI	A
		UNK26	-		SPII	SPII	SPIII	ADX	SPI	ADX	SPVI	SPVI	A
		UNK27	-		SPII	SPII	ADX	ADX	ADX	ADX	ADX	ADX	A
		UNK28	-		SPI	SPI	SPIV	SPIV	SPII	SPII	SPIV	SPIV	-
		UNK29	-		SPII	SPII	ADX	ADX	ADX	ADX	SPVI	ADX	A
		UNK30	-		SPII	SPII	SPIII	ADX	SPI	ADX	SPVI	SPVI	A

Table 3.18.Populations structure results for the germplasm for *K*= 2, 4, 5 and 8. Sections belonging to each subpopulation and the admixed group are indicated. Expected heterozygosity (*He*) and gene flow (F_{ST}) values are also included.

_K_=2 Best number of _K_ suitable for the germplasm according to Structure Harvester database

SP	*Ns*	Sections	∑*Ni*	%	*He*	F_{ST} values
SPI	4	*ANA, BRE, MAJ, ORG, UNK*	29	22	0.20	0.43
SPII	4	*ANA, BRE, MAJ, ORG, UNK*	79	61	0.30	0.13
Admixed	3	*ANA, MAJ, ORG, UNK*	22	17	-	-

_K_=4 Four main taxa of sections in collection (*ANA, BRE, MAJ, ORG*)

SP	*Ns*	Sections	∑*Ni*	%	*He*	F_{ST} values
SPI	3	*ANA, MAJ, ORG, UNK*	14	11	0.30	0.18
SPII	1	*MAJ*	16	12	0.22	0.39
SPIII	3	*ANA, MAJ, ORG, UNK*	15	12	0.16	0.55
SPIV	3	*ANA, MAJ, ORG, UNK*	24	18	0.21	0.41
Admixed	4	*ANA, BRE, MAJ, ORG, UNK*	61	47	-	-

_K_=5 Considering unknown specimens as a member of any of the four sections or a member of a fifth section

SP	*Ns*	Sections	∑*Ni*	%	*He*	F_{ST} values
SPI	3	*ANA, MAJ, ORG, UNK*	13	10	0.21	0.43
SPII	3	*ANA,MAJ, ORG, UNK*	23	18	0.21	0.43
SPIII	1	*MAJ*	11	8	0.29	0.28
SPIV	1	*MAJ*	7	5	0.22	0.38
SPV	2	*MAJ, ORG, UNK*	7	5	0.16	0.57
Admixed	4	*ANA, BRE, MAJ, ORG, UNK*	69	53	-	-

_K_=8 The number of oregano sections that naturally grow in Turkey

SP	*Ns*	Sections	∑*Ni*	%	*He*	F_{ST} values
SPI	1	*MAJ*	2	2	0.17	0.56
SPII	1	*MAJ*	5	4	0.22	0.40
SPIII	1	*MAJ*	4	3	0.20	0.44
SPIV	3	*ANA, MAJ, ORG, UNK*	21	16	0.15	0.59
SPV	1	*MAJ*	8	6	0.17	0.64
SPVI	3	*ANA, MAJ, ORG, UNK*	13	10	0.19	0.49
SPVII	2	*MAJ, ORG*	2	2	0.23	0.37
SPVIII	2	*MAJ, ORG, UNK*	6	4	0.28	0.32
Admixed	4	*ANA, BRE, MAJ, ORG, UNK*	69	53	-	-

3.4.3.4. Comparison of population structure and dissimilarity clustering

The dendrogram clustering pattern was compared to the subpopulation grouping for *K*=2 and *K*=4 (Table 3.19). For *K*=2, SPI individuals were only found in cluster B whereas SPII individuals were located in all three clusters (Figure 3.18). The admixed individuals were also dispersed over all three clusters with the most samples in cluster B (Figure 3.18). The PCoA graph was also colored according to *K*=2 (Figure 3.21). According to this plot, admixed individuals in SPIII were placed between the clusters of SPI and SPII which fell in separate halves of the graph: SPII samples were located in the +X part of the graph while SPI samples were in the –X part of the graph. SPI samples were more dispersed while SPII accessions were more concentrated.

For *K*=4, SPI and SPIII corresponded to cluster A. SPII and the admixed accessions were spread over all three clusters (Figure 3.19 and Figure 3.20). SPIV accessions formed a nearly exclusive subcluster within cluster B. When admixed accessions were excluded from the dendrogram analysis (Figure 3.20), the agreement between the population structure and NJ clustering analysis is very evident. The PCoA graph also showed a strong correspondence with the population structure results (Figure 3.22). SPI and SPII specimens were located in the lower right quadrant of the plot quite close together but not overlapping. SPIII samples were predominantly placed in the upper right quadrant closest to SPI individuals. SPIV samples were highly dispersed in the upper left quadrant and very distinct from the rest of the subpopulations. The admixed specimens were also spread out in the plot and located between all four subpopulation clusters.

Table 3.19. Comparison of subpopulation membership (%) among total number of individuals (∑*Ni*) per cluster and for unknown (UNK) species for each number of *K*; 2, 4, 5 and 8 for each Cluster (A, B, and C) in dissimilarity analyses.

SP	***K***	**Cluster A**		**Cluster B**		**Cluster C**		**Not examined**	
		∑Ni=67		∑Ni=43		∑Ni=9		∑Ni=11	
		Ni	%	Ni	%	Ni	%	Ni	%
SPI	2	-	-	18	42	-	-	-	-
SPII		64	96	7	16	8	89	11	100
Admixed		3	4	18	42	1	11	-	-
Total %			100		100		100		100
SPI	4	14	21	-	-	-	-	-	-
SPII		12	18	3	7	1	11	-	-
SPIII		15	22	-	-	-	-	-	-
SPIV		-	-	13	30	-	-	11	100
Admixed		26	39	27	63	8	89	-	-
Total %			100		100		100		100

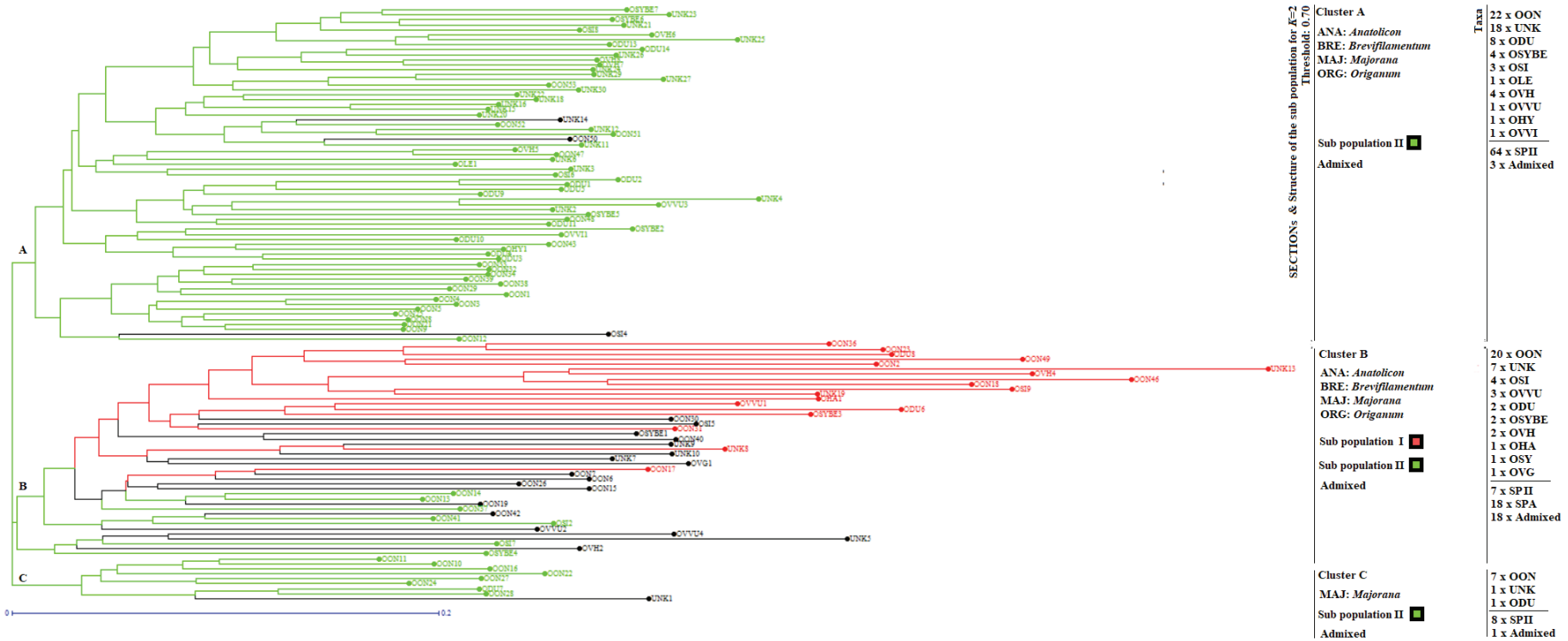

Figure 3.18. NJ Dendrogram colored according to assumed *K*=2 for oregano populations (threshold: 0.70). The dendrogram consisted of three main clusters: Cluster A, B and C. Colors indicate the subpopulation to which accessions belonged according to population structure analysis: Lime green: Sub population II, red: Sub population I, and black: Admixed individuals.

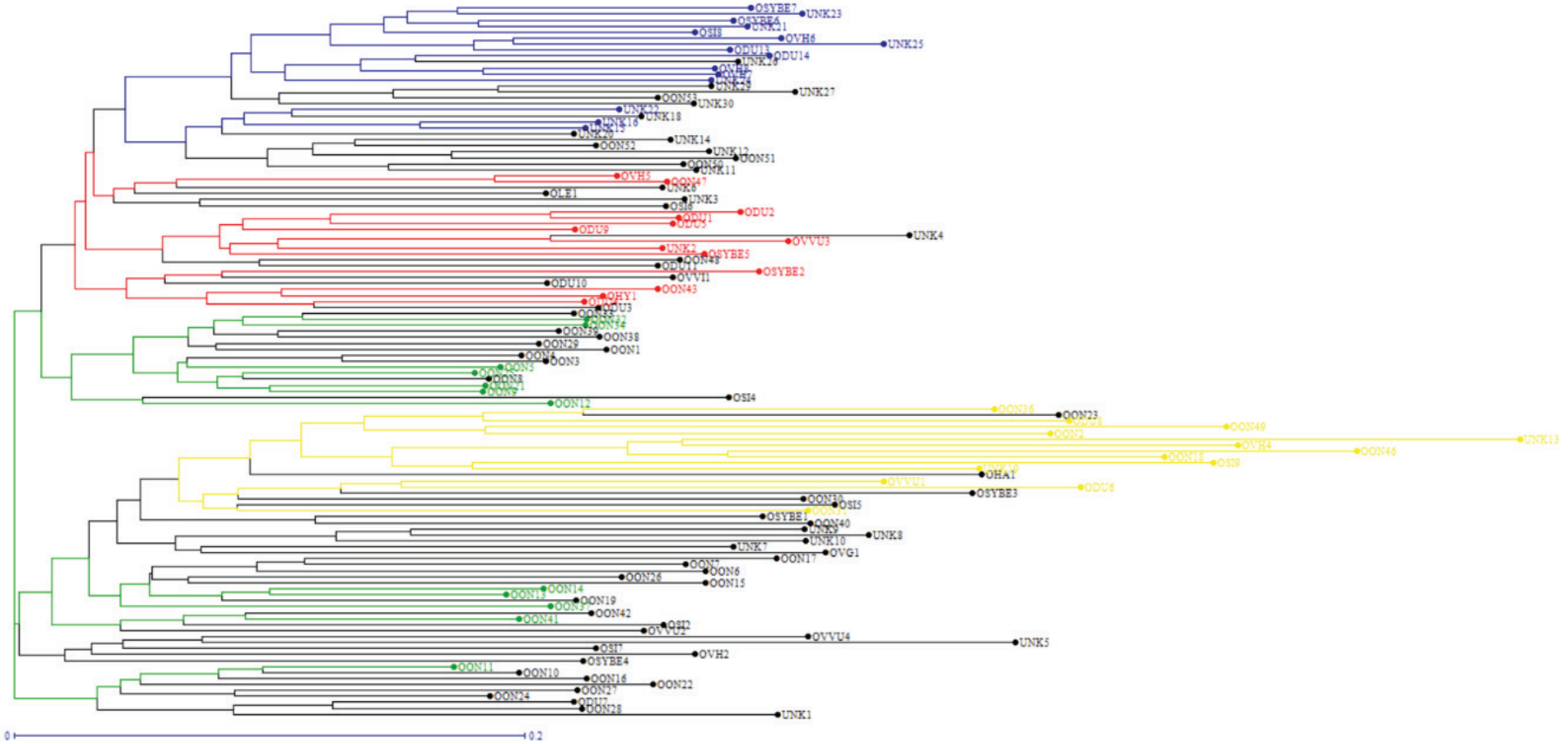

Figure 3.19. NJ Dendrogram colored according to assumed *K*=4 for oregano populations (threshold: 0.70). The dendrogram consisted of three main clusters: Cluster A, B and C. Colors indicate the subpopulation to which accessions belonged according to population structure analysis: Red: SPI, Green: SPII, Blue: SPIII, Yellow: SPIV, and black: Admixed individuals.

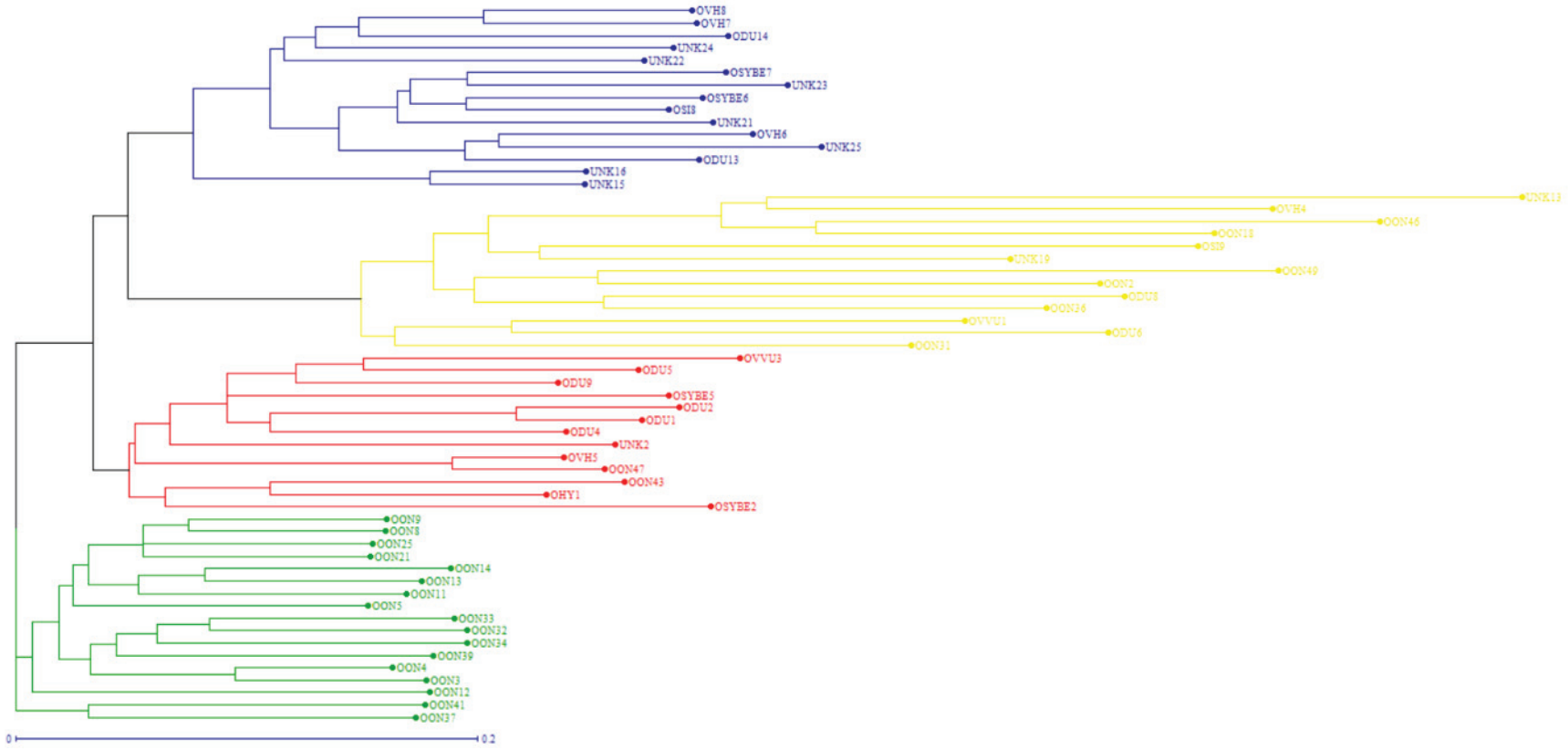

Figure 3.20. NJ Dendrogram colored according to assumed *K*=4 for oregano populations except the admixed individuals (threshold: 0.70). The dendrogram consisted of three main clusters: Cluster A, B and C. Colors indicate the subpopulation to which accessions belonged according to population structure analysis: Red: SPI, Green: SPII, Blue: SPIII, and Yellow: SPIV.

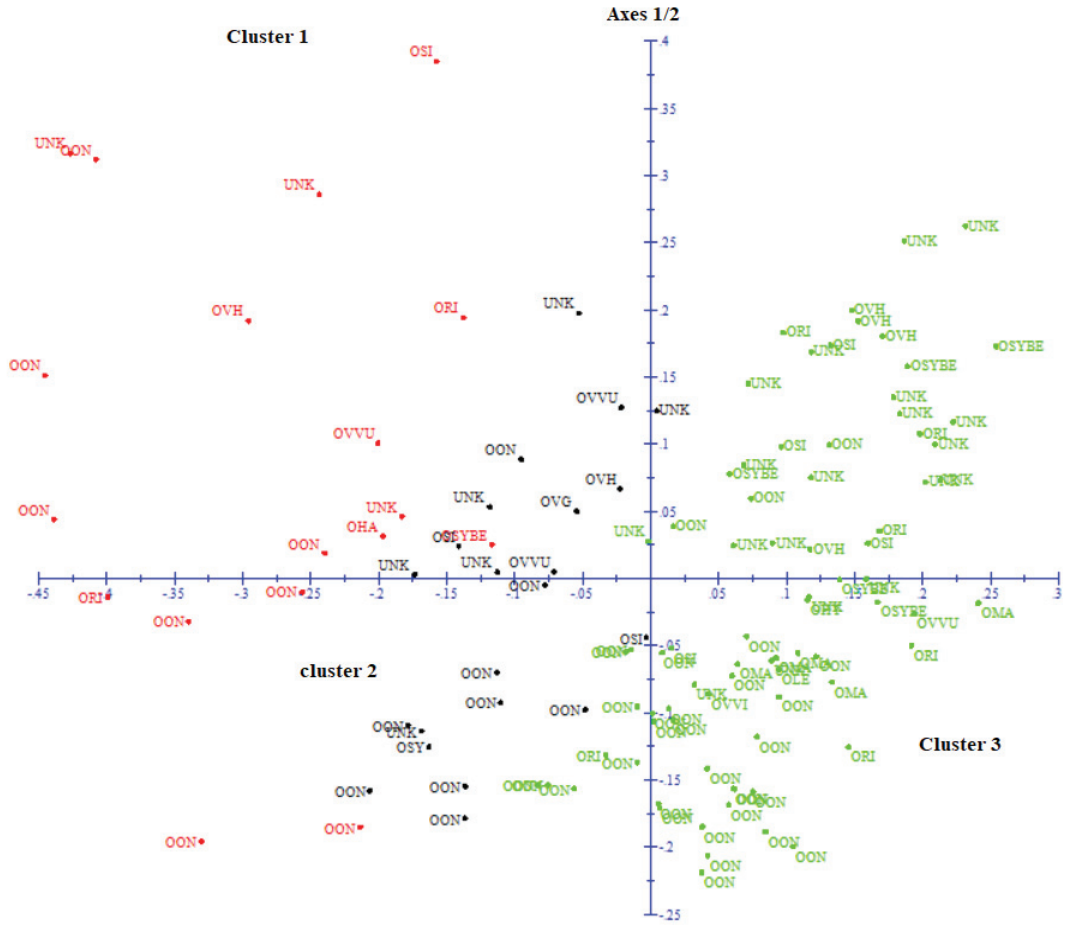

Figure 3.21. PCoA graph colored according to population structure for *K*=2 (threshold: 0.7).Colors indicating structure subpopulations as red is SPI (cluster I), green is SPII (cluster II), and black referring to admixed individuals.

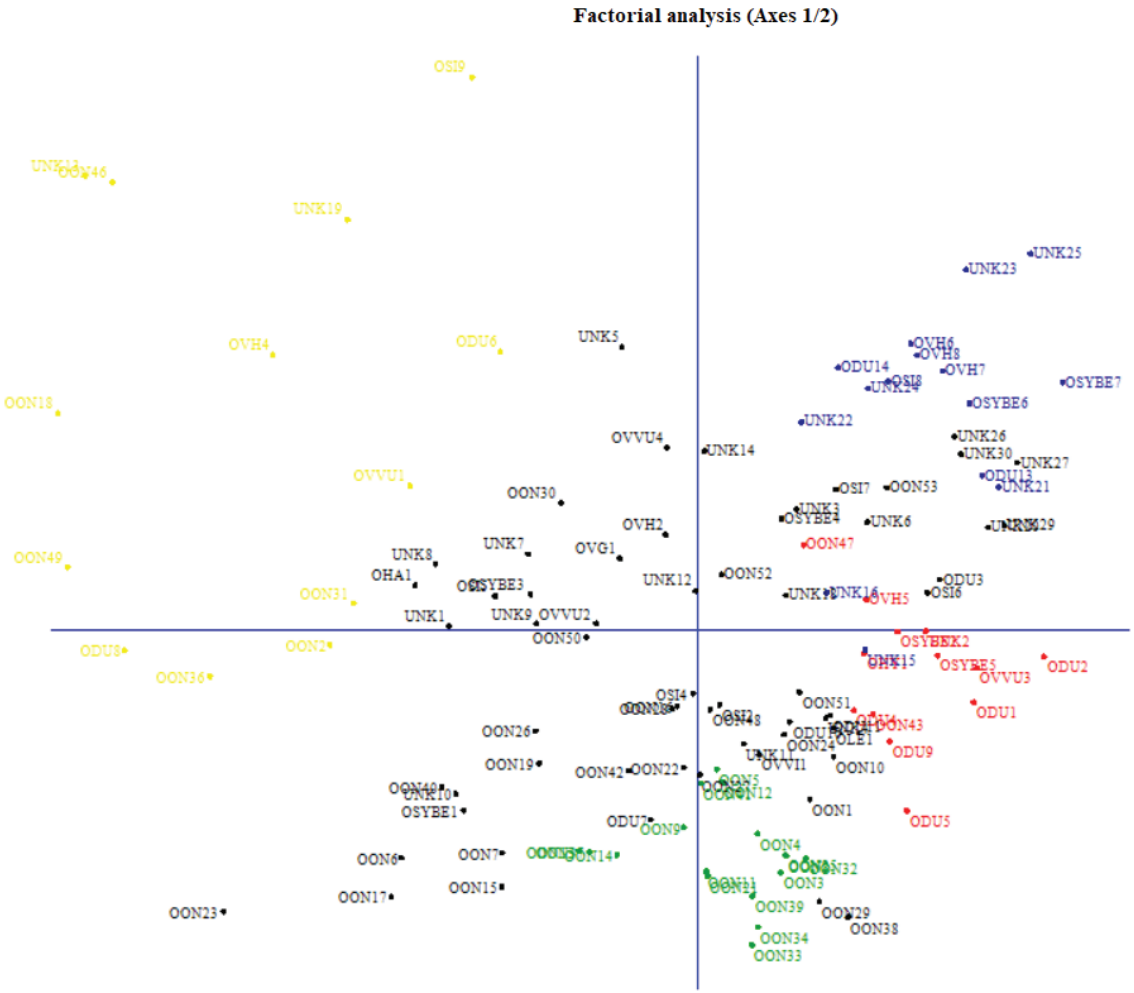

Figure 3.22. PCoA graph colored according to population structure for *K*=4 (threshold: 0.7). Colors indicating structure subpopulations as red is SPI (cluster I), green is SPII (cluster II), blue is SPIII, yellow is SPIV and black referring to admixed individuals

3.5. Discussion

Metabolic pathways and their abundant products are important for medicinal and aromatic plants to defend and adapt themselves to various environmental conditions. The diversity of bioactive compounds within the genus *Origanum* has the most chemotype-rich variation compared to the closely related *Thymus, Thymbra,* and *Satureja* genera in family, Lamiaceae. In Türkiye, the most frequently traded "oregano" species are OON, OVH, OVVU, OMI, *Thymus capitatus*, *Thymus spicata*, *Corydothymus capitatus*, *Satureja hortensis*, *Satureja cuneifolia* (Gürbüz et al., 2011). There was an approximate four fold increase in oregano production in the last two decades which creates potential for Türkiye as an important exporter as indicated in IX. Türkiye Agriculture Engineers Technical Congress Declaration Book (Source: Url8). Despite the undeniable importance of oregano species, critical warnings are not being held by people, creating danger for the conservation of this genus in its own habitat. In addition, there are several other factors that must be considered in the protection of oregano genetic resources in isolated locations such as ongoing hybridization events leading to continuous homoploid speciation. The cultivation and clonal production of some widely distributed oregano species such as *O. vulgare* subsp. *hirtum*, *O. onites*, and *O. syriacum* is widely performed and is important for the selection and improvement of better-quality clones containing various economically important bioactive compounds (Arslan, 2016; Tan, 2022). However, at the same time it is crucial that endemic oregano species are not overlooked. The highly endemic nature of oregano results in chemotypic and genetic richness and creates the necessity to promote and conserve more oreganos via domestication, cultivation and even breeding applications. Thus, there is a need for the management of sustainable oregano biodiversity depending on *in situ* and *ex situ* conservation actions as well as responsible use of Türkiye's native flora.

Gene banks often measure the morphological characters of their specimens; however, it is also important to characterize oregano species molecularly to understand their complex taxonomic relationships. Molecular markers reveal the genetic structure of species diversity and may help to identify certain specimens within a gene pool by associating metabolome diversity with genetic factors. Selected accessions can then be used as parents for breeding or clonal production programs. In Türkiye, there are two main gene banks (Aegean Agriculture and Research Institute – AARI and Ankara

University) that conserve, cultivate, and investigate MAPs. According to Karık et al. (2016), preparing collections, conservation acts and characterizations of medicinal and aromatic plants in AARI has been applied since 1979. These actions determined as crucial for gathering high quality yields independent of nature. There is already determined two cultivars of oregano (Taysi 2002 and Ceylan 2002; both OON) with high quality and yield properties, yet one more (*O. onites*) oregano line was suggested to be assumed as a new trading cultivar (Karık et al., 2016; Lukas and Novak, 2020). The vegetative or generative operations already applied to oregano in AARI Institute due to requirement of both settled and rich genetic resources; however, oregano as being a cross-pollinating species, require specialized planting lands for vegetatively produced oreganos while generatively produced oreganos can serve as a good source of allelic variation for breeding programs (Karık et al., 2007; Karık et al., 2016). Due to the complexity in their reproductive biology, conventional breeding is insufficient for a precise estimation and development of novel economically important oregano cultivars; so, there is a need for a quicker and alternative method for breeding practices (Tan, 2022). Investigation of individual oregano seeds and clones interdisciplinary including the molecular genetic level will help to develop new oregano species easier via Marker Assisted Selection (MAS). Some of the oregano materials from the AARI gene bank were investigated at the DNA level by Ayanoğlu et al. (2006). Moreover, other karyological and molecular studies have been conducted to investigate the oregano genome worldwide (Ayanoğlu et al., 2006; Novak et al., 2008; Katsiotis, 2009; Marieschi et al., 2010; Amar and Wahab, 2013; Karaca et al., 2013; Ince et al., 2014; Lukas et al., 2013; Aboukhalid et al., 2017; Jedrzejczyk, 2018; Taşcıoğlu et al., 2018; Taş, 2019; Arabacı et al., 2021; Karagöz et al., 2022; Antaloudaki et al., 2022; Tan, 2022). Molecular characterization is important for a better understanding of the level of genetic diversity and speciation dynamics within genus *Origanum*. In this chapter, the genetic diversity in the *Origanum* collection conserved in AARI Gene Bank was investigated.

In this study, 130 *Origanum* specimens from the AARI collection were examined. These specimens belong to eleven oregano taxa including four endemic species (OHY, OSI, OHA and OLE) from sections *Anatolicon*, *Brevifilamentum*, *Majorana*, and *Origanum.* Samples with unknown information were also included.

3.5.1. SRAP markers in *Origanum*

Plant growth, development, and viability is affected by the ability of the plant to adjust to various environmental constraints such as climate, altitude, sea level, flooding events, fertilizers, or predators each season and throughout their life span. Changes in the environment caused by natural or by human forced may cause plants to have diversified or specialized morphological (phenotypic) or biochemical (chemotypic) features to adapt themselves individually or collectively (as a population) over time. However, these characters are prone to change and are mostly variably at the transcriptome level. Therefore, DNA markers are more reliable sources of variation and taxonomic identification. Every plant genome has chromosomes which contain packaged DNA molecules which are very well conserved and may not be affected by slight environmental changes. As a result, DNA-based molecular markers have been developed and have become an important method to characterize and investigate plant species. Molecular markers are reliable and advantageous due to their reproduciblity, polymorphism, cost friendliness, and applicability to whole genomes.

In the genus *Origanum*, different types of molecular markers have been used to investigate single oregano taxa or admixed populations. In this dissertation, SRAP markers were used to screen species in the genus *Origanum* for the first time. In the work described in chapter 2, 46 specimens from 22 Origanum species representing eight sections were screened with 25 SRAP primer combinations (Tascioglu et al. 2018). A total of 325 polymorphic alleles were detected for the germplasm with 3 to 20 alleles per SRAP marker. The highest genetic diversity (*GD*) value, 0.35, was obtained with primer pair me3-em4 and the lowest, 0.14, was seen for me3-em11. The average *GD* across all markers was 0.27. For the AARI collection, the highest number of alleles was observed for me3-em2 marker with a *GD* value of 0.42 and 11 alleles. On the contrary, the lowest *GD* value was measured for marker me4-em1 marker with a *GD* of 0.22 and 10 alleles. Thus, the same primer pairs were not equally polymorphic in both sets of materials as might be expected given that the materials for each study were quite distinct. For example, germplasm spanning eight sections was examined in Chapter 2 while only four sections were included in the AARI collection. In the study of Tanhaş (2019), 18 oregano taxa were investigated with SRAP markers and the mean GD value was calculated as Polymorphism Information Content (PIC). The PIC value obtained was similar to that of

the AARI material, 0.28. In the study of Amar and Wahab (2013), an oregano population consisting of OVVU (*ORG*), OVH (*ORG*), OSYSI (*MAJ*), and OMA (*MAJ*) taxa was screened with ISSR and SRAP markers. As a result, the PIC value (threshold=1) was 0.97 with high polymorphism value. This value translated to a value of 0.48 according to our calculation method. In the study of Alexseeva et al. (2021), an OVH population consisting of 239 individuals from Bulgaria was investigated with 11 SSR and eight SRAP markers. The SRAP markers were suggested as more informative than microsatellites. Genetic differentiation was observed at low and high ranges (F_{ST}= 0.0047 – 0.11) and ten genetic clusters within the OVH population were identified. However, with the use of biochemical diversity calculations, the number of populations was reduced to six chemotypic subpopulations. This finding reflects the importance of interdisciplinary characterization of *Origanum* specimens.

In this work, the selected SRAP markers generated 100% polymorphic alleles for oregano and were very informative for the evaluation of genetic diversity for both AARI and herbarium oregano germplasm. Overall, previous research as well as our work indicate that SRAP markers are effective for the study of genetic diversity in *Origanum*.

In the AARI material, within section diversity was high (96%) when compared to among population diversity (4%). This high value of within population diversity was also observed at the species level (95%). These findings are supported by SRAP analyses conducted by Zagorcheva et al. (2020) who found 97% within population diversity for two lavender populations from the same subfamily, Nepetoideae. On the contrary, Sarfaraz et al. (2021) investigated thyme species with SRAP markers and the within population diversity was observed as 63% for their population which contained eleven taxa. This might be due to differences in their speciation processes. Oregano prefers homoploid hybridization and therefore mostly undergoes sympatric speciation while thyme undergoes allopatric speciation via natural selection (Molins et al. 2011). As a result, the within population diversity among oregano species is higher when compared to its ancestral family, thyme.

3.5.2. Hybridization in *Origanum* and genetic diversity in sections *ANA*, *BRE*, *MAJ*, and *ORG*

The genus *Origanum* is monophyletic but hypothesized to have originated from hybridization events among the polyphyletic genus *Satureja* and the paraphyletic genus *Thymus* (Ietswaart, 1980; Brauchler, 2010; Celep et al., 2021; Antaloudaki et al., 2022). Thus, it was concluded by Ietswaart that *Origanum* is closely related to these two genera. In addition, it is closely related to the monophyletic genus *Micromeria* (Ietswaart, 1980; Brauchler, 2010). According to Brauchler et al. (2010), oregano specimens from six section (*AMA, BRE, CAMPA, CHI, ELON*, and *ORG*) are paraphyletic with *Thymus* species according to plastid marker sequence analysis but not for nuclear marker analysis. According to Brauchler, this finding may indicate that plastid introgression rather than common ancestry may explain their grouping pattern. However, the authors also admit that more work needs to be done in this area. This example emphasizes the need for further research on *Origanum's* ancestry as well as the relationships between *Origanum* sections.

Türkiye is known as the gene center and origin for oregano species with eight of ten sections found in the country. In Türkiye, there are also a total of 13 intermediate hybrid species that have been reported to be naturally present (Dirmenci et al., 2021a). According to Table 1.3 (Ietswaart, 1980; Dirmenci et al., 2021a), sections *ORG, MAJ and AMA* have the most cross-sectional hybridizations with five instances each. These are followed by *ANA*, *BRE* with four instances each and *PRO* with three cross-hybridizations. *CHI* and *LONG* have only had cross-sectional hybridizations with a single section. The highest number of viable intermediate hybrids was reported between section *MAJ* and two main sections, *ANA* and *ORG*, in nature (Dirmenci et al., 2021a). The three reported intermediate hybrids between *ANA* and *MAJ* (OLI x OSYBE; OSI x OON; OVET x OON) suggest that there might be specimens in the herbarium and AARI collections which are named as OSI, ODU, OSYBE or OON but are actually the result of hybridization. This hypothesis is supported by the high *Nm* values between sections *ANA* and *MAJ* (Ietswaart, 1980; Taşcıoğlu et al., 2018; Dirmenci et al., 2021a).

New hybrid oregano species are frequently being identified and reported in other regions of the world as well as Türkiye. The generation and diversification of new species are also suggested to be a consequence of species-specific mating systems and the

reproductive strategies of individual plants (Barret, 2010; Van Looy et al., 2011). Ietswaart stated that the sections *CHI, ELON, MAJ, ORG*, and *PRO* have gynodioecy that can affect the speciation dynamics of oregano. Gynodioecious plants have both bisexual and female only (male sterile) flowers. Thus, such species may be more open to cross-hybridization which should be reflected in gene flow values. For example, in nature, taxon OVH from sect. *ORG* was the most abundantly reported parental species among hybrids (Dirmenci et al., 2021a). OVH was followed in frequency as a parental line of hybrids by OSYBE and OON from sect. *MAJ*. OSI from sect. *ANA* and OLA from sect. *PRO* have also participated in the generation of hybrids in nature. In the results presented in Chapter 2, the highest levels of gene flow were within and between species in sections *MAJ*, *ORG* and *PRO*, all of which are gynodioecious. In contrast, lower levels of gene flow were observed between the strictly bisexual species in sect *BRE* (OHU and ORO) with other species.

According to Ietswaart, sections *ORG*, *MAJ* and *ANA* are the ancestral species which led to the speciation and divergence of other oregano species (Ietswaart, 1980). The frequent occurrence of natural hybrids and high gene flow between species from these sections supports this claim. In the AARI collection, the OVH specimens had the highest gene flow with OVVU (*ORG*) and OSI (*ANA*), while OSI had the highest gene flow with OVVU and OVH (both *ORG*). These results are in agreement with what is observed in natural hybrids. Moreover, the highest number of migrants was observed between taxa OSI – OVH suggesting that some of the OSI or OVH individuals in the AARI germplasm might be intermediate hybrids. *O. x aytacii* (OSI x OVH) is such an intermediate hybrid which has phenotypic features that are predominantly similar to OSI in nature. This makes the hybrid difficult to identify morphologically as it can be mistaken for either parent. Molecular genetic data can aid in the identification of such hybrids and also help detect materials which have been misclassified based on morphology. Although OLA (*PRO*) and OSYBE (*MAJ*) were reported as having a viable hybrid in nature (*O. x haradjanii*, Hatay, 1952), the genetic material from the herbarium collection (Chapter 2) showed low gene flow between these two species (*PhiPT*: 0.23, *Nm*: 0.84). It is important to examine gene flow between species and to identify potential hybrids as hybridization is a source of genetic diversity and contributes to bioactive compound richness.

3.5.2.1. *Section Anatolicon (ANA)*

In the AARI collection, section *ANA* had ten accessions from two *Origanum* taxa, *O. sipyleum* (OSI) and *O. hypericifolium* (OHY). The overall diversity value (*h*) for the section was 48%. *ANA* was equally identical (96%) to all other sections: *BRE*, *MAJ*, and *ORG*. The highest gene flow from section *ANA* was to section *ORG*. These results are in agreement with the hybridation patterns for these two sections in nature and throughout evolutionary time.

Within the section, OSI with nine accessions had the highest genetic identity (0.95) to OVVU from section *ORG*. The highest number of migrants (*Nm*) for OSI was observed as 24.75 with OVH, while the *Nm* value between OSI and OVVU was observed as zero. This might be due to the high genetic similarity between OSI and OVVU (0.95). The high genetic identity between these two taxa from sections *ANA* and *ORG* might be due to the fact that OVH and OVVU are the most widely distributed and abundant taxa in Türkiye.

3.5.2.2. *Section Brevifilamentum (BRE)*

The AARI material contained two specimens from *BRE*, OHA and OLE. Genetic diversity (*h*) of these samples was 48%. The genetic identity of section *BRE* was equal for all other sections (0.96). In the study of Karagöz et al. (2022), *NGI* was observed as 0.62 for *O. acutidens* (OAC) accessions that were collected from five different provinces in the same section, *BRE*. However, because of the limited number of samples in the AARI material, we cannot make a fair comparison with these previous results. Individual gene flow within OHA and OLE could not be calculated due to single individuals per taxon.

3.5.2.3. *Section Majorana (MAJ)*

The AARI collection contained 74 specimens from OON, ODU, and OSYBE in section *MAJ*. Similar to the other sections, MAJ had 96% genetic identity with each of the other sections. Gene flow for *MAJ* was highest with *BRE*. At the species level, the

genetic identity values were nearly the same for all pairs of taxa (93 to 94%). In our work, OON had the lowest gene flow with OVH (*ORG*) (F_{ST}= 0.10). However, according to their *NGI* values, these taxa are almost identical (0.94). In the study of Tanhaş (2019), OON (*MAJ*) was determined to be the closest to OVH from sect. *ORG* supporting our observation. In the study of Lukas et al. (2013), gene flow was observed as higher between Sicilian ODU and Turkish OON (F_{ST}=0.28-0.33) when compared to Greek OON and ODU (F_{ST}=0.47-0.56) which they suggest reflects the geographical overlap of ODU and Turkish OON. Our data agree with these results as the Turkish accessions of OON and ODU had high gene flow. OON was suggested to be the ancestral lineage for generation of ODU and OSY (spread over three different locations: Northwestern, South, Eastern Mediterranean), while OSY was assumed to be the ancestor for OMA (which is distributed mostly in Cyprus, not Türkiye). In agreement with this, the highest gene flow for the AARI *MAJ* material was observed between ODU and OSYBE (syn. OSY).

3.5.2.4. *Section Origanum (ORG)*

The AARI material contained 14 specimens from section *ORG* including species OVG, OVH, OVVI, and OVVU. Section ORG had 96% genetic identity with the other sections. Genetic diversity in the section was 48%. At the species level, genetic identity of the *ORG* species varied from 93 to 95% with the other species in the collection. In our work, only two species had more than one accession, OVH and OVVU. The genetic identity for these species ranged from 0.93 to 0.95 for each taxon. Across taxa, OVH had the highest gene flow with OSI from section *ANA*. The highest gene flow within section *ORG* was observed for the species pairs: OVVU – ODU and OVVU – OVH (F_{ST}= 0). There are no observations of OVVU as a parent of natural oregano hybrid species according to Table 1.3 (Dirmenci et al., 2021). However, according to observations by Ietswaart (1980), there were hybridizations between OVVU with OSI, OMA, and OVH, in addition to artificial hybrids with ODU (Ietswaart, 1980). In the AARI collection, no allele migration was evident between OVVU (*ORG*) and ODU (*MAJ*), OSI (*ANA*), or OVH (*ORG*). On the contrary, OVVU specimens collected from nature in the herbarium collection described in Chapter 2 had relatively higher numbers of migrants with OSY (OSYBE – *MAJ*) and OVH (*ORG*). This may indicate that OVVU actually does hybridize

with other species in nature without resulting in a hybrid with intermediate morphological characters.

3.5.2.5. The effect of geographical distribution on oregano diversity

Over time, cross-hybridization among individuals led to gene flow among populations and species. At the same time, reproductive barriers caused by geographic isolation can limit the tendency for cross-taxon hybridization. According to Karagöz et al. (2020), many types of geographical and ecological circumstances have great influence on gene flow among cross-pollinating *Origanum*. Türkiye has a 783,562 km² surface area with 74.6% of land covered by mainly seven categories of mountains and landforms across various heights above sea level (Duran, 2013; Dal and Gönençgil, 2018). The mountains in the coastal regions are exposed to various climatic conditions and their surface structures and height affect the transfer of the climate towards the inner geographic regions. Especially, the mountains lying perpendicular to the Aegean coast allows the coastal climate to affect the inner regions. In contrast, mountains that are parallel to coastal regions inhibit the dominancy of the coastal climate on inner regions. This is true in the Black Sea, Mediterranean and Central Anatolian regions (Apaydin et al., 2011). These types of geographical indices force plants to evolve and adapt to different altitudes above sea level. In the study of Kokkini et al. (1994), the researchers associated glandular/non glandular trichome morphology of OVVU with geographical location from eastern to central and northern Greece. Evolutionary migration of *O. compactum* (OCO) between Northern Morocco and Southern Spain was described (Aboukhalid et al., 2017) and it was concluded that the mountains had a major role in hindering the migration of this species. In addition, according to Van Looy et al. (2009), extreme and regular flooding events changes geological structures and resulted in genetic diversity of *O. vulgare* over evolutionary time. These types of specializations to climate and geo-structural variability may increase biodiversity richness at the same time that geographical isolation limits hybridization.

The distribution of *O. vulgare* accessions from sect. *ORG* was associated with phytogeographical location (Celep et al., 2021). For example, OVVIR and OVVU were found to be growing in the Euro-Siberian region, while OVG was suggested to be occurring in the Irano-Turano region. In addition, OVH was found originally in the

Mediterranean region in Türkiye (Celep et al., 2021). The occurrence of species diversity between OVH and OVVU in *ORG* is hypothesized to be due to their adaptation to different altitudes creating chemical diversity, specialized flower features, and restricted geographical localization in nature (Mertzanidis et al., 2022). According to this study, OVH is called as "white oregano" and OVVU was called as "black oregano" due to the white and purple colors of their flowers. OVH was found at lower altitudes while OVVU was found at higher altitudes above sea level. Their findings suggested that OVH had higher biochemical diversity when compared to OVVU. In contrast, the lower diversification of OVVU might be the result of genetic adaptation to poorer lands. In our work, there was no information about the altitude of collection locations of samples OVH and OVVU. However, it was observed that some of the OVH specimens formed a tight cluster while OVVU samples were more dispersed. This does not agree with the findings of Mertzanidis et al. (2022). However, it is important to note that OVVU accessions were from four different provinces in three regions (Marmara, Mediterranean, and Aegean; all coastal regions) while the clustered OVH specimens were all from Kahramanmaraş. Thus, the geographic distribution of these accessions corresponds with their distribution in the genetic diversity analysis. Moreover, the distinct genetic diversity between OVH and OVVU was also supported by observation of zero *Nm* value; which might also reflect their geographical isolation generates genetic barriers for hybridization between these taxa from the same section, *ORG*.

The effect of restricted distribution of species in genus *Origanum* was also evident in the OLA specimens from the herbarium collection. These accessions formed their own subcluster in PCoA and structure analyses. This may be due to the fact that all of the samples were collected from two adjacent provinces in the Mediterranean region. Thus, geographical isolation from the other species would prevent wide cross-hybidization. The accessions that were most closely related to the OLA specimens were from the Mediterranean region and representec sections *MAJ* and *ORG*. This agrees with the report that *PRO* can form hybrids with sect. *MAJ* (Dirmenci et al., 2021). Hybridization with sect. *ORG* might be due to geographical proximity allowing them to hybridize without a geographical or molecular barrier.

Both geographic and genetic barriers can inhibit the generation of viable hybrids due and result in relatively pure plantlets. In the study of Lukas et al. (2013), it was concluded that there was gene flow from ODU to OON, while OON has also a relatively "pure" population in certain geographical locations. In the AARI material, exclusive

genetic clustering was observed for a few of the species suggesting that they might be relatively pure lines. For example, seven OON accessions clustered apart from all but one other known accession in dendrogram analysis (Figure 3.9). These specimens could be considered purer than the OON samples that were genetically similar to other species. There were also instances of two or more specimens of OVH, ODU and OON from the same provinces that formed exclusive clusters.

3.5.2.6. Possible taxonomic origins of unknown specimens

The unknown specimens from the AARI collection that were screened with SRAP markers were mostly found in dendrogram subcluster A.1 with 20 accessions followed by seven accessions in cluster B, and a single UNK accession placed in subcluster C.2. The most likely identities for UNK specimens are given in Table 3.20 and are based on their closest neigboring taxa (Table 3.20). UNK3 was closest to OSI accessions suggesting that it belongs to this species. UNK11, UNK12, UNK14, UNK15, UNK16, UNK18, UNK20, and UNK22 grouped together and were most closely related three OON accessions which suggests they might be OON or a closely related species. UNK27, UNK29 and UNK30 were also most closely related to OON. UNK1 was equally distant to OON and ODU which might reflect its shared genetic identity with both accessions as a hybrid specimen or a single genotype having gene flow in both directions. As Lukas et al. suggested, there is gene flow from ODU towards OON (Lukas et al., 2013), a situation that might result in individuals like UNK1. UNK2, UNK4 and UNK5 were most like OVVU accessions. UNK6 was equally distant to OVH and OON suggesting that it could be a native hybrid, *O. intercedens* (OON x OVH, 1961), or either OVH or OON. In addition, a small group of UNK individuals were clustered together with a single OON accession which was found in a cluster with accessions from several different taxa: OSYBE and ODU (*MAJ*), OSI (*ANA*), and OVH (*ORG*) reflecting an admixed genetic structure. Thus, these unknown specimens cannot be categorized as any specific taxon. UNK13 and UNK25 were most genetically similar OVH accessions suggesting them to be a member of this taxon. UNK24 was equally related to both OVH and ODU suggesting an intermediate hybrid between them, while UNK26 was closest to ODU representing its most probable identity is ODU. Accessions UNK7, UNK8, UNK9, and UNK10 clustered together with the single OVG specimen from section *ORG* suggesting that these unknown

accessions can be OVG or closely related to OVG. Both UNK21 and UNK23 were most closely related to OSYBE accessions reflecting a high probability that these specimens are OSYBE. Although the dendrogram analysis can provide clues as to the identity of the unknown samples, they should be classified as a given taxon based on an interdisciplinary effort that combines both molecular and morphological studies. For example, DNA sequencing or barcoding could be combined with an analysis of characters that are indicative of each species and their hybrids.

Table 3.20.Known neigboring taxa of UNK specimens in unweighted NJ dendrogram constructed based on Dice coefficient for AARI oregano collection.

	Known neighboring taxa	Section
UNK1	OON*, ODU*	*: *MAJ*
UNK2	OVVU*, OSYBE**	*: *ORG*, **: *MAJ*
UNK3	OSI*, OLE**, OON***	*: *ANA*, **: *BRE*, ***: *MAJ*
UNK4	OVVU*, OSYBE**	*: *ORG*, **: *MAJ*
UNK5	OVVU*, OSI**, OVH*, OSYBE***	*: *ORG*, **: *ANA*, ***: *MAJ*
UNK6	OVH*, OON**, OLE***, OSI****	*: *ORG*, **: *MAJ*, ***:*BRE*, ****:
UNK7	OVG*	*: *ORG*
UNK8	OVG*	*: *ORG*
UNK9	OVG*	*: *ORG*
UNK10	OVG*	*: *ORG*
UNK11	OON*	*: *MAJ*
UNK12	OON*	*: *MAJ*
UNK13	OVH*, OON**, OSI***	*: *ORG*, **: *MAJ*, ***: *ANA*
UNK14	OON*	*: *MAJ*
UNK15	OON*	*: *MAJ*
UNK16	OON*	*: *MAJ*
UNK17	-	-
UNK18	OON*	*: *MAJ*
UNK19	OVH*, OON**, OSI***	*: *ORG*, **: *MAJ*, ***: *ANA*
UNK20	OON*	*: *MAJ*
UNK21	OSI*, OSYBE**	*: *ANA*, **: *MAJ*
UNK22	OON	*: *MAJ*
UNK23	OSI*, OSYBE**	*: *ANA*, **: *MAJ*
UNK24	ODU*, OVH**	*: *MAJ*, **: *ORG*
UNK25	ODU*, OVH**	*: *MAJ*, **: *ORG*
UNK26	ODU*, OVH**	*: *MAJ*, **: *ORG*
UNK27	OON*	*: *MAJ*
UNK28	-	-
UNK29	OON*	*: *MAJ*
UNK30	OON*	*: *MAJ*

3.6. Conclusions and Further Recommendations

Utilizing molecular genetic tools allows a clearer understanding of speciation and determination of taxonomic variation within the genus *Origanum* and other plant species.

This dissertation validates the effectiveness of molecular genetic research to understand *Origanum* taxonomy and speciation. In this dissertation, the usefulness of SRAP markers to evaluate genetic relationships among Turkish oregano collections (Narin Sadıkoğlu oregano herbarium and gene bank germplasm) was shown. The coverage of each collection at the genus level was uneven indicating the need for enhancement of the national AARI germplasm collection to include specimens from all eight sections.

This research also indicates the undeniable importance of morphological classification in the assessment of oregano diversity and in the identification of unkown specimens. Thus, there is an urge to improve the conservation and improvement strategies for oregano by employing more comprehensive and complementary genotypic, phenotypic and chemotypic analyses. Moreover, chemotypic and phenotypic analyses must be performed in different flowering periods, seasons and years because these traits can be variable under different climate conditions. Such an interdisciplinary and comprehensive approach will allow better exploitation of the potential genetic richness of endemic oregano species in Türkiye. Moreover, the best bioactive compounds and traits can be selected efficiently and propagated for commercial use. This will reduce uncontrolled harvesting from nature and help protect Türkiye's endemic flora.

This research indicates that there are overlapping hybridization patterns among different *Origanum* taxa which creates high gene flow within and between species. This work also suggests that the gynodiecious nature in some oreganos and restriction in some geographical locations can affect the hybridization tendencies among native and *ex situ* conserved oregano specimens. Thus *ex situ* cultivation practices should consider these tendencies and take measures to prevent uncontrolled cross-hybridization if the goal is to conserve genetic materials as they were when collected from nature.

While the work presented in this dissertation highlights the usefulness of molecular markers in studying medicinal and aromatic plants, more research must be conducted on these valuable species. Sequencing of *Origanum* genomes will open the door to barcoding which will allow validation and protection of Türkiye's oregano germplasm. It will also expedite forward and reverse genetic approaches for oregano improvement and help guide conservation and cultivation of taxa from the genus *Origanum*.

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APPENDICES

APPENDIX A

Appendix A. Suggestion for a global abbreviation of nomenclature in the genus *Origanum* L.

In this dissertation, the use of three to five letter abbreviations of species names for oregano was successful. In the literature, oregano species have been abbreviated in different styles such as Ori, oni, dubium, Or, OR, and OH. This nomenclature creates confusion between name of species and molecular marker abbreviations. In this work, the species were abbreviated with capital letters according to the first letter of the genus name followed by the first one to two letters of the species name, and, if necessary, the addition of one or two letters of the subspecies name. For example, *O. onites* was abbreviated with three letters: *O. onites*, OON. OVVU was used as an abbreviation for *O. vulgare* subsp. *vulgare*. In the case of *O. syriacum* subsp. *bevanii,* it was abbreviated as OSYBE. For *O. vulgare* subsp. *gracile* and *O. vulgare* subsp. *glandulosum*; the abbreviations OVG and OVGL or OVGLAN are suggested for global recognition of oregano species names. To avoid any conflict or confusion, species with similar epithets were abbreviated differently. For instance, *O. micranthum, O. microphyllum*, and *O. minutiflorum* have similar names. In this dissertation, it is recommended to use the abbreviation: OMICRA, OMICRO, and OMI, for these species.In addition, the use of numbers at the end of abbreviation will allow differentiation of individual specimens of material from the same species such as "OON1" or "OSYBE2". In addition, the use of an initial letter "c" or "w" is helpful to discriminate cultivated or wild accessions as Lukas et al. (2013) first applied to their germplasm. These initials can be used as lower-case initials preceding the abbreviation of species names such as "cOON" or "wOON". Moreover, the ten sections in the genus *Origanum* can be abbreviated in three to five capital letters. Thus, the sections *Amaracus, Anatolicon, Brevifilamentum, Longitubus*,

Chilocalyx, Majorana, Campanulaticalyx, Elongatispica, Origanum, and *Prolaticorolla* were abbreviated as *AMA*, *ANA*, *BRE*, *LONGI*, *CHI*, *MAJ*, *CAMPA*, *ELON*, *ORG*, and *PRO*, respectively, in this dissertation. An italic representation is recommended to allow easier discrimination of section names from abbreviated species names. Section *Origanum* was abbreviated as "*ORG*" to avoid any conflict at the abbreviation of the genus *Origanum*. We believe that a standardized abbreviated nomenclature for the genus will reduce confusion in studies that use global perspectives to examine multiple *Origanum* species with either molecular, morphological or biochemical traits.

APPENDIX B

Appendix B. Numerical values of inferred subpopulations for population structure of AARI germplasm

Membership of AARI specimens for inferred subpopulations in structure analyses for *K*=2, 4, 5, and 8 was given in numerical values in Table B.1.

Table B.1. The numerical values for inferred sub populations identities in population structure analyses for *K*=2, 4, 5 and 8.

RNo (TR_____)	Code	Inferred Populations																		
		SP I	SP II	SP I	SP II	SP III	SP IV	SP I	SP II	SP III	SP IV	SP V	SP I	SP II	SP III	SP IV	SP V	SP VI	SP VII	SP VIII
		K=2		*K*=4				*K*=5					*K*=8							
TR 53149	ODU1	0.01	0.99	0.95	0.02	0.03	0.01	0.03	0.01	0.01	0.49	0.47	0.01	0.01	0.50	0.00	0.02	0.01	0.10	0.37
TR 53153	ODU2	0.02	0.99	0.95	0.02	0.02	0.01	0.03	0.01	0.02	0.06	0.89	0.01	0.01	0.07	0.01	0.01	0.02	0.05	0.83
TR 53209	ODU3	0.03	0.97	0.57	0.04	0.38	0.02	0.12	0.01	0.01	0.86	0.01	0.01	0.01	0.77	0.00	0.01	0.10	0.10	0.01
TR 53194	OON1	0.02	0.98	0.24	0.62	0.13	0.01	0.07	0.01	0.48	0.02	0.42	0.05	0.21	0.02	0.01	0.08	0.02	0.01	0.61
TR 53162	ODU4	0.02	0.98	0.88	0.08	0.03	0.02	0.02	0.01	0.03	0.77	0.18	0.01	0.05	0.69	0.00	0.01	0.01	0.17	0.06
TR 53169	ODU5	0.01	0.99	0.78	0.20	0.01	0.01	0.01	0.01	0.11	0.04	0.83	0.01	0.07	0.03	0.00	0.01	0.01	0.01	0.88
TR 53244	ODU6	0.95	0.05	0.07	0.03	0.07	0.83	0.06	0.78	0.02	0.11	0.02	0.05	0.01	0.07	0.75	0.01	0.07	0.03	0.01
TR 54467	OON2	0.97	0.03	0.03	0.09	0.01	0.87	0.01	0.83	0.07	0.08	0.01	0.06	0.08	0.03	0.80	0.00	0.01	0.03	0.01
TR 54471	OON3	0.01	0.99	0.03	0.92	0.05	0.01	0.05	0.01	0.88	0.04	0.03	0.01	0.86	0.01	0.00	0.04	0.02	0.02	0.03
TR 54473	OON4	0.02	0.98	0.03	0.92	0.04	0.01	0.03	0.01	0.88	0.01	0.08	0.02	0.80	0.00	0.00	0.01	0.02	0.01	0.13

(Cont. on the next page)

Table B.1. (cont.)

TR 54480	OON5	0.08	0.92	0.08	0.81	0.03	0.08	0.02	0.05	0.62	0.28	0.02	0.01	0.70	0.17	0.06	0.02	0.02	0.03	0.01
TR 54483	OON6	0.56	0.44	0.01	0.56	0.01	0.42	0.01	0.40	0.58	0.01	0.01	0.01	0.54	0.01	0.43	0.01	0.00	0.00	0.00
TR 54484	OON7	0.55	0.45	0.05	0.49	0.03	0.44	0.02	0.41	0.47	0.07	0.03	0.32	0.25	0.03	0.33	0.03	0.01	0.03	0.01
TR 54477	OON8	0.01	0.99	0.07	0.90	0.02	0.01	0.01	0.01	0.58	0.40	0.01	0.01	0.68	0.27	0.00	0.01	0.01	0.02	0.01
TR 54485	OON9	0.03	0.97	0.02	0.93	0.03	0.02	0.02	0.01	0.75	0.21	0.01	0.06	0.76	0.12	0.01	0.01	0.01	0.03	0.00
TR 54490	OON10	0.03	0.97	0.29	0.54	0.15	0.02	0.02	0.01	0.08	0.89	0.01	0.01	0.14	0.82	0.00	0.01	0.01	0.01	0.00
TR 54502	OON11	0.04	0.96	0.02	0.92	0.03	0.03	0.02	0.01	0.66	0.30	0.01	0.06	0.69	0.20	0.01	0.01	0.02	0.01	0.01
TR 54493	OON12	0.03	0.98	0.24	0.71	0.04	0.02	0.03	0.01	0.66	0.03	0.27	0.23	0.34	0.02	0.01	0.04	0.01	0.02	0.34
TR 54512	OON13	0.12	0.88	0.01	0.94	0.02	0.04	0.01	0.03	0.92	0.03	0.01	0.51	0.46	0.01	0.01	0.01	0.00	0.01	0.00
TR 54513	OON14	0.16	0.84	0.02	0.85	0.03	0.10	0.02	0.06	0.76	0.14	0.01	0.49	0.43	0.04	0.02	0.01	0.01	0.01	0.01
TR 54514	OON15	0.39	0.61	0.03	0.64	0.02	0.31	0.02	0.31	0.63	0.02	0.03	0.13	0.48	0.01	0.31	0.01	0.01	0.01	0.03
TR 54516	OON16	0.19	0.81	0.22	0.45	0.19	0.15	0.08	0.08	0.12	0.70	0.02	0.06	0.15	0.62	0.07	0.02	0.06	0.01	0.02
TR 54517	OON17	0.71	0.30	0.01	0.46	0.01	0.52	0.01	0.49	0.48	0.01	0.01	0.52	0.11	0.01	0.33	0.01	0.01	0.00	0.01
TR 54522	OON18	0.98	0.02	0.02	0.04	0.02	0.92	0.02	0.92	0.03	0.02	0.01	0.09	0.02	0.01	0.86	0.01	0.01	0.01	0.01
TR 54525	OON19	0.39	0.61	0.02	0.67	0.02	0.30	0.02	0.28	0.67	0.02	0.01	0.42	0.38	0.01	0.15	0.02	0.01	0.01	0.01
TR 54530	OON20	0.85	0.15	0.10	0.11	0.05	0.75	0.03	0.73	0.07	0.09	0.08	0.16	0.05	0.05	0.58	0.02	0.02	0.05	0.07
TR 54531	OON21	0.02	0.98	0.01	0.97	0.01	0.01	0.01	0.01	0.96	0.02	0.01	0.04	0.92	0.01	0.00	0.01	0.01	0.01	0.01
TR 54536	OON22	0.23	0.77	0.40	0.36	0.03	0.21	0.02	0.15	0.12	0.63	0.08	0.03	0.11	0.61	0.14	0.01	0.01	0.03	0.06
TR 54544	OON23	0.82	0.18	0.02	0.34	0.01	0.64	0.01	0.63	0.28	0.07	0.01	0.01	0.32	0.02	0.63	0.01	0.00	0.01	0.00
TR 54545	OON24	0.04	0.97	0.34	0.48	0.15	0.03	0.02	0.01	0.07	0.89	0.01	0.04	0.11	0.80	0.01	0.01	0.01	0.01	0.01
TR 54547	OON25	0.02	0.98	0.13	0.83	0.03	0.01	0.02	0.01	0.75	0.05	0.17	0.02	0.70	0.03	0.01	0.03	0.01	0.02	0.19
TR 54548	OON26	0.39	0.61	0.10	0.47	0.11	0.32	0.07	0.27	0.31	0.30	0.05	0.12	0.21	0.32	0.24	0.02	0.03	0.01	0.04
TR 54561	OON27	0.18	0.82	0.34	0.45	0.09	0.12	0.03	0.08	0.18	0.70	0.02	0.16	0.08	0.67	0.04	0.01	0.02	0.01	0.01
-	UNK1	0.70	0.30	0.21	0.15	0.03	0.61	0.01	0.44	0.02	0.52	0.01	0.02	0.01	0.54	0.41	0.01	0.01	0.01	0.00
TR 54442	OON28	0.18	0.82	0.68	0.15	0.02	0.15	0.01	0.07	0.03	0.81	0.08	0.02	0.01	0.83	0.05	0.01	0.01	0.03	0.04
TR 54435	ODU7	0.11	0.89	0.53	0.41	0.02	0.05	0.01	0.03	0.22	0.53	0.21	0.09	0.07	0.56	0.01	0.01	0.01	0.01	0.24
TR 54497	OON29	0.01	0.99	0.36	0.62	0.01	0.01	0.01	0.01	0.48	0.12	0.39	0.02	0.32	0.09	0.00	0.01	0.01	0.03	0.52
TR 54520	OON30	0.66	0.35	0.23	0.07	0.16	0.55	0.12	0.46	0.05	0.34	0.03	0.45	0.01	0.09	0.36	0.01	0.05	0.02	0.01
TR 54563	OON31	0.84	0.16	0.03	0.22	0.05	0.71	0.04	0.69	0.23	0.02	0.02	0.45	0.01	0.01	0.49	0.02	0.01	0.01	0.01
TR 54476	OON32	0.02	0.98	0.20	0.76	0.02	0.02	0.02	0.01	0.64	0.03	0.31	0.02	0.50	0.02	0.01	0.01	0.01	0.03	0.41

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Table B.1. (cont.)

TR 57124	OSI1	0.94	0.06	0.04	0.03	0.07	0.86	0.05	0.86	0.03	0.03	0.03	0.01	0.01	0.02	0.87	0.02	0.03	0.02	0.02
TR 54445	OON33	0.06	0.94	0.02	0.92	0.02	0.04	0.02	0.03	0.92	0.01	0.03	0.12	0.68	0.01	0.02	0.09	0.01	0.01	0.06
TR 54596	OON34	0.04	0.96	0.15	0.79	0.02	0.04	0.02	0.03	0.71	0.08	0.17	0.02	0.61	0.03	0.03	0.06	0.01	0.02	0.23
TR 54549	OON35	0.99	0.01	0.01	0.02	0.01	0.96	0.01	0.96	0.02	0.01	0.01	0.01	0.01	0.00	0.95	0.01	0.01	0.01	0.00
TR 54552	OON36	0.94	0.06	0.03	0.15	0.01	0.81	0.01	0.79	0.14	0.04	0.02	0.13	0.07	0.02	0.72	0.01	0.01	0.04	0.01
TR 54554	OON37	0.26	0.74	0.03	0.81	0.03	0.13	0.03	0.10	0.82	0.03	0.02	0.52	0.41	0.01	0.02	0.01	0.01	0.01	0.01
TR 54557	OON38	0.02	0.99	0.33	0.64	0.01	0.01	0.01	0.01	0.59	0.07	0.33	0.02	0.53	0.02	0.00	0.01	0.01	0.01	0.41
TR 54562	OON39	0.05	0.95	0.07	0.86	0.02	0.05	0.01	0.03	0.81	0.02	0.14	0.01	0.69	0.01	0.01	0.03	0.01	0.20	0.05
TR 54595	OON40	0.57	0.43	0.02	0.49	0.04	0.44	0.04	0.43	0.49	0.02	0.03	0.65	0.03	0.01	0.23	0.01	0.01	0.05	0.02
TR 54594	OON41	0.12	0.88	0.07	0.69	0.18	0.06	0.18	0.04	0.66	0.09	0.03	0.88	0.02	0.02	0.00	0.02	0.04	0.01	0.02
TR 54579	OON42	0.32	0.68	0.17	0.57	0.03	0.23	0.03	0.17	0.55	0.19	0.06	0.94	0.01	0.01	0.01	0.01	0.01	0.01	0.01
TR 54441	OVVU1	0.96	0.04	0.04	0.07	0.06	0.84	0.03	0.76	0.04	0.16	0.01	0.18	0.02	0.06	0.69	0.01	0.01	0.03	0.01
TR 58558	ODU8	0.95	0.05	0.03	0.12	0.02	0.84	0.01	0.81	0.09	0.08	0.01	0.08	0.05	0.05	0.77	0.01	0.01	0.02	0.01
TR 60214	OLE1	0.03	0.97	0.36	0.45	0.17	0.02	0.09	0.01	0.34	0.49	0.07	0.10	0.32	0.25	0.01	0.02	0.05	0.24	0.02
TR 60424	OSYBE1	0.49	0.51	0.03	0.51	0.07	0.39	0.05	0.38	0.51	0.03	0.03	0.68	0.03	0.01	0.10	0.07	0.01	0.09	0.01
TR 60427	OSI2	0.15	0.85	0.46	0.35	0.08	0.11	0.08	0.12	0.34	0.08	0.39	0.67	0.02	0.02	0.01	0.05	0.01	0.06	0.16
TR 60516	OSI3	0.95	0.05	0.04	0.03	0.04	0.89	0.03	0.87	0.03	0.04	0.03	0.07	0.01	0.03	0.84	0.02	0.02	0.01	0.01
TR 60561	OHA1	0.75	0.25	0.24	0.05	0.03	0.68	0.03	0.67	0.04	0.10	0.15	0.12	0.01	0.05	0.56	0.01	0.02	0.20	0.04
TR 61058	ODU9	0.01	0.99	0.77	0.19	0.03	0.01	0.03	0.01	0.11	0.03	0.82	0.03	0.03	0.02	0.00	0.02	0.02	0.02	0.86
TR 61104	OHY1	0.06	0.94	0.79	0.05	0.11	0.04	0.12	0.03	0.03	0.59	0.22	0.06	0.03	0.47	0.02	0.01	0.11	0.26	0.05
TR 69502	OON43	0.03	0.97	0.78	0.14	0.05	0.03	0.04	0.01	0.11	0.72	0.12	0.09	0.06	0.60	0.01	0.01	0.03	0.12	0.07
TR 73007	ODU10	0.04	0.96	0.62	0.26	0.08	0.04	0.08	0.04	0.24	0.20	0.44	0.43	0.03	0.08	0.01	0.02	0.04	0.02	0.37
TR 73014	OVVI1	0.15	0.85	0.51	0.32	0.05	0.12	0.05	0.07	0.33	0.44	0.11	0.46	0.04	0.31	0.02	0.03	0.02	0.06	0.08
TR 73548	OVVU2	0.46	0.54	0.51	0.07	0.05	0.38	0.06	0.33	0.05	0.36	0.20	0.63	0.01	0.10	0.05	0.03	0.02	0.13	0.04
TR 73563	OSI4	0.31	0.69	0.65	0.07	0.02	0.27	0.02	0.27	0.05	0.21	0.46	0.14	0.02	0.11	0.21	0.01	0.02	0.03	0.46
TR 76974	OSYBE2	0.10	0.91	0.70	0.06	0.18	0.06	0.18	0.04	0.03	0.61	0.14	0.09	0.02	0.49	0.02	0.03	0.13	0.15	0.07
TR 76979	OSYBE3	0.75	0.25	0.29	0.02	0.03	0.66	0.03	0.60	0.01	0.31	0.05	0.02	0.01	0.32	0.58	0.01	0.02	0.01	0.03
TR 76983	OSI5	0.60	0.40	0.19	0.16	0.11	0.55	0.08	0.55	0.11	0.02	0.24	0.13	0.01	0.01	0.48	0.13	0.01	0.01	0.21
TR 76984	OON44	0.90	0.10	0.08	0.02	0.09	0.81	0.08	0.80	0.02	0.03	0.08	0.01	0.01	0.02	0.82	0.02	0.05	0.02	0.05

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Table B.1. (cont.)

TR 76985	OSI6	0.03	0.97	0.69	0.03	0.26	0.02	0.21	0.01	0.02	0.51	0.25	0.01	0.01	0.54	0.01	0.07	0.09	0.11	0.16
TR 76988	OON45	0.97	0.03	0.03	0.02	0.02	0.93	0.01	0.92	0.02	0.03	0.02	0.06	0.01	0.01	0.89	0.01	0.01	0.01	0.01
TR 76990	OSYBE4	0.15	0.85	0.23	0.15	0.54	0.09	0.55	0.06	0.14	0.18	0.07	0.41	0.02	0.05	0.01	0.01	0.46	0.02	0.02
-	UNK2	0.04	0.96	0.72	0.14	0.10	0.03	0.14	0.03	0.15	0.08	0.60	0.07	0.06	0.07	0.01	0.12	0.10	0.16	0.42
-	UNK3	0.29	0.71	0.36	0.06	0.36	0.22	0.35	0.14	0.04	0.43	0.05	0.02	0.02	0.41	0.11	0.18	0.13	0.12	0.02
TR 76997	OVVU3	0.06	0.94	0.89	0.04	0.04	0.04	0.02	0.02	0.01	0.01	0.94	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.92
TR 76999	OVH1	0.88	0.12	0.12	0.08	0.03	0.77	0.02	0.73	0.04	0.18	0.03	0.03	0.08	0.09	0.74	0.01	0.02	0.03	0.02
-	UNK4	0.27	0.73	0.70	0.03	0.02	0.25	0.01	0.21	0.01	0.01	0.76	0.01	0.00	0.00	0.20	0.01	0.01	0.01	0.76
	UNK5	0.66	0.34	0.21	0.02	0.19	0.58	0.19	0.57	0.02	0.15	0.09	0.04	0.01	0.08	0.56	0.02	0.17	0.09	0.04
TR 77673	OVH2	0.50	0.50	0.25	0.04	0.26	0.45	0.18	0.36	0.02	0.42	0.03	0.07	0.01	0.37	0.36	0.03	0.13	0.02	0.02
TR 77675	OVH3	0.97	0.03	0.05	0.01	0.04	0.90	0.04	0.89	0.01	0.03	0.02	0.01	0.01	0.02	0.87	0.01	0.02	0.05	0.01
TR 77676	OVH4	0.88	0.12	0.17	0.03	0.03	0.78	0.03	0.78	0.02	0.02	0.16	0.02	0.01	0.02	0.77	0.01	0.02	0.09	0.08
TR 82335	OVG1	0.48	0.52	0.45	0.04	0.10	0.41	0.09	0.42	0.04	0.06	0.39	0.05	0.01	0.03	0.36	0.04	0.04	0.25	0.22
TR 82855	OSI7	0.19	0.81	0.24	0.08	0.52	0.15	0.54	0.14	0.07	0.18	0.07	0.02	0.03	0.12	0.04	0.28	0.24	0.26	0.01
TR 80181	ODU11	0.05	0.95	0.61	0.30	0.05	0.04	0.03	0.03	0.19	0.01	0.74	0.02	0.05	0.01	0.02	0.06	0.02	0.04	0.79
TR 80182	OVVU4	0.55	0.45	0.38	0.03	0.07	0.52	0.05	0.43	0.02	0.47	0.04	0.01	0.01	0.39	0.42	0.01	0.05	0.10	0.01
-	UNK6	0.15	0.85	0.63	0.02	0.25	0.10	0.19	0.06	0.01	0.65	0.09	0.02	0.01	0.53	0.02	0.01	0.09	0.31	0.01
-	UNK7	0.61	0.39	0.05	0.32	0.12	0.51	0.10	0.48	0.29	0.10	0.03	0.47	0.02	0.04	0.32	0.07	0.04	0.03	0.02
-	UNK8	0.70	0.30	0.12	0.18	0.08	0.63	0.06	0.61	0.16	0.03	0.14	0.14	0.04	0.02	0.58	0.02	0.03	0.01	0.15
-	UNK9	0.64	0.36	0.03	0.29	0.14	0.55	0.11	0.53	0.31	0.01	0.04	0.06	0.15	0.01	0.52	0.16	0.05	0.02	0.03
-	UNK10	0.55	0.45	0.03	0.50	0.02	0.45	0.02	0.43	0.51	0.02	0.03	0.08	0.35	0.01	0.41	0.10	0.01	0.02	0.02
TR 77668	OON46	0.99	0.01	0.01	0.01	0.02	0.96	0.02	0.96	0.01	0.01	0.01	0.01	0.00	0.01	0.96	0.01	0.01	0.01	0.00
TR 77669	OON47	0.17	0.84	0.80	0.03	0.08	0.10	0.10	0.10	0.03	0.09	0.68	0.01	0.01	0.01	0.01	0.02	0.01	0.91	0.04
TR 77672	OVH5	0.04	0.96	0.88	0.04	0.06	0.02	0.07	0.02	0.03	0.18	0.70	0.01	0.01	0.02	0.00	0.01	0.01	0.89	0.06
TR 77674	OSYBE5	0.03	0.97	0.92	0.02	0.04	0.02	0.06	0.02	0.01	0.06	0.85	0.02	0.01	0.04	0.01	0.01	0.06	0.20	0.65
TR 54475	OON48	0.22	0.78	0.43	0.32	0.04	0.21	0.03	0.21	0.25	0.01	0.50	0.02	0.11	0.01	0.19	0.11	0.01	0.02	0.53
TR 54488	OON49	0.98	0.02	0.02	0.02	0.01	0.96	0.01	0.93	0.01	0.04	0.01	0.02	0.01	0.03	0.92	0.01	0.01	0.01	0.01
-	UNK11	0.05	0.95	0.03	0.64	0.31	0.03	0.29	0.02	0.63	0.01	0.05	0.17	0.14	0.01	0.01	0.55	0.03	0.01	0.09
TR 54491	OON50	0.40	0.60	0.01	0.52	0.16	0.31	0.14	0.29	0.55	0.01	0.01	0.14	0.03	0.01	0.06	0.74	0.01	0.00	0.01

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Table B.1. (cont.)

TR 54499	OON51	0.07	0.93	0.03	0.48	0.44	0.04	0.44	0.03	0.48	0.02	0.03	0.01	0.06	0.01	0.01	0.85	0.02	0.03	0.01
-	UNK12	0.24	0.76	0.04	0.32	0.52	0.12	0.53	0.09	0.34	0.02	0.02	0.01	0.01	0.01	0.01	0.95	0.01	0.01	0.00
TR 54556	OON52	0.09	0.91	0.02	0.41	0.54	0.03	0.52	0.02	0.43	0.01	0.01	0.01	0.01	0.00	0.01	0.95	0.01	0.00	0.01
TR 63126	ODU12	0.98	0.02	0.01	0.01	0.05	0.93	0.04	0.93	0.01	0.01	0.01	0.01	0.00	0.00	0.93	0.03	0.02	0.00	0.01
-	UNK13	0.98	0.02	0.01	0.01	0.07	0.92	0.06	0.92	0.01	0.01	0.01	0.01	0.00	0.01	0.91	0.06	0.01	0.00	0.00
-	UNK14	0.33	0.67	0.03	0.17	0.66	0.14	0.65	0.12	0.20	0.02	0.02	0.07	0.01	0.02	0.04	0.83	0.03	0.01	0.01
-	UNK15	0.02	0.98	0.03	0.25	0.71	0.01	0.69	0.01	0.24	0.04	0.02	0.02	0.04	0.06	0.00	0.79	0.07	0.01	0.01
-	UNK16	0.03	0.97	0.04	0.23	0.72	0.01	0.71	0.01	0.23	0.02	0.04	0.01	0.02	0.01	0.00	0.92	0.02	0.01	0.01
-	UNK17	0.95	0.05	0.02	0.01	0.15	0.82	0.15	0.82	0.01	0.01	0.01	0.01	0.00	0.01	0.76	0.20	0.01	0.01	0.00
-	UNK18	0.11	0.89	0.04	0.30	0.59	0.08	0.56	0.05	0.29	0.01	0.09	0.05	0.01	0.01	0.02	0.78	0.03	0.01	0.09
-	UNK19	0.95	0.05	0.02	0.01	0.20	0.77	0.20	0.76	0.01	0.01	0.01	0.02	0.00	0.01	0.77	0.03	0.14	0.02	0.01
-	UNK20	0.01	0.99	0.35	0.03	0.62	0.01	0.62	0.01	0.02	0.04	0.32	0.01	0.01	0.04	0.00	0.27	0.41	0.05	0.21
-	UNK21	0.05	0.95	0.06	0.02	0.90	0.03	0.83	0.02	0.01	0.11	0.02	0.02	0.01	0.07	0.01	0.02	0.82	0.03	0.01
-	UNK22	0.05	0.95	0.03	0.03	0.93	0.02	0.92	0.01	0.03	0.03	0.02	0.02	0.00	0.02	0.01	0.54	0.41	0.01	0.01
-	UNK23	0.06	0.94	0.01	0.01	0.97	0.01	0.97	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.13	0.84	0.01	0.01
-	UNK24	0.09	0.91	0.05	0.08	0.84	0.03	0.84	0.03	0.08	0.02	0.04	0.02	0.04	0.01	0.02	0.03	0.84	0.01	0.02
-	UNK25	0.14	0.86	0.01	0.01	0.96	0.02	0.96	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.04	0.90	0.02	0.00
TR 71538	OSI8	0.09	0.91	0.01	0.02	0.95	0.01	0.95	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.94	0.01	0.01
TR 76973	OSYBE6	0.05	0.95	0.02	0.01	0.96	0.01	0.94	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.94	0.01	0.01
TR 76975	OVH6	0.20	0.81	0.04	0.02	0.91	0.04	0.81	0.03	0.01	0.14	0.01	0.02	0.01	0.06	0.02	0.02	0.84	0.03	0.00
TR 76976	OVH7	0.03	0.97	0.14	0.02	0.83	0.01	0.81	0.01	0.01	0.02	0.16	0.01	0.01	0.01	0.01	0.01	0.84	0.02	0.10
TR 76977	OVH8	0.06	0.94	0.02	0.01	0.96	0.02	0.95	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.04	0.91	0.02	0.01
TR 76978	OSYBE7	0.02	0.98	0.02	0.01	0.97	0.01	0.97	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.96	0.01	0.01
TR 64495	ODU13	0.03	0.97	0.18	0.03	0.78	0.01	0.65	0.01	0.02	0.30	0.02	0.01	0.01	0.17	0.01	0.01	0.73	0.05	0.01
-	UNK26	0.10	0.90	0.26	0.03	0.66	0.06	0.68	0.05	0.02	0.03	0.22	0.01	0.01	0.03	0.04	0.02	0.73	0.04	0.13
TR 64641	ODU14	0.19	0.81	0.04	0.03	0.84	0.09	0.85	0.07	0.02	0.02	0.04	0.01	0.01	0.01	0.04	0.32	0.59	0.02	0.01
-	UNK27	0.03	0.97	0.51	0.01	0.47	0.01	0.50	0.01	0.01	0.23	0.25	0.00	0.01	0.17	0.01	0.01	0.58	0.07	0.16
-	UNK28	0.98	0.02	0.02	0.01	0.03	0.94	0.03	0.93	0.01	0.02	0.01	0.01	0.00	0.01	0.94	0.00	0.03	0.01	0.01
-	UNK29	0.02	0.98	0.40	0.03	0.56	0.01	0.58	0.01	0.02	0.33	0.06	0.02	0.02	0.18	0.01	0.01	0.68	0.08	0.02

(Cont. on the next page)

Table B.1. (cont.)

-	UNK30	0.05	0.95	0.34	0.02	0.62	0.02	0.68	0.02	0.02	0.05	0.24	0.02	0.01	0.02	0.01	0.01	0.71	0.16	0.07
TR 76824	OSI9	0.94	0.06	0.03	0.01	0.21	0.76	0.20	0.76	0.01	0.01	0.02	0.01	0.01	0.01	0.77	0.01	0.18	0.01	0.01
TR 69501	OON53	0.06	0.94	0.31	0.02	0.65	0.03	0.65	0.02	0.01	0.28	0.04	0.03	0.01	0.17	0.01	0.02	0.69	0.05	0.02

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PhD., Molecular Biology and Genetics (GPA: 3.57/4.00) | Izmir Institute of Technology | Izmir-Türkiye, 2021,
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Çakir, G., **Taşcioğlu, T.***, Çavdar, A., Doğanlar, S., Frary, A., & Frary, A. 2021. "Molecular Genetic Characterization of the Turkish National Green Plum (*Prunus cerasifera* Ehrh.) Collection." *Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi*, *31*(1), 61-73.

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