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BUDAPEST

Genetic diversity of Basil (*Ocimum basilicum* L.) cultivars based on SCOT markers

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ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism

CTAB: Cetyl Trimethyl Ammonium Bromide

DMSO: Dimethyl Sulfoxide

DNA: Deoxyribonucleic acid

ISSR: Inter-Simple Sequence Repeat

PCoA: Principal Coordinate Analysis

PCR: Polymerase Chain Reaction

PIC: Polymorphic Information Content

RAPD: Random Amplified Polymorphic DNA

RFLP: Restrictions fragment length polymorphism

SCoT: Start Codon Targeted

SSR: Simple Sequence Repeat

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I. INTRODUCTION

1.1. Background

Ocimum basilicum L. belongs to the Lamiaceae family. It's a fragrant annual herb that's a significant commercial crop with a broad range of biological and pharmaceutical uses (Abdollahi Mandoulakani et al, 2017). Basil is found in mountainous areas of Africa, Asia, and South America. This plant is a significant essential oil crop in Iran, India, and other tropical Asian countries, and it is extensively utilized in the culinary, perfume, pharmaceutical, cosmetic, and aromatherapy sectors (Pirmoradi et al, 2013). Basil leaf is often used as a daily spice and cooking component in its fresh form. Basil is also used to enhance digestion, increase respiratory circulation, reduce cold symptoms, and treat stomach disorders in traditional medicine due to the plant's anti-inflammatory, antioxidant, and antibacterial qualities (Izadiyan et al, 2016).

Despite the importance of *O. basilicum* with humans, there is currently no complete classification system for basil that serves to exploit precious genes, identifying and tracing the origin and relation of varieties of basil cultivated. In addition, the identification of varieties of basil based on morphological characteristics, quality, color, and shapes are difficult because they are highly similar and depend heavily on the geographical area...At the same time, applied research in molecular biology on basil has been done but is not yet well-known. This necessitates a more thorough examination of genetic diversity as well as morphological and molecular markers to provide knowledge that can be used to develop future basil breeding techniques that are both successful and efficient. Hence, this research was initiated with the following objectives:

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1.2. Objectives.

The goal of this research was to study the genetic diversity of the *Ocimum basilicum* L. and a few other *Ocimum* species cultivated in the test field of the university by the use of SCoT primers. The aim was to test the efficiency of SCoT markers for this species and its capability to differentiate *Ocimum* species.

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II. LITERATURE REVIEW

2.1. Bontany, nutritive value and uses of *Ocimum basilicum*.

2.1.1. Taxonomy

Ocimum basilicum L. belongs to:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: *Ocimum*

Species: *O. basilicum*

(Purushothaman et al, 2018)

Ocimum is a member of the Lamiaceae family, *Ocimum* has roughly 150 different species (Simon et al, 1999). *O. basilicum*, *O. gratissimum*, *O. xcitriodorum*, *O. americanum* L., *O. minimo* L., and *O. tenuiflorum* L. are the most extensively grown plants worldwide (Egata, 2021).

2.1.2. Morphology, physiology, and genetics of basil

Morphology of some important species in the genus *Ocimum* is presented based on the research of Singh, (2012):

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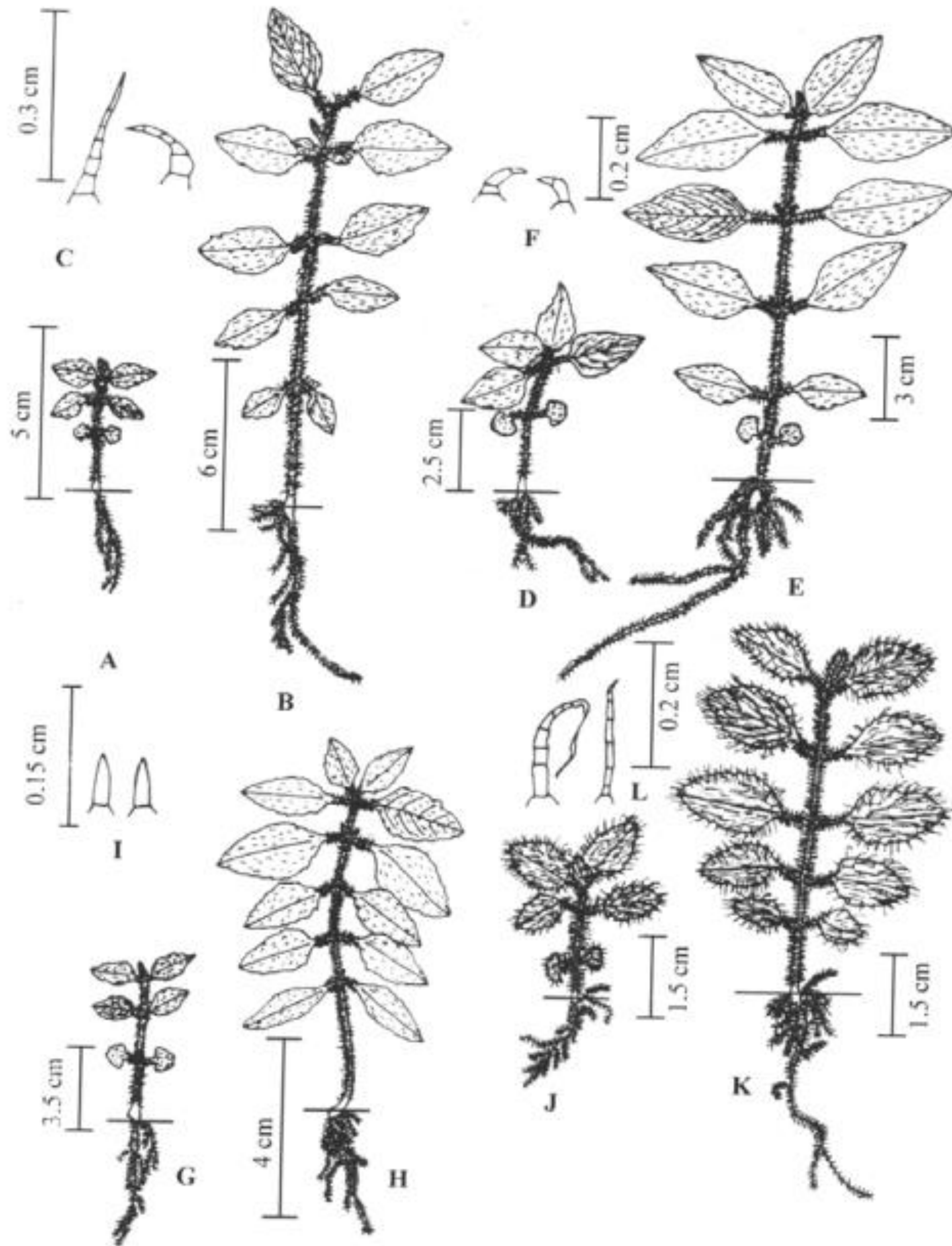

Figure.1: Seedlings and hairs: *Ocimum americanum* L.- A. Second leaf stage; B. Fifth leaf stage; C. Hairs; O. *basilicum* L.- D. Second leaf stage; E. Fifth leaf stage; F. Hairs; O. *gratissimum* L.- G. Second leaf stage; H. Fifth leaf stage; I. Hairs; O. *tenuiflorum* L.- J. Second leaf stage; K. Fifth leaf stage; L. Hairs. (Singh., 2012)

Figue 1 describes four different types of basil plants (*O. americanum*, *O. basilicum*, *O. gratissimum*, and *O. tenuiflorum*) at different stages of growth (second and fifth leaf stages) and with details about their hairs.

The genus *Ocimum* includes several species with similar characteristics, such as non-fibrous taproots, smooth secondary roots, and isocotylar, leafy para-cotyledons. However, there are some differences in the characteristics of each species, such as the presence of a clear ring in the collet of *Ocimum americanum*, the unique and smooth collet with a golden ring in *Ocimum tenuiflorum* and *Ocimum gratissimum*, and the terete and purplish-green hypocotyl with a hairy surface in *Ocimum basilicum*. Additionally, the leaves of each species differ slightly in shape, venation, and pubescence, with *Ocimum tenuiflorum* having fewer veins and *Ocimum basilicum* having a distinct deltoid shape.

2.1.3. Origin and distribution

O. basilicum originates from India (Miraj et al, 2016). Annual *basil* plants grow in the wild in temperate, tropical, and subtropical climates (Rubab et al, 2017). *O. basilicum* has been introduced and cultivated in many parts of the world, including the Mediterranean, North and South America, and various islands in the Pacific and Indian Oceans. It is typically grown as an annual plant in temperate regions and as a perennial in tropical regions (Simon et al, 1999).

2.1.4. Agro-ecology

Basil is a plant that contains essential oils and may be cultivated in a variety of climates and on a variety of surfaces. Basil is one of the crops for using sodic wasteland among a variety of aromatic and medicinal plants because of its resistance to greater salt, pH, and exchangeable sodium percentage. As a result, *O. basilicum* is found in tropical regions of Asia, Africa, Central America, and South America.

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However, Iran, Japan, China, and Turkey are the countries with the highest volume of cultivation (Purushothaman et al, 2018).

Currently, basil is grown mostly in Asia, Africa, and the Mediterranean areas of Europe. It is, nevertheless, also cultivated in temperate regions. (Bączek et al, 2019) It is grown as a short-lived annual crop throughout Europe. Because of this, plantations in temperate regions are often formed by nurturing seedlings before transplanting them into the field, which typically occurs in the second part of May (Darrah, 1974).

Ocimum basilicum L. is a plant native to warm climates. The ideal temperature for growth is 25°C, and at this temperature, leaves have a more volatile oil content. Compared to plants cultivated at different temperatures, those that were grown at 25°C for two weeks were taller, had more dry matter, and had bigger leaves (Chang et al., 2005).

2.1.5. Nutrient value, chemical content and pharmaceutical value

O. basilicum produces essential oils that are high in phenolic components like flavonoid and antocyanin (Juliani et al., 2002). The total amount of volatile chemicals found was 79.21% of the oil. Aldehyde (41.56%), alcohol (19.85%), heterocyclic compounds (0.06%), aliphatic unsaturated hydrocarbons (2.42%), cyclic unsaturated hydrocarbons (7.45%), esters (0.22%), aromatic compounds (6.96%), ethers (0.04%), acids (0.36%), and ketones (0.36%) were the most common volatiles found in basil (0.29 percent). The primary abundant chemicals in essential oil were discovered to be (E)-3,7-dimethyl-2,6-octadienal and (Z)-3,7-dimethyl-2,6-octadienal, among the substances identified (Widyawati et al, 2013).

The essential oils of the *Ocimum* genus comprise monoterpene, oxygenated monoterpene, sequisterpene, oxygenated sequisterpene, and phenylpropanoid,

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according to Bunrathep et al. (2007) (the most important components are eugenol, methyl eugenol, and methyl chavicol).

According to Alipour et al. (2014) research, the administration of basil aqueous extracts exhibited a protective effect on bones against osteoporosis induced by glucocorticoids in rats. The essential oil derived from *O. basilicum* has been found to possess antinociceptive properties both peripherally and cerebrally. At a dosage of 200 mg/kg, it has demonstrated an analgesic effect that is comparable to that of aspirin. Choudhury Golak et al published in 2010. According to Subhasree et al. (2009), *O. basilicum* contains polyphenols that exhibit antioxidative properties in food. According to Benedec et al. (2007), the administration of *O. basilicum* tincture (1:10) resulted in a decrease in leukocyte count, circulating phagocyte activation, and monocyte percentage in rats that were subjected to acute inflammation caused by turpentine oil.

According to Amrani et al's (2006) findings, the aqueous extract of basil has demonstrated hypoglycemic and hepato-protective properties, as well as a significant reduction in total cholesterol, LDL-cholesterol, and triglyceride levels. According to Kathirvel et al's (2012) research, *O. basilicum* essential oil exhibits anti-proliferative properties against human oral epidermal carcinoma (KB) and murine leukemia (P388) cell lines.

2.2. CULTIVATION AND BREEDING OF BASIL

2.2.1 Cultivation

Since there is such a high demand for *O. basilicum* on the global market, several private businesses have begun growing it for export to other nations as a fresh or dried herb (Masresha Yimer, 2010)

The positive impact of co-inoculating *Dietzia natronolimnaea* and *Glomus intraradices* with vermicompost on the growth of *Ocimum basilicum* and the

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structure of resident microbial communities in low fertility soils affected by salinity has been observed by Bharti et al. (2016)

Alternative farming methods like aquaponics, which includes both plants and aquatic life, and hydroponics, which only includes plants, have the potential to deliver large yields per unit of land area while using little water and no land (Saha et al., 2016)

2.2.2. Breeding

O. basilicum breeding aims to develop new varieties that have desirable traits such as obtaining a good-quality yield of basil herb (Sajjadi, 2006), having extraction of valuable compounds, such as antioxidant phenolics and essential oil components, is a crucial process (Makri et al., 2008) or researching genome editing utilizing CRISPR: Cas9 technology. The study present exhibits potential for the advancement of efficient and precise breeding techniques, aimed at delivering superior cultivars to farmers and enhanced products to consumers. (Gonda et al., 2020)

Italy: Italy is one of the largest producers of basil and has a long history of breeding for various basil varieties. The Italian company, Vitalis Organic Seeds, is one of the leading companies in the production of organic basil seeds.

United States: In the United States, several companies and universities are involved in basil breeding, including Johnny's Selected Seeds, Ball Horticultural, and the University of California, Davis.

Israel: Israel has a thriving agricultural industry, and companies such as Hazera Genetics and Origene Seeds are involved in breeding basil varieties for both domestic and international markets.

India: India is the largest producer of basil in the world and has a growing demand for high-quality basil varieties. Some of the companies involved in basil breeding in India include Namdhari Seeds and Rasi Seeds.

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2.3. OVERVIEW OF RESEARCH AND DEVELOPMENT OF BASIL

2.3.1. Genetic diversity study

The term "genetic diversity" may refer to both the vast number of distinct species as well as the variations that can be found within each species. According to Farook and Azam (2002), it is described as the diversity of alleles and genotypes that are present in a population, and this is represented in the morphological, molecular, physiological, and behavioral variations that exist between individuals and populations. It is also referred to in a more general sense as variation in the nucleotide, genes, chromosomes, or genomes of a species on the level of an individual, population, species, or area throughout a certain period time.

The information provided on the genetic diversity within and across closely related crop species is crucial for the logical use of genetic resources, and it is essentially the first stage in a plant breeding effort. In order to learn more about the genetic variety of the varieties/stocks and maybe alter the course of breeding programs, genetic diversity analysis can be a beneficial technique (Khleshtkina et al., 2004). It is very helpful for investigating the evolutionary ecology of populations, describing populations, plant variations, and species, as well as finding genetic material duplicates in germplasm collections. Similar to this, genetic diversity is necessary to achieve a variety of plant breeding objectives, including breeding for higher yield, greater adaptability, and desired quality. A species has a higher probability of long-term survival and thriving if its genetic variety is higher (Frankel et al., 1995).

2.3.2 Studies of basil genetic diversity

Several studies have been performed to evaluate the genetic diversity of *Ocimum basilicum*. Vieira et al. (2003) and Al-Mashri et al. (2013) with RAPD markers. Chen et al (2013) used ISSR, RAPD and SRAP markers. And with a

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combination of 3 markers showing clear genetic diversity information, especially on SRAP, it can be a better tool for diversity analysis, and genetics of basil compared with RAPD and ISSR markers.

2.4. MOLECULAR/DNA MARKERS

Molecular markers are indicators that function based on naturally occurring polymorphisms in the DNA sequence. These markers may be used to differentiate between individuals. It includes a wide range of DNA molecular markers, each of which has the potential to be used in separate analyses of variation. They are superior to morphological and biochemical markers due to the fact that they occur often throughout the genome, possess a highly polymorphic character, are unaffected by environmental factors, and have the potential to be detected at any stage of plant growth (O'Neill et al., 2003).

Since the 1980s, an enormous variety of molecular markers has been found, and ongoing research is leading to the development of brand-new kinds of markers. They may be caused by several types of DNA mutations, such as point mutations, substitution mutations, rearrangements, insertion, and deletion mutations; mistakes that occur during the replication of tandemly repeated DNA; or a combination of these factors. According to Weising et al. (2005), molecular markers may be divided into two categories: those that are based on hybridization (non-PCR) and those that are based on PCR.

Molecular markers provide the possibility of characterizing types and measuring genetic linkages with a greater degree of precision than is possible with other markers (Soller & Beckmann, 1983). In addition, according to Weising et al. (2005), molecular markers may be used in the process of identifying cultivars and clones, as well as in genetic mapping, marker aided selection, and molecular systematics. The characteristics of an ideal molecular "marker" include

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polymorphism, co-dominance, the absence of phenotypic plasticity, frequent occurrence in the genome, even distribution throughout the genome, high reproducibility, an easy and quick assay, and the capacity to easily exchange data with other research facilities. These characteristics were identified by Karp et al. (1997) and Weising et al. (2005).

Non-PCR based molecular markers: Restrictions fragment length polymorphism (RFLP) is the most used non-PCR based molecular marker. This was created in 1980 by Botstein et al. Its foundation is the differential hybridization of cloned DNA to DNA fragments in a sample of DNA that has been processed by restriction enzymes. A single clone/restriction enzyme combination is required for the marker to function. In linkage analysis, genetic mapping, genetic fingerprinting, and variation analysis, RFLP is a trustworthy marker. The abundance of the markers in the genomes of most species, excellent repeatability, and co-dominance nature are only a few benefits of the RFLP marker system (Karp et al., 1996).

Contrarily, their usefulness has been limited by the need for large amounts of DNA for Southern blotting and restriction enzyme analysis, probe characterization, the need for radioactive isotopes, which makes the analysis expensive and dangerous, the labor-intensive and time-consuming nature of the assay, and the possibility that one out of multiple markers may be polymorphic, which is extremely inconvenient, especially for crosses between closely related species. (Swati et al., 1999)

PCR-based molecular markers: Numerous molecular approaches have been created as a consequence of the advancement of polymerase reaction (PCR). The method may be divided into two major categories: site-targeted primed sequences and arbitrary/or semi-arbitrary primed sequences (Karp et al., 1997; Weising et al., 2005).

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A variety of markers, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and inter simple sequence repeats (ISSR), are used in the arbitrary or semi-arbitrary (i.e., without knowledge of the flanking sequence of the area to be amplified) approach. All of the aforementioned markers have similar methods, resulting in their multi-locus, dominance, and dispersion over the whole genome (Lowe et al., 2004).

Start Codon Targeted (SCoT): is a type of DNA marker that targets start codons, which are the sites on a DNA molecule where protein synthesis begins. SCoT markers are used in genetic research and are derived from DNA sequences flanking the start codons.

SCoT markers were first developed by Collard and Mackill (2009) and have since been used in various applications such as genetic diversity analysis, gene mapping, and marker-assisted breeding.

Random Amplified Polymorphic DNA (RAPD): Welsh & McClelland and Williams et al. (1990) described this technique. A single oligonucleotide is employed as the primer for the amplification of genomic DNA when RAPD markers are utilized (10 nucleotides in length). It is a dominant marker that may be used for phylogenetic investigations, linkage map creation, and cataloging of desirable features for marker aided selection (Williams et al., 1990). It has some benefits, such as not requiring sequence data for random primer design, not requiring the development of probes, not requiring the use of radioactive materials, and having high genomic abundance and random distribution throughout the genome (Williams et al., 1990). Contrarily, RAPD has significant drawbacks including its dominant nature, the inability to discriminate between different loci and species, the low reproducibility caused by mismatch annealing, inconsistent results depending on the source of the tissue analyzed, and the chance co-migration of bands at different loci (Black, 1993).

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Inter Simple Sequence Repeats (ISSR): Zietkiewicz et al. (1994) first described inter simple sequence repeats (ISSR). ISSR markers are DNA pieces of about 100–300 bp that are found between neighboring, oppositely orientated microsatellites regions. It is a dominant marker with several uses, including gene mapping in closely related species, clone and strain identification, phylogenetic and taxonomic investigations, DNA fingerprinting, and studies of genetic variation. When compared to other dominant markers, ISSR markers have a number of advantages, including the fact that they do not require genomic sequence information, a high level of polymorphism and cost effectiveness per polymorphism, reproducibility, random distribution throughout the genome, and the provision of a less demanding methodology, especially for organisms with incomplete genetic information.

However, it has significant limitations, such as dominating nature, the potential for nonhomology of pieces with identical sizes, and issues with repeatability (Zietkiewicz et al., 1994).

Microsatellite (SSR): Akkaya et al. (1992) coined the term "microsatellites" for the first time. They are brief tandem repetitions, with the amount of repeats varying, and are often made up of 1-6 bp. The polymorphism revealed by the PCR amplification products resolved on gels is recognized based on size differences resulting from variations in the number of repetitions. It may be used for a broad variety of purposes, including as identifying variations between and within species, DNA fingerprinting, marker-assisted selection, genetic linkage mapping, examining population structure, and identifying hybrids. Mendelian inheritance as a co-dominant marker, high reproducibility and polymorphism at each locus, high abundance and wide distribution throughout the genome, locus-specificity, revealing of high allelic diversity, and enabling rapid generation of data from relatively small amounts of plant tissue are some of the benefits of SSR markers (Tautz, 1989;

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Mohan et al., 1997). Despite their benefits, SSR markers are costly to produce, time-consuming in certain species, need sequence information, and may not be acceptable for use across species owing to species-specific primers.

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CHAPTER 3: MATERIALS AND METHODS

3.1. Materials, site and time

3.1.1. Plant Materials

Ocimum basilicum and few other *Ocimum* species cultivated in the test field of the university (19 samples) has been studied in course of my work (Table 1).

Table1. *Ocium* Species used in this study

Number	Name	Spieces
1	'Purple Ruffles'	*Ocimum basilicum*
2	'Red Rubin'	*Ocimum basilicum*
3	'Rubin Kaira (Stavia)'	*Ocimum basilicum*
4	'Fine Verde Compacto'	*Ocimum basilicum*
5	'Penang Lemon (Pinang)'	*O. africanum*
6	'Tulsi Temperate'	*O. sanctum/tenuiflorum/americanum*
7	'Rama Tulsi'	*O. sanctum/tenuiflorum*
8	'K/Cardinal'	*Ocimum basilicum*
9	'Feleny'	*Ocimum basilicum*
10	'Red Boza'	*Ocimum basilicum*
11	'Christmas'	*Ocimum basilicum*
12	'Pepper scented'	*O. selloi*
13	'Barhat'	*Ocimum basilicum*
14	Tulsi Vana	*O. gratissimum*
15	Vietnamise	*Ocimum basilicum*
16	Aromatto	*Ocimum basilicum*
17	Corsican	*Ocimum basilicum*
18	Anisse	*Ocimum basilicum*
19	O.b. mrihani	*Ocimum basilicum*

3.1.2. DNA extraction

DNA was extracted from leaves of species in the genus *Ocimum* by CTAB method (Doyle et al., 1991): Approximately 100 mg of leaf tissue was cleaned and ground in a mortar with a porcelain pestle and then placed in a 1.5 mL ependoff tube and

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750 µL buffer. CTAB (2% CTAB, 20mM EDTA, 1.4M NaCl 1% beta-mercaptoethanol, 100mM Tris-HCl pH 8.0). Samples were incubated at 65ºC for 15 minutes, then mixed with the same volume of 24:1 chloroform isoamylalcohol mixture. Samples were centrifuged at 14,000 rpm for 5 mins. The DNA was precipiated from the supernatant with 500 µL of cold absolute alcohol and 3M sodiumacetate, then centrifuge at 14,000 rpm for 5 minutes, then a precipitate is obtained and washed with 70% alcohol. Dry and dissolve in 30 µL of deionized distilled water.

3.1.3 SCOT primers

SCoT markers have been developed to generate plant DNA markers using short conserved regions around the ATG start codon. It uses 18-mer primers in single primer PCR with an appropriate annealing temperature. SCoT primers were chosen based on the publication of Collard et al. (2009), and they are listed in Table 2.

Table 2. SCoT primers senquences used in this study

Primer	Sequence (5'-3')	% GC
SCoT6	CAACAATGGCTACCACGC	56
SCoT8	CAACAATGGCTACCACGT	50
SCoT11	AAGCAATGGCTACCACCA	50
SCoT23	CACCATGGCTACCACCAG	61
SCoT25	ACCATGGCTACCACCGGG	67
SCoT26	ACCATGGCTACCACCGTC	61
SCoT27	ACCATGGCTACCACCGTG	61
SCoT29	CCATGGCTACCACCGGCC	72
SCoT32	CCATGGCTACCACCGCAC	67
SCoT34	ACCATGGCTACCACCGCA	61

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3.2. Method

3.2.1. PCR (Polymerase Chain Reaction)

All PCR reactions were performed in a total volume of 20 µl in a Swift MaxPro thermocycler (Esco Healthcare Pte, Singapore). PCR mixtures contained 10x PCR buffer, 2.5 mM $MgCl_2$, 0.02 mM deoxyribonucleotide triphosphates (dNTP) mixture, 0.5 unit of DreamTaq polymerase, 5 µmol of primer, 2% BSA, 1% DMSO and sterile distilled water. Each reaction contained 20-80 ng of template DNA (2 µl). A standard PCR cycle was used: an initial denaturation step at 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 49°C for 1 min, and 72°C for 2 min; the final extension at 72°C was held for 5 mins.

3.4.1. Electrophoresis

All PCR amplification products were separated on 1% agarose gels in Tris-borate buffer stained with 1% ethidium bromide in 60 – 80 minutes running time at 120 Volts. 1kbp DNA ladder was used to determine the size and quality of DNA fragments. The results were visualized under UV light by the Gel documentation system. Banding pattern of each gel was analysed and information was collected in a binomial table where each fragment were scored as absent (0) or present (1)

3.4.2. Statistical analysis

The software PAST was utilized for the purpose of conducting an examination of principal coordinate analysis (PCoA) and allelic frequency (Pi). The calculation of Polymorphic Information Content (PIC) was performed on Microsoft Excel by applying the formula $PIC_i = 2f_i(1 - f_i)$. In this formula, PICi represents the polymorphic information content of marker "i", f_i denotes the frequency of the amplified allele (i.e., the band that is present), and $(1 - f_i)$ represents the frequency of the null allele (i.e., the band that is absent). This methodology is in accordance with the approach described by Roldan-Ruiz et al. (2000). The maximum value for

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Polymorphic Information Content (PIC) in the case of dominant markers is 0.5. The dendrogram figures depicting the results of cluster analysis were generated using the scientific data analysis software PAST version 4.03.

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CHAPTER 4: RESULT

4.1. The amplification of SCoT markers

All 19 samples showed good amplification using the 10 primers. In the course of the evaluation of the gels only clear, well-distinguishable bands were scored. Based on the presence-absence tables, data are summarized in Table 3

Table 3. Results of 10 SCoT primers achieved in 19 cultivars of *Ocimum* species

Primer	Total number of amplified bands	Number of polymorphic bands	Percentage of polymorphism (%)	Polymorphism information content (PIC)
SCoT6	10	10	100	1
SCoT8	4	4	100	1
SCoT11	5	5	100	1
SCoT23	10	10	100	1
SCoT25	9	9	100	1
SCoT26	6	5	83.33333333	0.4445
SCoT27	4	4	100	1
SCoT29	5	5	100	1
SCoT32	12	12	100	1
Average	7.3	7.2	98.33333333	0.94445

Overall, the utilization of SCoT primers resulted in the production of DNA fingerprints within the range of 250-3000 bp for *Ocimum* species cultivars, as depicted in Figure 2. The presence of polymorphism was identified, exhibiting an average polymorphism rate of 98.33%. The study revealed that almost primers, such

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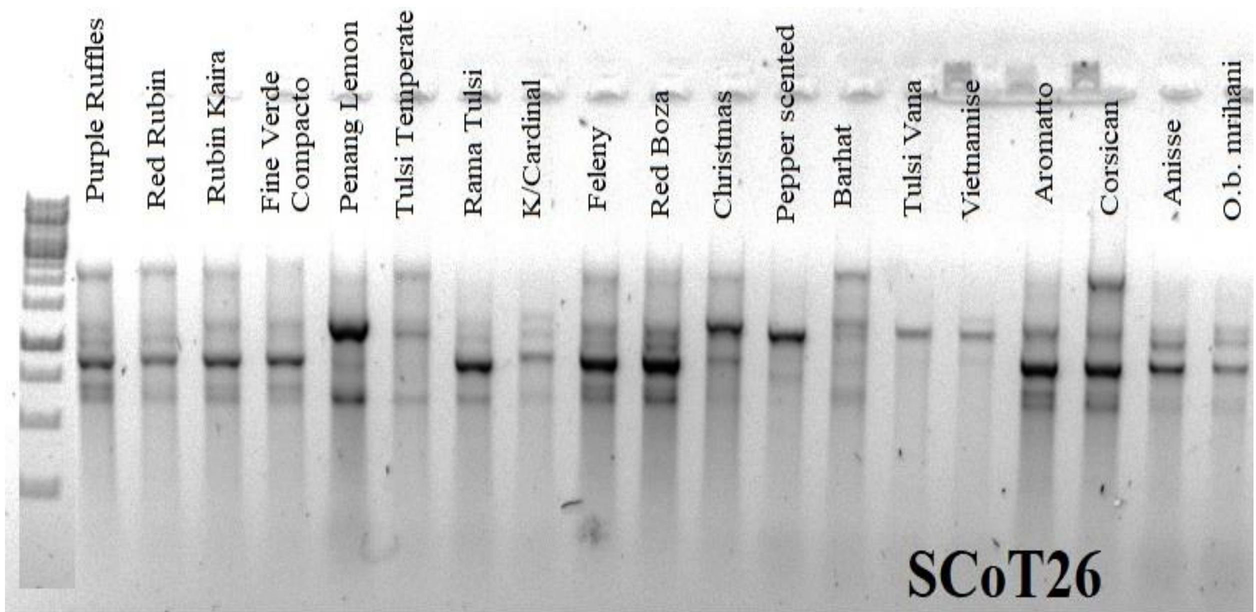
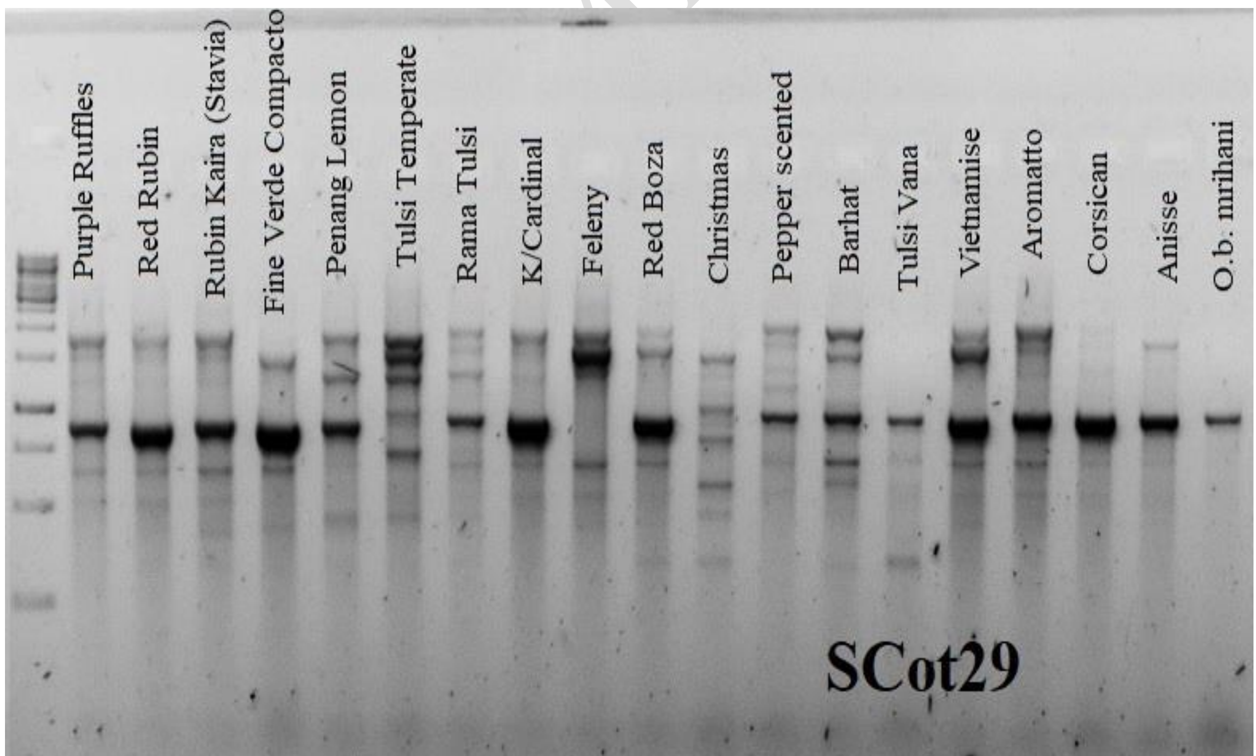
as SCoT26, SCoT29 and SCoT32, resulted in the highest number of amplified bands, all of which were found to be polymorphic. The dataset's PIC values exhibited a range of 0.4445 to 1, with a mean value of 0.94445.





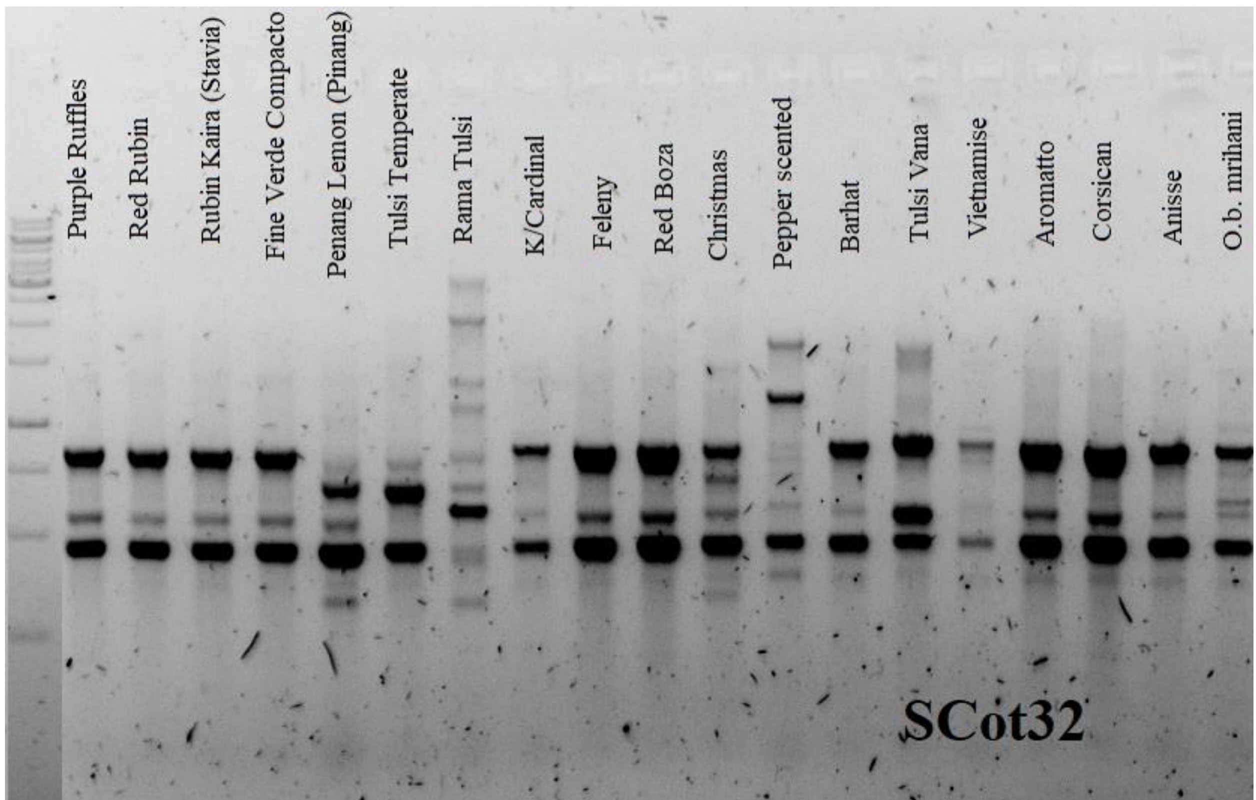


Figure 2. SCoT results achieved in 19 cultivars of *O. basilicum* and other *Ocimum* species. Left is 1kbp DNA ladder

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4.2. Cluster and Principal coordinate analyses

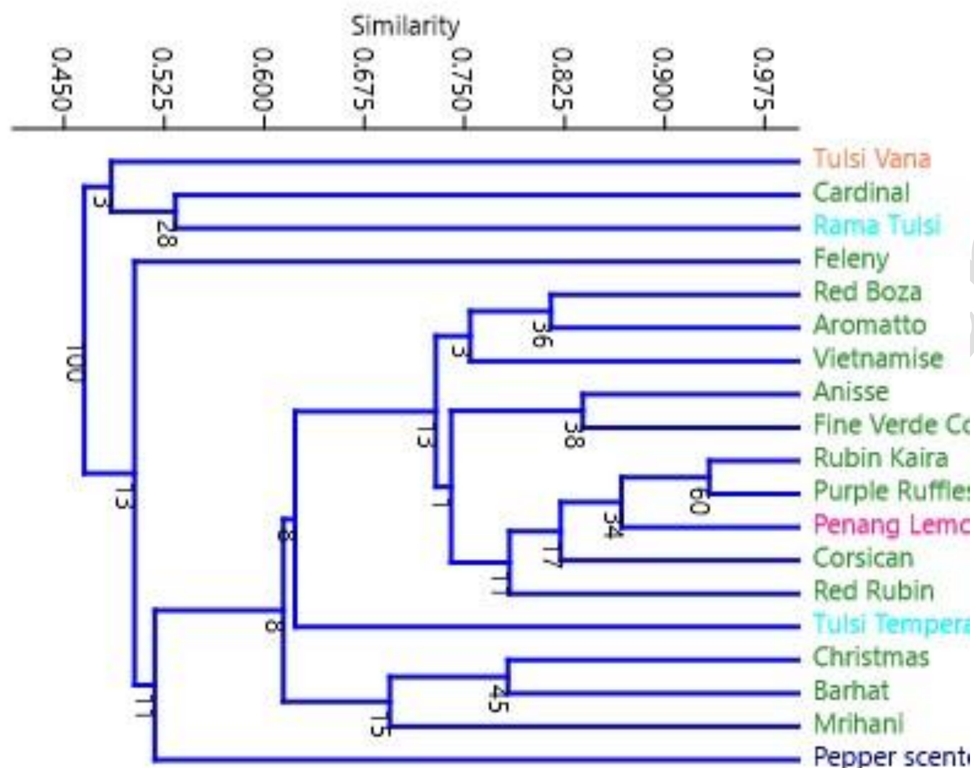

Figure 3. Clusters generated in *O.basilicum* L. and other *Ocimum* species based on SCoT markers. Different colors correspond to different species.

When the similarity value reached 0.525, four primary clusters emerged namely 'Tulsi Vana' (a cultivar of *O. gratissimum*), 'Feleny' (a cultivar of *O. basilicum*), 'Pepper scented' (a cultivar of *O. selloi*), and the remaining cultivars. The obtained results indicate a noteworthy degree of similarity among the samples of *O.basilicum*. 'Rubin Kaira (Stavia)' and 'Purple Ruffles' exhibit a similarity ratio of 0.93, whereas other species within the *Ocimum* genus display a comparatively low similarity ratio, suggesting a notable genetic diversity, as observed in 'Rama Tusil' and 'Tulsi Temperat' samples.

The clustering pattern of genotypes in the UPGMA dendrogram was strongly supported by the Principal Coordinate Analysis (PCoA) of SCoT marker data

(Figure 4). The cultivars ‘Tusil Vana", "Rama Tusil’, ‘Pepper scented’ and ‘Tulsi Temperate’ were observed to be significantly distant from the other cultivars. Similarly, the cultivar pairs ‘Rubin Kaira’– ‘Purple Rufles’, ‘Red Boza’– ‘Aromatto’, and ‘Christmas’– ‘Mrihani’ were found to be closely situated to each other.

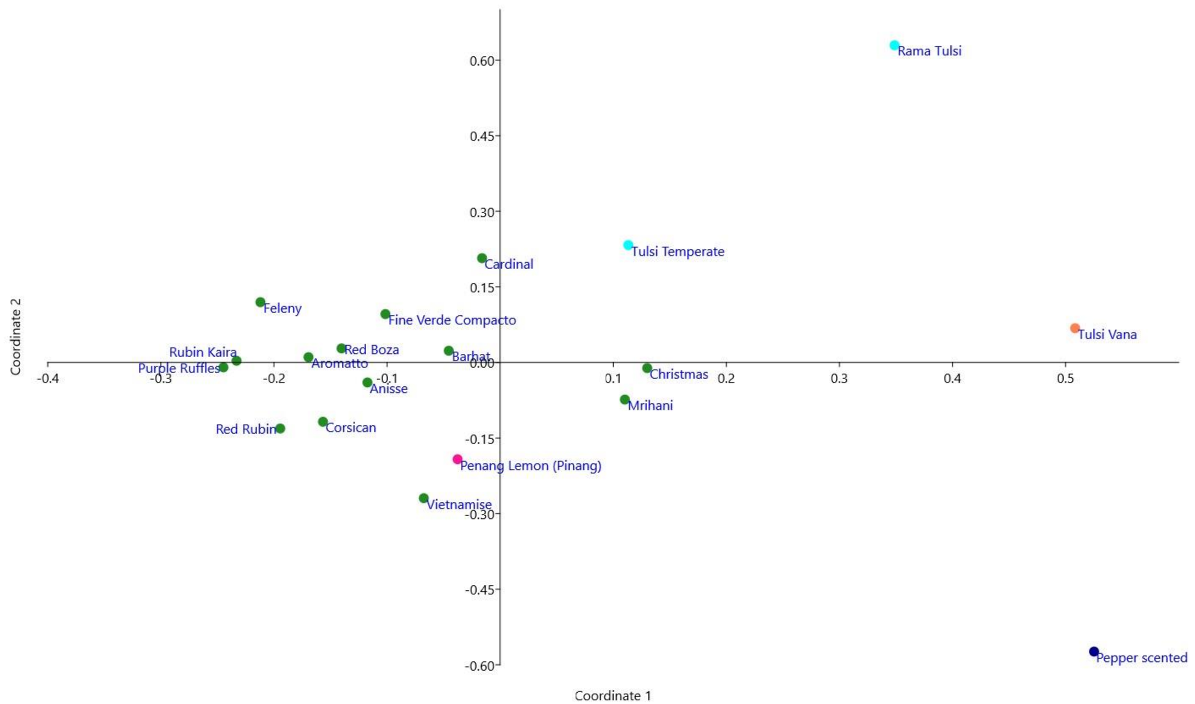

Figure 4. Principal Coordinate Analysis (PCoA) plot of 19 cultivars of *O.basilicum* L. and other *Ocimum* species based on SCoT markers. Different colors correspond to different species

CHAPTER 5: CONCLUSIONS

5.1. Generation of polymorphic DNA markers:

In conclusion, this study successfully utilized SCoT markers to produce DNA fingerprints for various cultivars of *Ocimum* species, including *O. basilicum*. The amplification of all 10 SCoT primers yielded well-defined and distinguishable bands, with a high rate of polymorphism observed across the dataset. Notably, primers SCoT26, SCoT29, and SCoT32 produced the largest number of amplified bands, all of which displayed polymorphic traits. The PIC values obtained ranged from moderate to high, with a mean value indicating good discriminatory power among the studied cultivars. Cluster and Principal Coordinate analyses revealed a significant level of genetic diversity within the *Ocimum* species, with *O. basilicum* cultivars displaying a degree of similarity. Specifically, certain cultivars, such as 'Tulsi Vana', 'Rama Tusil', 'Pepper scented', and 'Tulsi Temperate', were found to be notably distant from other cultivars. Additionally, some cultivars, such as 'Rubin Kaira' and 'Purple Ruffles', exhibited a higher degree of similarity, while other species within the *Ocimum* genus displayed comparatively lower similarity ratios. These findings can have important implications for the conservation and breeding of *Ocimum* species and contribute to our understanding of their genetic diversity.

5.2. Genetic diversity in basil

The results of this study have important implications for the conservation and breeding of *Ocimum* species. The use of SCoT markers provides a fast, reliable, and cost-effective method for assessing genetic diversity within the *Ocimum* genus. By identifying the most polymorphic primers, SCoT markers can be used to efficiently generate DNA fingerprints and identify genetic variability among different cultivars.

The high degree of genetic diversity observed among the *Ocimum* species suggests that there is significant potential for breeding and developing new varieties.

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Breeding programs that aim to enhance the yield, quality, and resistance to biotic and abiotic stresses of *Ocimum* species can benefit from the genetic variability observed in this study. Additionally, the genetic diversity of *Ocimum* species can provide new opportunities for the development of novel pharmaceutical, nutraceutical, and cosmetic products.

The genetic diversity observed in this study can be threatened by factors such as habitat loss, climate change, and the use of uniform cultivars in commercial agriculture. Conservation efforts can involve the maintenance of genetic diversity in seed banks, the identification and protection of wild populations, and the development of sustainable cultivation practices that preserve genetic variability.

This study demonstrates the utility of SCoT markers in assessing genetic diversity in *Ocimum* species and provides valuable insights into the genetic variability of this important genus. The findings of this study can be used to inform breeding programs, conservation efforts, and the development of new pharmaceutical and nutraceutical products.

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SUMMARY

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Genetic diversity of Basil (*Ocimum basilicum* L.) cultivars based on SCOT markers

MSc in Agricultural Biotechnology (Plant)

Ocimum basilicum, commonly known as sweet basil, is a herb that is widely cultivated and used in various culinary and medicinal applications. Despite its importance, there is limited information available on the genetic diversity of this plant species. Therefore, the aim of this study was to generate polymorphic DNA markers using SCoT primers to assess the genetic diversity of *O. basilicum* cultivars and other *Ocimum* species. The SCoT technique is a PCR-based molecular marker system that can generate highly polymorphic DNA fingerprints. The method involves the use of short, arbitrary primers that anneal to random genomic regions, resulting in the amplification of multiple DNA fragments. By comparing the presence and absence of amplified fragments, SCoT markers can be used to assess genetic diversity and construct genetic maps. In this study, 10 SCoT primers were used to amplify genomic DNA from 19 cultivars of *Ocimum* species, including 14 cultivars of *O. basilicum* and 5 other *Ocimum* species. The amplification of SCoT markers resulted in the production of DNA fingerprints with a range of fragment

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sizes from 250 to 3000 bp. The gels showed clear, well-distinguishable bands that were scored to generate presence-absence tables. The study shows the number of amplified bands, the number of polymorphic bands, the percentage of polymorphism, and the polymorphism information content (PIC) for each primer. The results of this study showed that all 10 SCoT primers generated a good amplification of DNA in all 19 samples, with clear and distinguishable bands. The percentage of polymorphism ranged from 83.33% to 100%, with an average of 98.33%. The PIC values ranged from 0.4445 to 1, with a mean value of 0.94445. These results indicate a high level of genetic diversity among the *O. basilicum* cultivars and other *Ocimum* species, which is consistent with previous reports on the genetic diversity of this genus. To further analyze the genetic diversity among the *O. basilicum* cultivars and other *Ocimum* species, cluster and principal coordinate analyses were performed. The clustering pattern of genotypes in the UPGMA dendrogram was strongly supported by the principal coordinate analysis (PCoA) of SCoT marker data. The results showed that there was a high degree of similarity among the *O. basilicum* cultivars, while other *Ocimum* species displayed a comparatively low similarity ratio, suggesting a notable genetic diversity. The cultivars 'Tusil Vana', 'Rama Tusil', 'Pepper scented', and 'Tulsi Temperate' were found to be significantly distant from the other cultivars, indicating that these cultivars may be genetically distinct and could be used for breeding programs aimed at developing new varieties. In general, this study demonstrates the utility of SCoT markers in assessing the genetic diversity of *O. basilicum* and other *Ocimum* species. The results showed that there is significant genetic diversity among the *Ocimum* species, which can be used for breeding programs and the development of new pharmaceutical and nutraceutical products. The generated SCoT markers can be used to identify and conserve the genetic diversity of *Ocimum* species, as well as to develop new varieties with improved characteristics.

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APPENDIX

Annex: Similarity and distance indices

	Purple Ru	Red Rubin	Rubin Kair	Fine Verd	Penang Le	Tulsi Tem	Rama Tuls	Cardinal	Feleny	Red Boza	Christmas	Pepper sc	Barhat	Tulsi Vana	Vietnamis	Aromatto	Corsican	Anisse	Mrihani
Purple Ru	1	0.877193	0.862069	0.827586	0.741935	0.666667	0.45614	0.76	0.821429	0.785714	0.618182	0.45283	0.766667	0.481481	0.678571	0.793651	0.794118	0.779661	0.714286
Red Rubin	0.877193	1	0.77193	0.736842	0.655738	0.603774	0.428571	0.693878	0.690909	0.690909	0.618182	0.490566	0.711864	0.415094	0.678571	0.774194	0.746269	0.758621	0.690909
Rubin Kair	0.862069	0.77193	1	0.827586	0.612903	0.592593	0.45614	0.64	0.714286	0.75	0.581818	0.415094	0.733333	0.444444	0.678571	0.761905	0.735294	0.745763	0.714286
Fine Verd	0.827586	0.736842	0.827586	1	0.612903	0.666667	0.526316	0.68	0.75	0.714286	0.690909	0.45283	0.8	0.518519	0.678571	0.761905	0.735294	0.847458	0.785714
Penang Le	0.741935	0.655738	0.612903	0.612903	1	0.586207	0.459016	0.592593	0.6	0.666667	0.610169	0.491228	0.59375	0.413793	0.7	0.727273	0.75	0.698413	0.566667
Tulsi Tem	0.666667	0.603774	0.592593	0.666667	0.586207	1	0.45283	0.652174	0.615385	0.615385	0.588235	0.408163	0.714286	0.52	0.538462	0.62069	0.5625	0.618182	0.538462
Rama Tuls	0.45614	0.428571	0.45614	0.526316	0.459016	0.45283	1	0.489796	0.436364	0.472727	0.481481	0.307692	0.440678	0.339623	0.363636	0.52459	0.447761	0.482759	0.509091
Cardinal	0.76	0.693878	0.64	0.68	0.592593	0.652174	0.489796	1	0.666667	0.708333	0.553191	0.4	0.692308	0.521739	0.625	0.690909	0.6	0.666667	0.625
Feleny	0.821429	0.690909	0.714286	0.75	0.6	0.615385	0.436364	0.666667	1	0.777778	0.566038	0.392157	0.655172	0.423077	0.592593	0.721311	0.666667	0.666667	0.555556
Red Boza	0.785714	0.690909	0.75	0.714286	0.666667	0.615385	0.472727	0.708333	0.777778	1	0.716981	0.431373	0.655172	0.5	0.740741	0.819672	0.69697	0.77193	0.62963
Christmas	0.618182	0.618182	0.581818	0.690909	0.610169	0.588235	0.481481	0.553191	0.566038	0.716981	1	0.44	0.736842	0.509804	0.679245	0.644068	0.615385	0.714286	0.679245
Pepper sc	0.45283	0.490566	0.415094	0.45283	0.491228	0.408163	0.307692	0.4	0.392157	0.431373	0.44	1	0.472727	0.408163	0.470588	0.448276	0.444444	0.444444	0.54902
Barhat	0.766667	0.711864	0.733333	0.8	0.59375	0.714286	0.440678	0.692308	0.655172	0.655172	0.736842	0.472727	1	0.464286	0.62069	0.707692	0.742857	0.688525	0.724138
Tulsi Vana	0.481481	0.415094	0.444444	0.518519	0.413793	0.52	0.339623	0.521739	0.423077	0.5	0.509804	0.408163	0.464286	1	0.461538	0.474576	0.46875	0.509091	0.5
Vietnamis	0.678571	0.678571	0.678571	0.678571	0.7	0.538462	0.363636	0.625	0.592593	0.740741	0.679245	0.470588	0.62069	0.461538	1	0.721311	0.636364	0.736842	0.592593
Aromatto	0.793651	0.774194	0.761905	0.761905	0.727273	0.62069	0.52459	0.690909	0.721311	0.819672	0.644068	0.448276	0.707692	0.474576	0.721311	1	0.794521	0.8125	0.655738
Corsican	0.794118	0.746269	0.735294	0.735294	0.75	0.5625	0.447761	0.6	0.666667	0.69697	0.615385	0.444444	0.742857	0.46875	0.636364	0.794521	1	0.753623	0.69697
Anisse	0.779661	0.758621	0.745763	0.847458	0.698413	0.618182	0.482759	0.666667	0.666667	0.77193	0.714286	0.444444	0.688525	0.509091	0.736842	0.8125	0.753623	1	0.736842
Mrihani	0.714286	0.690909	0.714286	0.785714	0.566667	0.538462	0.509091	0.625	0.555556	0.62963	0.679245	0.54902	0.724138	0.5	0.592593	0.655738	0.69697	0.736842	1

DECLARATION

Me, as the undersigned Nguyen Minh Tuyen (Code-Neptun: E22AZK) declare, that the Diploma Thesis entitled Genetic diversity of Basil (*Ocimum basilicum* L.) cultivars based on SCOT markers submitted on April 28, 2023 is my own intellectual property.

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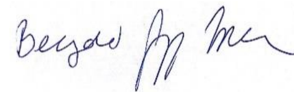

Internal supervisor

Nguyen Minh Tuyen