MASTER THESIS

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Studying the origin of lavender cultivated in Tihany by molecular markers.

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MSC. In Agricultural Biotechnology

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Abbreviations:

AFLP: Amplified Fragment Length Polymorphism

AMV: Alfalfa mosaic virus

BSA: Bovine Serum Albumin

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic Acid

dNTP: Deoxyribonucleotide Triphosphate

EMA: European Medicines Agency

EO: Essential Oil

EST-SSRs: Expressed Sequence Tag - Simple Sequence Repeats

GAD: Generalized Anxiety Disorder

ISO: International Organization for Standardization

NF: French Norms (Normes Françaises)

PCR: Polymerase Chain Reaction

Ph. Eur.: Pharmacopoeia Europaea

PNISO: Polish Norms (Polska Norma)

RAPD: Random Amplified Polymorphic DNA

RFLP: Random fragment length polymorphism

SCoT: Start Codon Targeted

SNP: Single Nucleotide Polymorphisms

SSR: Simple Sequence Repeat

1. INTRODUCTION

Lavender belongs to the Lamiaceae family, it is an aromatic plant with medicinal uses that is cultivated in arid habitats with calcareous sedimentary rocks, supports drought and has a great capacity for adaptation. It is endemic to the Mediterranean (Despinasse et al.2020).

For many centuries, lavender oil has been used to wash wounds and in their recovery. Lavender was used by Greek and Roman armies to rinse and bandage their wounds. During World War I, several hospitals and military doctors even tried using lavender to treat wounded soldiers. A 2016 study found that lavender oil improved healing by encouraging the formation of scar tissue (Mori et al. 2016).

This fabulous plant has a long history of cultivation and a great economic importance being one of the most important crops grown worldwide because of the essential oils' extraction (Adal et al. 2015). From a long time ago, lavender have been used in the cosmetic and pharmaceutical industries, in the elaboration of fragrances, as a natural food flavoring agent, in alternative medicine, among others (Aprotosoaie et al. 2017; Benabdelkader et al. 2011). The quality of it´s essential oils is determined by the flowers, which contain a high concentration of monoterpenoids, linalyl acetate, and linalool, which give this plant its distinctive aroma. (Hassanpouraghdam et al. 2011). The market value of lavender oils depends directly on the species or cultivars that have been used for its extraction. Due to this, different approaches have been considered in the development of lavender genomic resources, molecular markers, comparative analysis of flower volatiles, and specific breeding and marker-assisted selection (Stanev et al. 2016).

To improve yield productivity, the application of molecular approaches and metabolic engineering is becoming increasingly popular, which has made it possible to improve other genetic qualities such as oil quality, resistance to adverse growing conditions, protein content. linalyl acetate and diseases (Wells et al. 2018).The testing and improvement process of the analyzed varieties must consider the growth parameters, the adaptability, the crop lines, the flower yield, and the quality of the essential oils (Stanev 2010). As a result, there is a clear need to investigate the origins of lavender using molecular markers. Through the taxonomic classification of lavender seeks to develop adequate DNA-based markers that help in the genetic identification, fingerprinting application, diversity studies and to exploit the *Lavandula* gene pool for specific breeding (Adal et al. 2015).

Given the great demand for essential oils, programs have currently been developed in France focused on improving certain characteristics of lavender, selecting and propagating plants derived from seeds that have better development. After hybridization, the heterosis of the different

varieties and lines is analyzed. of improvement, finally applying chemical and radiation mutagenesis. The increase in floral yield, as well as the content and quality of essential oils, linalyl acetate, and cold tolerance has increased significantly (Stanev et al. 2016).

At present, agricultural practices in lavender crops have been modernized, mechanizing most of the tillage and flower-picking processes; Therefore, growing lavender varieties with better characteristics, such as long stems that remain upright, facilitates the implementation of these new technologies (Singh et al. 1989). In a study conducted with Bulgarian lavender, the cultivation of selected varieties of superior lavender that have been perfectly adapted to environmental conditions was tested, rather than seed-derived lavender, and showed a greater number of lavender flowers from 6% to 30 % and double and triple the yield of essential oils, in addition to showing a variation in the composition of the essential oils obtained from these varieties (Adal et al. 2015).

Lavender holds immense economic, cultural, and touristic significance in Hungary, and the plant is associated with numerous local customs. It has been grown since the 18th century in Pannonhalma and since the 1920s on the Mecsek Hills and the Tihany peninsula near Lake Balaton, one of Europe's largest lakes. In Hungary, its lavender is used to make artisanal and therapeutic goods. This lavender hungaricum is regarded as a therapeutic plant (HelloVidek. 2020).

1.1. Aim of the study

The objective of this research is to study the origin of lavender that has been grown in the Tihany region in Hungary, through the use of two different molecular markers such as EST-SSR and SCoT for the analysis of genetic variability.

2. LITERATURE REVIEW

2.1. Distribution

Most fields of lavender can be found in the Mediterranean and certain areas in Asia, Europe, India, South America, and North Africa. In Provence, located in southeastern France, the lavender fields are famous, because are one of the world's largest lavender producers (Détár et al.,2020).

Figure 1 illustrates the distribution of lavender in Europe. Lavender is native to southern Europe in the countries of Italy, Andorra, Spain, and France and has become naturalized in other areas of the continent and in Africa. (USDA, Agricultural Research Service, National Plant Germplasm System. 2023).

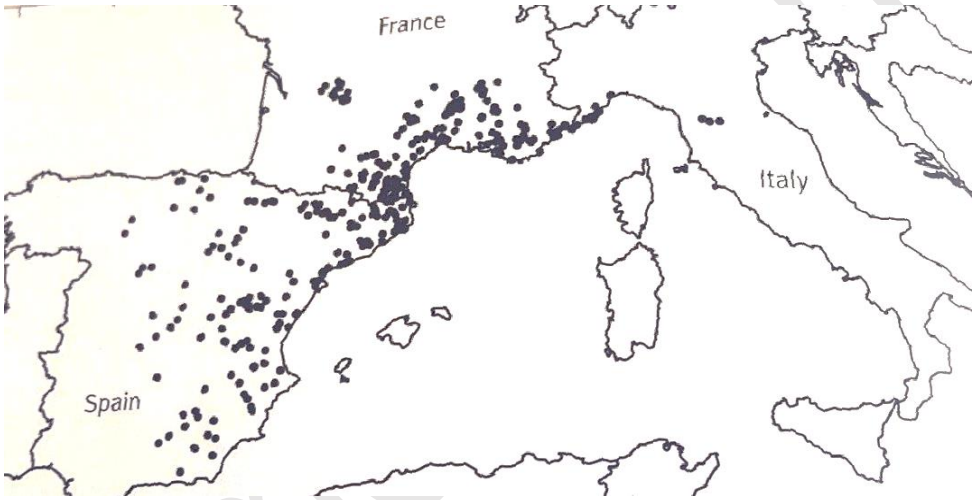

Figure 1. Distribution of lavender around Europe (Upson T. 2002).

2.2. Morphology

The genus is a rather heterogeneous and divergent group in terms of its general morphology. The genus *Lavandula* in the family Lamiaceae includes aromatic plants such as lavender, mint, basil, and sage. In all the Lamiaceae the nectary lobes are opposite those of the ovary instead of alternating, which defines them. Numerous species include woody shrubs up to one meter high and evergreen shrubs with a woody base as shown in Figure 2, the leaves may be fully or partially separated. In the Figure 3 it can be observed that the lavender inflorescences are grouped in a dense, spreading structure with one or several flowers, at the top of each branch called a "cyme" and each pair of floral whorls at right angles to the pair of lower and upper ones or in an alternating spiral configuration (Lis-Balchin, M. 2002).

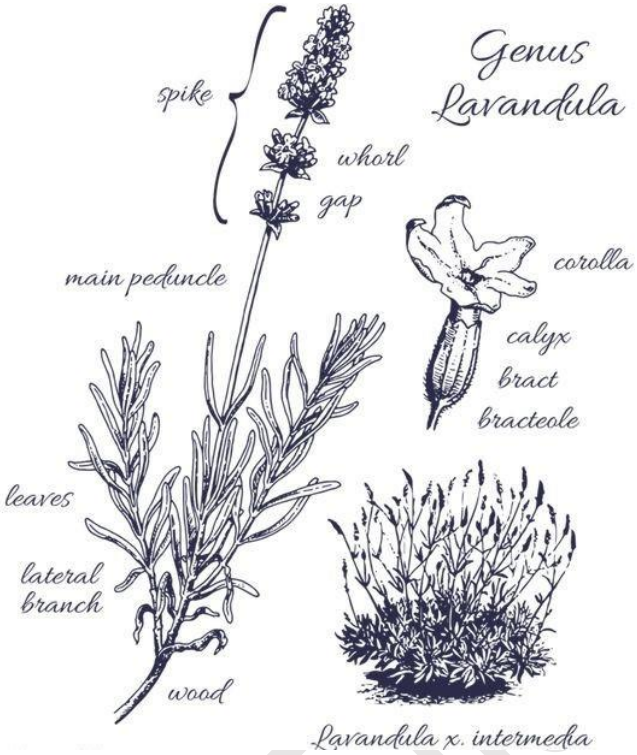

Figure 2. Representation of the structure of the *Lavandula x. intermedia* (Legarre, 2020).

The multifloral crest of lavender flowers, which grows in a spike on a separate peduncle and is usually purple to violet in color, may also feature white or pink flowers depending on the variety. On the other hand, the leaves of these plants are thin and develop in opposite directions, they present a great variety of shapes according to the species, as can be seen in Figure 4. Although both the leaves and the flowers create oils essential, only floral EO has commercial importance because flowers produce a much greater amount of EO than leaves (Erland et al.2016).

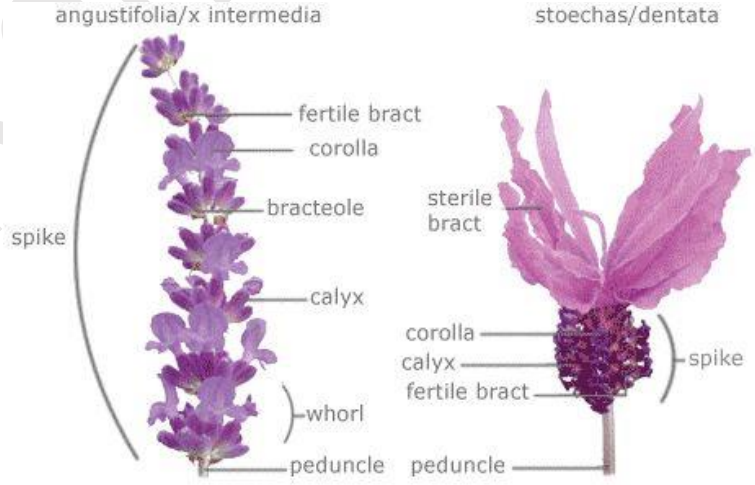

Figure 3. Morphology of the flowers in L. *angustifolia and x intermedia* and *stoechas/ dentata* (Downderry Nursery 2018).

The name lavender comes from its exceptional floral morphology: Figure 3 demonstrates the peculiarity of the petals of Lamiaceae plants, which join together to form an upper and lower "lip" that blooms in the last days of spring and early spring. of summer, although flowering is prolonged. until the end of August (Erland et al. 2016).

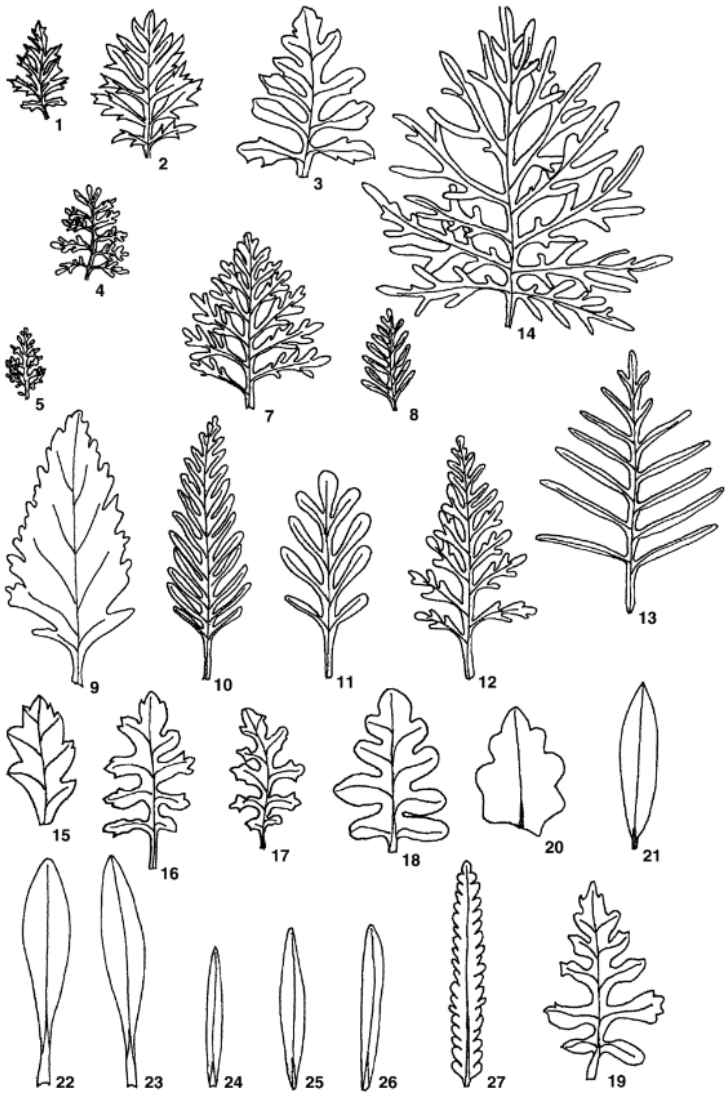

Figure 4. Diversity of leaf shape and forms found within the genus *Lavandula* (Lis-Balchin, M. 2002). 1. *L. mairei var. Mairei*, 2. *L. pubescens*, 3. *L. citriodora*, 4. *L. maroccana*, 5. *L. antineae*, 7. *L. multífida*, 8. *L. coronopifolia*, 9. *L. rotundifolia*, 10. *L. minutolii var. Minutolii*, 11. *L. pinnata*, 12. *L. canariensis*, 13. *L. buchii var. Gracile*, 14. *L. bipinnata*, 15. *L. subnuda*, 16. *L. dhofarensis*, 17. *L. nimmoi*, 18. *L. somaliensis*, 19. *L. galgalloensis*, 20. *L. hasikensis*, 21. *L. atriplicifolia*, 22. *L. lanata,* 23. *L. latifolia,* 24. *L. angustifolia*, 25. *L. stoechas subsp. Stoechas,* 26. *L. viridis*, 27. *L. dentata var. Dentata.*

2.3. Taxonomy

Lavandula sp. is a shrub belonging to the Nepetoideae subfamily and the Lamiaceae family (Labiatae). Currently, it is believed that *Lavandula* is a unique group that is distinct from Nepetoideae, and that just one genus*, Lavandula,* belongs to the tribe Lavanduleae (Cantino et al., 1992). The taxonomy of lavender is described in Table 1.

Table 1. Taxonomy of genus Lavandula L. (USDA, Agricultural Research Service, National Plant Germplasm System. 2023).

KINGDOM	Plantae
DIVISION	Tracheophyta (Vascular plants)
CLASS	Magnoliopsida (Dicotyledons)
ORDER	Lamiales
FAMILY	Lamiaceae (Lamiaceae or Labiates)
SUBFAMILY	Nepetoideae
TRIBE	Lavanduleae
GENUS	*Lavandula*

There are about 39 species of the genus *Lavandula* around the world, several hybrids and at least 400 registered cultivars; *Lavandula angustifolia* is the best-known species of this genus for its use in the extraction of essential oils due to its chemical properties, some other well-known species that are also cultivated are: *L. officinalis* Chaix ex Vill sin. *L. vera* DC syn. *L. spica* L., *Lavandula* × *intermedia* Emeric ex Loisel syn*. L. hybrida* L. and *Lavandula latifolia* Medicus (Lesage-Meessen et al. 2015).

Around the world, oils are extracted mainly from *L. angustifolia*, *L. latifolia* and its natural hybrid *L. x intermedia* due to its high market value due to its monoterpene profile (Cavanagh and Wilkinson 2002).

The cultivation of *Lavandula* species has grown significantly in Hungary in last years. The most popular cultivars are from two species, particularly ‘Silver’, ‘Beate’, ‘Budakalászi’, ‘Hidcote Blue’, ‘Mailette’, and ‘Munstead’ from the species *L. angustifolia* and ‘Grappenhall’, ‘Judit’ and ‘Grosso’ from the species *L. x intermedia* (Détár et al, 2020).

2.3.1. ‘Budakalászi’ Lavender

It is a cultivar of *L. angustifolia* that was developed in the Budakalász district of Hungary around 1974 (Lis-Balchin 2002). This cultivar develops into a little, 30- to 40-cm-tall bush. Similar to other kinds, it has spikes of fragrant, soft flowers that are violet to pale blue in color, which is a

distinctive trait of the *L. angustifolia* genus. It is grown extensively for its essential oil (EO), which is used in perfumes, cosmetics, and pharmaceutical items. This cultivar, according to research by Détár et al (2020), contains a high concentration of essential oils, about 8.2 ml/100 g, and exhibits significant levels of linalool.

1. Figure 5. *L. angustifolia* var. 'Budakalászi' in Hungary district Budakalsz (https://kultgarden.hu/kertepites/a-levendulak-8-varazslatos-fajtaja/).

2.3.2. 'Hidcote Blue' Lavender

According to Lis-Balchin (2002) 'Hidcote Blue' is another *L. angustifolia* cultivar that is endemic to the UK, was developed in 1950. 'Hidcote Blue' is one of the most attractive lavenders in terms of color because, unlike the 'Budakálszi' lavenders, its flowers develop into a spike of vibrant purple to deep blue tones in early July. Between 30 and 60 cm tall and around 50 cm wide, the branched growth of this variety contains several branches. It can be multiplied from cuttings or seedlings. It is quite valuable both as an ornament and as a scent (Renaud et al. 2001).

Figure 6. *L. angustifolia* var. 'Hidcote Blue' (https://kultgarden.hu/kertepites/a-levendulak-8-varazslatos-fajtaja/).

2.3.3. 'Maillette' Lavender

Another well-known *L. angustifolia* cultivar that originates from France is called 'Maillette'. The 'Maillette' lavender cultivar exhibits the characteristics of a compact shrub that grows to a height of 30 to 60 cm. The inflorescence stem is of average length, usually unbranched, and has a compact spike with pale violet to light blue flowering cymes. Linalool and linalyl acetate make up

more than 70% of the chemical makeup of 'Maillette' EO, which is of high quality and has a pleasant scent (Lafhal et al. 2016 & Pistelli et al. 2017).

Figure 7. Lavender 'Mailette' France Organic. (http//labofflowers.com/store/ols/products/lavender-mailette-lavender-ang-var-mailette-france-organic)

2.3.4. 'Munstead' Lavender

The 'Munstead' variety of lavender, which is a native of the United Kingdom, was developed from *L. angustofolia* in 1916. The blossom can be either light purple-blue or dark blue in color. It is an upright shrub with grayish-green leaves that is 65x65 cm in size. The flower stalk can grow as tall as 15 cm. The bloom smells delicious. Of all the varieties of English lavender, it is regarded as being the most heat tolerant. It is a well-liked cultivar of lavender that is cultivated for its fragrance, aesthetic appeal, and adaptability in gardening. It bears Gertrude Jekyll's name, a well-known landscape architect (Grbeša, 2016).

Figure 8. L. ang. var 'Munstead' (www.nichols.co.nz/Garden-Centre/Shop/Product-Details/Lavender/Lavender-Munstead-1L?ID=16624).

2.3.5. 'Grosso' Lavender

Lavender (*Lavandula x intermedia*) variety 'Grosso' is one of the most important varieties of the genus *Lavandula* and stands out for its cultivation in the Mediterranean area. It is a hybrid of *L. latifolia* and *L. angustifolia*, often known as lavandin (Bombarda et al. 2008). Three kinds are the most often cultivated, with the 'Grosso' hybrid standing out for its numerous inflorescences and high essential oil production rates. Monoterpenes, such as linalool and linalyl acetate, make up the

majority of its essential oil (Andriolli, 2014). They are woody-stemmed shrubs that grow to a height of about 150 cm, have lance-shaped, grayish-green leaves, and are hairy. Typically, the stems and inflorescences are divided. The varying size and form of the fertile bracts are oval or rhomboid. The bilateral corolla is symmetrical and has 13 veins on the calyx. The inflorescences are up to 75 cm long and have tiny violet flowers on them. It blooms between June and July (Upson, 2002).

Figure 9 Lavandula x intermedia Emeric ex Loisel. var. Grosso (https://www.magisterproducts.hu/lavendel-daytime-visage).

2.3.6. 'Grappenhall' Lavender

According to Grbeša (2016) 'Grappenhall' is tall, can grow up to 90 cm, grows quickly and spreads just as quickly and is a stable cultivar. Lavender 'Grappenhall' flowers are purple-lilac and grow on spikes at the top of thin stems 30-40 cm long without leaves. It has green foliage; it has a high antioxidant capacity. This is a variation originated from *L. x intermedia* in the United Kingdom in 1902. This variety of *Lavandula x intermedia* is a tall and resistant lavender that tolerates temperatures as low as -15°C in the winter but does not tolerate humidity. (Détár et al.2020).

Figure 10. *Lavandula x intermedia* 'Grappenhall' (https://www.megyeriszabolcskerteszete.hu/lavandula_x_intermedia_grappenhall_halvanykek_provanszi_levendul).

2.3.7. 'Judit' Lavender

Lavender 'Judit' is an Italian-bred hybrid of *Lavender x intermedia* and *L. latifolia* that has been grown in Hungary since 2002. The bushes develop slowly and have a spherical form, with

ash blue leaves in the dormant stage and dark green foliage in the vegetative stage. It blooms earlier than the intermediate and for a longer period of time. It has violet-blue flowers that are darker and narrower lanceolate and symmetrically branching. Its blossoms smell great and have a long-lasting scent. It has a nice, powerful, and subtle aftertaste from its essential oil (https://prezentbudapest.hu/products/bio-levendula-illoolaj-judit).

Figure 11. Lavandula x intermedia 'Judit' – ("https://www.megyeriszabolcskerteszete.hu/nehany_tanacs_az_angol_levendula_ultetesere_gondozasara).

2.3.8. 'Super' Lavender

This cultivar features enormous flower heads and extremely long stalks. It is grown for the quality of its oil because it is regarded as one of the best essential oils among lavandins. The calyx is light violet green, and the corolla is violet. It has an evergreen shrub with a diameter of 1.2m. A wonderfully eye-catching display of pale violet spikes 7 to 10 cm long.Because of the cineole content found in most *L. x intermedia* hydrosols, the hydrosol obtained from the 'Super' cultivar has a pleasant herbaceous honey aroma with undertones of eucalyptus (http://snowyriverlavender .com.au/lavandin-cultivar-profile-super/).

Figure 12 Lavandula 'Super' Cultivar. (http://snowyriverlavender.com.au/lavandin-cultivar-profile-super/).

2.4. Cultivation Condition

Lavender has the ability to adapt to any type of soil, it usually grows on stony ground. However, to achieve optimal development, the conditions for its cultivation must have a moderate amount of organic matter. The soil must be a little sandy or sandy. loam, slightly alkaline with a pH of 6.4 to 8.2. Organic matter is very important in the early stages of development as it helps the formation of the root system. Lavender is very sensitive to very humid soils since it easily tends to rot the roots, so it must have soil with good drainage to avoid the accumulation of water. It develops best in sunny and warm climates, lavender can withstand temperatures as low as -10ºC, demonstrating the rustic and incredibly adaptive nature of this plant. Though the range of 15 to 30 °C is ideal for its growth. To stimulate flowering and promote compact growth, perform regular pruning. Pruning at the end of the season helps prevent plants from becoming woody (Fanlo et al., 2009).

2.5. Lavander Diseases

2.5.1. Rotten Roots and Botrytis

Fungi present in soils are the source of root rot, which can be a common issue for many bushes and hedges when planted improperly. If lavender plants are frequently overwatered and the soil permeability is especially poor around them, they may have root rot. The first signs of *Botrytis*, a disease caused by fungi, are the grayish color of the foliage at the base, the leaves turn yellow until they wither and fall. This disease is also caused by excess water in the soil. This can be controlled with less watering, removing the affected leaves and spraying a fungicide to prevent the disease from spreading.

2.5.2. Septoria leaf spot

This illness is brought on by *Septoria*-genus fungus. It is another fungus that grows in humid environments and destroys especially lavender. The earliest signs of this illness are little gray spots on the leaves, which eventually spread and damage the foliage. They can weaken the plant, which can have a significant impact on its growth. Keep the leaves as dry as possible to prevent this, as water that collects on them tends to promote Septoria leaf spot. In order to prevent this situation, it's important to space out the lavenders and occasionally trim the inner branches to allow for proper air circulation between the bushes. To stop the infection from spreading, remove the affected leaves, and use ecological fungicides like copper and neem oil to destroy the fungus.

2.5.3. Alfalfa Mosaic Disease

One of the most destructive diseases induced by AMV that can harm hedges and lavender bushes is alfalfa mosaic disease. usually transmitted by aphids that infest the plant or by infected farm or gardening gear. The leaves typically have a mosaic pattern that changes from green to yellow. They can sometimes have deformities, such as curling or distortion.

Pruning equipment should be cleaned and disinfected before and after use, and crops should be regularly weeded and checked for pests like aphids. Sadly, alfalfa mosaic disease has no known cure and should be removed from plants as soon as possible because it is extremely spreading to nearby plants. The best option to get rid of it is to burn it, and thus avoid possible spread (Stanković et al., 2014)

2.5.4. Lavender Shab

Lavender plants are fatally affected by a fungal disease spread by the wind. It turns woody branches and green stalks brown with black dots. Many fungi of the genus *Rhizoctonia,* especially *Rhizoctonia solani*, are the cause of lavender shab. Lavender plants affected by this disease may experience rotted roots and base, which may weaken and perhaps kill the plant. Nonetheless, resistant varieties of lavender are available; one such resistant variety is French lavender. There is no remedy; use gloves to dry them off and burn them afterwards (Salahudin et al., 2023).

2.6. Breeding

A few years ago, the production and distribution of lavender planting material in several European nations was free market since it was created by small producers in the area, therefore there were no well-established processes and services for the quality control of the material planting (Singh et al., 1989).

Since 1600, there have been more cultivars of lavender, and occasionally several names for the same genotype have been discovered and documented. According to some sources, the ‘Alba‘cultivar is the oldest (Lis-Balchin. 2002).

Currently, the International Union for the Protection of New Varieties of Plants has published a guideline for the conduct of tests for distinctness, uniformity, and stability prepared by experts from the European Union to be considered by the Technical Working Party for Ornamental Plants and Forest Trees as Lavandula/ lavender. It demonstrates the proper approach for harmonized assessment of distinctness, uniformity, and stability (DUS), as well as identifying acceptable features for DUS examination and generation of harmonized variety descriptions before distribution to farmers. (UPOV, 2022).

2.7. Lavandula Cultivar

Modern lavender breeding programs began in France and spread to Eastern Europe, the United Kingdom, and eventually the rest of the world. Initially, lavender breeding initiatives were centered on testing, selecting, and duplicating the highest performing plants. The effect of heterosis in Eastern Europe was then investigated through hybridization programs, experimental polyploidy and mutagenesis among many different species, and some locally adapted lavender cultivars were created. Selective breeding is increasingly important to perfect lavender breeding programs, and this is now possible thanks to the sequencing of the lavender genome (Crișan et al. 2023).

Currently, the major goals of lavender breeding are to increase oil production, improve the composition of fragrant compounds, and enhance cultivar resilience to drought and frost in the *Lavandula* species.

It has been possible to create interspecific hybrids of lavender by crossing different species, such as *L. intermedia* when *L. latifolia* and *L. angustifolia* were crossed, *L. losae* between *L. latifolia* and *L. lanata*, and lastly *L. chaytorae* when hybridizing *L. lanata* and *L. angustifolia*. There have also been obtained intersectional hybrids between *Lavandula* and *Dentatae*, such as *L. heterophylla*, *L. allardii*, and *L. ginginsii*. These hybrids, which are typically created by spontaneous hybridization, cannot thrive in cold locations because they do not have good frost resistance. Due to its huge floral spikes and lengthy flowering period, it has particularly eye-catching ornamental qualities (Upson & Andrews, 2004).

Improved understanding of interspecies compatibility could aid in successful hybridization. Lavandula reproduction might benefit from targeted breeding programs based on information such as genome size, chromosomal number, and genetic relationship research.Numerous studies demonstrate that knowledge of the genetic relationships between the two species that will be crossed can determine whether a hybridization will be successful. For instance, it was discovered in a study using *Sarcococca* that similar ploidy levels and the parents' 2C values are the primary influences on crossover efficiency, leading to higher crossing efficiency. The success of hybridization in *Salvia, Hydrangea,* and *Rhododendron* was significantly influenced by the genetic link (Van Oost et al.2021).

Understanding phylogenetic links and cytogenetic features can help with breeding methods. Understanding genetic factors, such as phylogenetic relationships, cytogenetic traits, and environmental factors that affect chemical biodiversity, can help with breeding strategies. For example, if you consider the different chemotypes found within the same species, you can see that they are important chemicals and that, when genetically inherited, they can be used to create high-yielding lavender cultivars (Crișan et al. 2023).

2.8. Lavanda essential oil composition

The process of steam distillation of fresh or dried lavender flower tops produces lavender essential oil. It is a practically colorless substance or, to be more precise, it has a mild yellow tint with a distinctive odor.

The high concentration of the two primary constituents—linalyl acetate, which ranges from 25% to 46% and R-enantiomers of linalool, which typically range from 20% to 45%—determines the oil's quality. Other compounds found in lesser ranges include lavandulol (<0.1%), lavandulyl acetate (<0.2%), camphor (>1.2%), limonene (>1.0%), eucalyptol (<2.5%), and α-terpineol (0.1–6.0). Lavender oil shouldn't include much ocimen, cineole, camphor, or terpin-4-ol due to their significant impact on aroma (Wińska et al., 2019).

Table 2 shows the main components of lavender species cultivated for commercial purposes, which make up over one percent of the oil composition. Here, we may examine in more depth how it varies among species.

Table 2. Composition of Floral Oil in Percentage for the Main Species of Lavender Grown Commercially for Oil (Lis-Balchin 2004).

	Lavandula angustifolia	***Lavandula latifolia***	***Lavandula × intermedia***
1,8-cineole	**-**	25–36%	4–10%
Borneo	**-**	0.8–4.9%	1.5–3.7%
Camphor	**-**	5.3–15.3%	6–12%
Lavandulyl acetate	0.1–14%	0.2–1.5%	0.5–3%
Linaloo	10–50%	26–44%	20–35%
Linalyl acetate	12–54%	0–1.5%	19–38%

The chemical composition is widely documented; PNISO 3515: 2002, Polish Pharmacopoeia VIII, and European Pharmacopoeia 9, both have thorough data on it.

Table 3 shows the parameters for the composition of the essential oil extracted of the blooming cymes of *L. angustifolia* for use in pharmaceuticals were set by the Ph. Eur. 10th edition. To aid in the assessment of their quality, ISO additionally lists certain attributes of lavender essential oils from different sources. Compared to the Ph. Eur, ISO 3515:2002 provides additional elements for the assessment of lavender essential oils. Additionally, ISO specifies various

permissible ranges for lavenders originating from various geographical locations. The origin has a major impact on these constraints (Pokajewicz et al., 2021).

The NF ISO publishes the international norms for *Lavandula angustifolia* Miller oil. This standard is intended for populations of lavender that are cultivated primarily in the south of France, or that are grown solely from seed. According to the NF ISO standard, lavender essential oil ought to smell "fresh and floral, reminiscent of the plant's inflorescence." (Despinasse et al., 2020).

Table 3. Comparative specifications (10th edition) for L. *angustifolia* essential oil between ISO 3515:2002 and Ph. Eur and NF ISO3515:2004 (Lis-Balchin et al., 1999 & Despinasse et al., 2020).

Components	Ph. Eur.	ISO 3515:2002 (Other Origin) [a]	NF ISO3515:2004
limonene	≤1%	≤1%	Max 0.5%
1.8-cineole [b]	≤2.5%	≤3%	-
β-phellandrene [b]	-	≤1%	Max 0.5%
***cis*-β-ocimene**	-	1-10%	4–10%
***trans*-β-ocimene**	-	0.5-6%	1.5–6.0%
3-octanone	0.1-5%	≤3%	Max 2%
camphor	≤1.2%	≤1.5%	Max 0.5%
linalool	20-45%	20-43%	25–38%
linalyl acetate	25-47%	25-47%	25–45%
terpinene-4-ol	0.1-8%	<8%	2–6%
lavandulyl acetate	min. 0.2%	≤8%	Min 2.0%
lavandulol	min. 0.1%	≤3%	Min 0.3%
α-terpineol	<2%	≤2%	Max 1.0%
eucalyptol	-	-	Max 1.0%

2.9. Medicinal Application

Since lavender has been utilized for so long, there are records of Greek and Roman cultures using the floral EO of lavender. The first mention of lavender's use dates back to the Middle Ages, when the abbess Hildegard used it between the years of 1098 and 1179 in what is now the country of Germany. Later, English herbalist and astrologer Nicolas Culpepper also mentioned lavender's use (Erland & Mahmoud, 2016).

They used it most frequently to treat dermatitis, paralysis, headaches, panic attacks, strokes, cardiac difficulties like heart attacks, influenza, lice, fleas, and mosquito bites, among other conditions. It was employed as a disinfectant even during the Second World War. As a result of the current interest in lavender and the creation of several investigations to discover its qualities, it has been possible to confirm its therapeutic, anti-inflammatory properties, antifungal, antibacterial, thymoleptic, anxiolytic, and sedative effects (Lis-Balchin, 2004 & Cavanagh & Wilkinson, 2002).

2.9.1. Antidepressive, anxiolytic and sedative properties

The European Medicines Agency (EMA) has authorized the traditional use of lavender essential oil as a natural remedy to reduce anxiety and tension. Using AE to moderate anxiety and depression has shown promising outcomes in several clinical and animal investigations. EO is widely used in herbal medicine as a stress and anxiety reliever (López et al. 2017).

Numerous research have made it clear that lavender essential oil (EO) has a major impact on the limbic system of the central nervous system. When inhaled or absorbed through the skin, the monoterpenes of lavender essential oil—linalyl acetate and linalool—have a neurological effect. Although the exact cellular mechanism of action of lavender essential oil components is still unclear, it is thought that the components influence serotonergic neurotransmission and ionotrophic γ-aminobutyric acid type A receptors (Chioca et al., 2013).

A product called Silexan®1 is currently available on the market. It is an essential oil derived from *Lavandula angustifolia* flowers and surpasses the quality standards set by Ph. Eur. Its primary active ingredients are linalool and acetate of linalyl, which has a prodrug that causes it to hydrolyze quickly to linalool *in vivo*. This medicine has shown excellent anxiolytic potential and has already received approval in Germany and several other countries where it has been used to treat patients with GAD and anxiety disorders (Müller et al. 2021).

2.9.2. Antimicrobial properties

According to Sienkiewicz et al. (2011), lavender essential oil (EO) possesses antibacterial qualities against clinical strains of bacteria obtained from patients suffering from respiratory tract infections. These infections may be caused by the gram-negative bacterium *Haemophilus influenzae*, which lavender EO treated inhibited the growth of strains resistant to amoxicycline or erythromycin. Researchers have shown that the major and minor components of this essential oil work synergistically to produce the antibacterial activity of the oil.

The essential oil obtained from *L. angustifolia* Mill. has been employed as an antiseptic against varieties of bacteria that are resistant to antibiotics, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus sp*. and challenging-to-treat fungi

illnesses, such Candida. Additionally, it has been shown that lavender essential oil works against the dysentery caused by *Shigella Flexneri* (Roller et al., 2009). Lavender essential oil (EO) possesses significant antibacterial qualities, including antiviral action against *herpes simplex* virus type 1, according to Minami et al. (2003).It has been found that EOs are significant antifungals in fungi because they prevent the growth of conidia and the formation of the germ tube.

However, lavender has been demonstrated to harm human cells in vitro, which indicates that extended exposure may induce negative effects. In vivo studies are necessary to evaluate potential side effects, as well as dose or application restrictions (Prashar et al., 2004).

2.10. Molecular markers

Recent research indicates that due to the value of lavender essential oil (EO) the market largely depends on the species and/or cultivars used, it is necessary to develop appropriate markers that can distinguish between different lavender species and their cultivars. regardless of their place of origin and environmental conditions. This happens because working with morphological markers is hampered by their dependence on environmental conditions and their inability to distinguish between cultivars and hybrids derived from genetically related species (Cavanagh & Wilkinson, 2002).

Several DNA-based molecular markers have already been employed, for instance, to carry out the genetic identification and taxonomy classification of lavender. In plant genetics and breeding studies, single nucleotide polymorphism and simple sequence repeats, or microsatellites, are the most preferred. In order to characterize a variety of crops, SSRs and SNPs offer the chance to utilize the lavender gene pool for targeted breeding and genetic conservation initiatives (Adal et al., 2015).

2.10.1. SSR (Simple Sequence Repeats) or Microsatellites

Microsatellites are 1-6 bp short tandem repeats that can be found in the eukaryotic genome, these repeats can vary the number of base pairs this make the SSR highly polymorphic. As the flanking areas are so extremely conserved, designing locus-specific primers is made easier.

SSR are among the DNA markers that are most helpful for analyzing population genetic structure, kinship, genetic resource evaluation, and molecular phylogeny. As co-dominant markers, microsatellites help to distinguish between homozygous and heterozygous individuals, and their hypervariability helps to distinguish between genotypes that are extremely similar (Karaca et al., 2013).

According to the original database, there are two different kinds of SSR markers: expressed sequence tag SSRs (EST-SSR) and genomic SSRs. The majority of SSRs in nature are genomic SSRs, which are derived from genomic libraries and typically found in non-coding areas of the genome. (Adal et al., 2015). SSR-enriched genomic DNA libraries are achieved utilizing SSR-specific primers to selectively amplify microsatellites containing genomic DNA fragments or selectively hybridizing genomic regions containing SSR. Conversely, random, or non-enriched libraries are made from cloned genomic DNA fragments at random (Senan et al., 2014).

Adal et al. (2015) reported that it has previously been demonstrated that SSRs are markers that lead to favorable results in genetic variation research, link and comparative mapping investigations, functional variation studies, and evolutionary relationship analyses of different crop species. Among other species, they have been utilized in *Salvia spp*., *Rosmarinus spp., Origanum vulgare, Triticum aestivum L., Medicago truncatula,* and *Citrus sp*.

2.10.2. SCoT (Start Codon Targeted)

The SCoT markers are commonly regarded as dominant, these markers are easy to use, reasonably priced, and repeatable. The polymerase chain reaction (PCR) is used to produce single SCoT primers, which are developed based on the short, conserved areas of plant genes that flank the ATG translation start codon. Genomic sequence information is not necessary for this process (Collard & Mackill, 2009). The fact that this method uses a single primer for both the forward and the reverse primer makes it conceptually comparable to RAPD, ISSR, or single primer amplification reactions (Agarwal et al., 2019).The technique is appropriate for most plant research facilities with conventional equipment since markers can be observed using standard gel electrophoresis with agarose gels and staining (Collard & Mackill, 2009).Numerous plants have been successfully analyzed for genetic diversity, stability, and structure, cultivar identification, quantitative trait loci (QTL) mapping, and DNA fingerprinting using this technique (Jedrzejczyk, 2020).

2.10.3. AFLP (Amplified Fragment Length Polymorphism)

The AFLP method is a high-quality fingerprinting method that generates hundreds of remarkably replicable markers from the DNA of any organism regardless of its origin or complexity, used to examine genetic diversity and evolutionary relationships in numerous species. Any DNA can be used to create patterns of DNA fragments without any prior knowledge of the sequence. AFLPs are superior or equal in terms of time and cost effectiveness, repeatability, and precision to other markers. (Mueller & Wolfenbarger, 1999).

2.10.4. ISSR (Inter-Simple Sequence Repeat)

The intersimple sequence repeat (ISSR) technique uses PCR to amplify a DNA fragment located between two identical microsatellite repeat regions running in opposite directions. ISSRs have great repeatability, which could be attributed to the application of lengthier primers. ISSRs generally segregate as dominant markers as a result of basic Mendelian inheritance (Pradeep et al., 2002). ISSR fingerprinting has been employed for genetic diversity research to identify the genomic origins of finger *Eleusine corocana*, cultivar recognition in chrysanthemum, genetic variety analysis of sorghum, maize, and species like *Pseudotsuga menzeisii* and *Ctyptomeria japonica* (Godwin et al., 1997).

2.10.5. RAPD (Random Amplified Polymorphic DNA)

RAPD molecular markers are dominant markers used in plant breeding initiatives and to map genetic resources. Its theory is based on hybridization with a complementary DNA sequence, therefore if there is no complimentary sequence, there will be no amplification and a polymorphism of band presence and absence will be noticed (Santoni et al., 2000). In this technology RAPD markers of approximately ten bp of random sequences are utilized as primers in the arbitrarily amplification of genomic DNA fragments through PCR. More complicated DNA fingerprint profiles have also been created using PCR amplification using primers of less than 10 nucleotides (Bardakci, 2001).

2.10.6. RFLP (Random fragment length polymorphism)

Most RFLP markers are co-dominant, relatively common in the genome, and arbitrarily dispersed, making them relatively polymorphic, highly locus-specific, and extremely reproducible. RFLPs have been used in genetic mapping research, as well as to identify variability in genes and phylogenetic connections in individuals. They are based on DNA sequence length variation produced randomly by restriction enzymes of genomic DNA with unique restriction enzyme recognition sites. The presence or absence of fragments of different sizes generated by certain endonucleases exposes variations in DNA sequences (Konzen et al., 2017).

2.10.7. Some molecular markers used in *Lavandula.*

Jug-Dujaković, et al. (2022). used AFLP molecular markers to examine the inter- and intracultivar diversity of local varieties of lavandin across the islands of Hvar and Vis. It enabled them to investigate the genetic variety and genetic structure of local *L. intermedia* varieties, whose contain exceptionally high genetic material adapted to the specific environmental conditions in the islands.

Talebi et al. (2022) led an investigation in *Lavandula angustifolia* Mill in which were chosen seven (ISSR) primers for PCR reactions and then calculated genetic diversity parameters such as effective allele number, Shannon index, polymorphism. These findings demonstrated a significant amount of genetic variation in the treated samples.

Gadouche et al. (2019) analyzed genetic variation across distinct populations of *Lavandula dentata* using RAPD molecular markers to create a molecular phylogeny of this plant. Furthermore, mentioned that because the environment can change the expression and function of a trait's gene, the polymorphism induced by RAPD markers could be attributed to mutations. It was discovered that it can create a wide variety of phenotypes known as genotype-environment interactions.

Additionally, Adal et al. (2015) detailed in his research the detection and description of 3,459 new EST-SSRs from Lavender *in silico*, determine 31 EST-SSRs have a high rate of cross-species transferability and polymorphism and the use of EST-SSR markers to analyze the genetic relationships between eight species and fifteen cultivars of *L. angustifolia* and *L. x intermedia.*

3. MATERIALS AND METHODS

3.1. Plant Materials

Table 4 presents the DNA that was extracted from the leaves of eight genotypes of lavender and eight cultivars of *L. angustifolia* that grown in Tihany, Hungary. The samples were obtained from MATE's gene banks.

Table 4. Samples used in this research.

Code	Name	Species
Bud	'Budakalászi' Lavender	*L. angustifolia*
Hid	'Hidcote Blue' Lavender	*L. angustifolia*
Grs	'Grosso' Lavender	*L. x intermedia*
Gpp	'Grappenhall' Lavender	*L. x intermedia*
Mai	'Maillette' Lavender	*L. angustifolia*
Spy	'Super' Lavender	*L. x intermedia*
Jud	'Judit' Lavender	*L. x intermedia*
Msd	'Munstead' Lavender	*L. angustifolia*
1/ut	cultivated in Tihany	*L. angustifolia*
2/ut	cultivated in Tihany	*L. angustifolia*
1/1	cultivated in Tihany	*L. angustifolia*
1/3	cultivated in Tihany	*L. angustifolia*
2/2	cultivated in Tihany	*L. angustifolia*
2/1	cultivated in Tihany	*L. angustifolia*
1/4	cultivated in Tihany	*L. angustifolia*
1/8	cultivated in Tihany	*L. angustifolia*

3.2. Molecular primers selected.

This study examined seven LAB primer pairs, six of which were derived from *Salvia* ESTs and one from *Stenogyne* ESTs. Table 5. shows the primer sequences and the annealing temperature that were used for PCR amplification.

Table 5. List of selected seven Cross-genera transferable EST-SSRs markers for Lamiaceae and the one selected EST-SSRs markers from the leaves (Adal et al., 2015 & Karaca et al., 2013).

Name	Sequences	Motif	Size (bp)	Tm[a] (°C)
Source*: Salvia ssp.*				
LAB008F	AGTATTTGTGCCGAGGGTTG	$[TC]_{15}$	134	55
LAB008R	ACAGGAAAGGGAGAGGGAGA			
LAB009F	GCATCAAGCCAGGTATTTTGG	$[AG]_{11}$	347	61
LAB009R	CGACGAAGGACAACAGCATAA			
LAB041F	CGCAATCCTCCCTCATAAAT	$[TC]_{19}$	249	61
LAB041R	GACCTTCTTCACGCTGGTG			
LAB042F	TCGGCACGAGGTATCAGTCT	$[TC]_{10}$	246	61
LAB042R	CCAGTTCCTCACCGACACTT			
LAB046F	GACGACGAGCAGATGGAC	$[TC]_{18}$	245	55
LAB046R	TGAGAAACAGGTGGAGTTGC			
LAB051F	GAATCCGAGTAGAGGGTGTTG	$[TACA]_5$	225	61
LAB051R	TGAAGCACATACCAAAATGG			
Source: *Stenogyne rugosa*				
LAB075F	GCACGAGGCTTAGATAGTGAT	$[TA]_{15}[AG]_{15}$	165	55
LAB075R	GTAATGGCAAGACTCCAACAC			
Source*: L. angustifolia* leaves				
LAL4F	AAGTTTCCCTCTGCCTCCTC	$[AAC]_8$	186	64- 54
LAL4R	AGAGGCCGTAGCTGTCTTCA			

Table 5. also shows the unique EST-SSRs markers that were used in this research which is derived from the L. angustifolia library of genomic DNA extracted from the leaves. Table 6. shows the seven SCoT primers used and their respective annealing temperature.

Table 6. SCoT primers used in *Lavandula* accessions (Collard & Mackill, 2009).

Name	Sequences	Tm[a] (°C)
Sc23	CACCATGGCTACCACCAG	55
Sc24	CACCATGGCTACCACCAT	55
Sc26	ACCATGGCTACCACCGTC	54
Sc27	ACCATGGCTACCACCGTG	54
Sc29	CCATGGCTACCACCGGCC	55
Sc32	CCATGGCTACCACCGCAC	56
Sc34	ACCATGGCTACCACCGCA	54

3.3. DNA extraction

The DNA was isolated from fresh lavender leaves. To speed cell lysis and avoid DNA degradation by environmental enzymes, cells were first crushed in a mortar with a pestle and liquid nitrogen. Following that, the methods indicated in the CTAB plant genomic DNA extraction kit was performed. The DNA was tested for quality and concentration using a Nano Drop-1000 spectrophotometer (Bioscience, Budapest, Hungary), and then placed into a freezer at -20°C to maintain its integrity and stability in the Department of Plant Biotechnology at MATE in Budapest. The DNA was also purified filtering the extracted DNA using the kit EZNA SP Plant DNA kit (VWR, Hungary) to eliminate impurities or contaminants that may affect PCR amplification.

3.4. PCR

PCR was performed with a standard solution that differs slightly depending on the primer used. In the case of the EST-SSRs markers, the quantities shown in Table 7 were carried out for a total volume of 15 µl and to perform the PCR with SCoT markers, the formula is also shown in Table 7 was used with a total volume of 20 µl and a variation that 1 µl of the primer was added. The solutions were then placed in pcr tubes and placed in a Swift MaxPro thermal cycler. The thermocycler was set up with each primer's optimal settings to amplify the relevant DNA fragment from genomic DNA isolated from lavender leaves. Because of the nature of the origin of the primers, the programs executed in the thermal cycler are different, for the EST-SSRs markers were touchdown PCR protocols and the programs executed to PCR amplification using SCoT primers were PCR standard.

Table 7. PCR solution for EST-SSRs markers and SCoT markers.

Component	EST-SSRs (15 µl)	SCoT (20 µl)
10x PCR buffer	1.5 µl	2 µl
dNTP	0.3 µl	0.4 µl
Primer F	0.5 µl	1 µl
Primer R	0.5 µl	
DNA	1 µl	1 µl
Dream Taq polymerase	0.03 µl	0.04 µl
H_2O	10.72 µl	15 µl
BSA	0.15 µl	0.2 µl
DMSO	0.3 µl	0.4 µl

The program executed for PCR with cross-genera transferable EST-SSRs markers began with 3 minutes of initial denaturation at 94°C, followed by 10 cycles of denaturation at 94°C for 20 seconds, annealing for 30 seconds in the first cycle set it with the temperature provided in Table 5 for each primer, lowering by 0.5 degrees Celsius in each cycle, and amplification at 72°C for 1 minute. An additional 30 PCR cycles were performed with the same cycling conditions and constant annealing temperature at 55°C or 61°C, depending on the primer. The denaturation and amplification onditions were the same as previously stated. Final reactions at 72°C for 10 minutes to finish the amplifications (Karaca et al., 2013).

The PCR mix with the LAL4 primer was amplified following the instruction of Adal et al., (2015), the PCR program was set with the initial denaturation at 95°C for 15 minutes, then 11 cycles of denaturation at 95°C for 30 seconds, annealing temperature decreased by 1°C per cycle from 64°C, and amplification at 72°C for 2 minutes. This was followed by a second round of 24 cycles of amplification with the following program: denaturation at 95°C for 30 seconds, annealing at 54°C, and extension at 72°C for 2 minutes, followed by a final extension at 72°C for 10 minutes.

The following conditions were employed for PCR amplification using SCoT primer an initial denaturation at 94°C for 3 minutes, followed by 35 cycles at 94°C for 1 minute, annealing for 1 minute taking in count the temperatures in the table 6, and extension at 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. The amplified products were preserved at 4 °C for electrophoresis analysis.

3.5. Gel Electrophoresis analysis

The amplified fragments were observed using 1% agarose gels with 1% ethidium bromide run in TBE buffer, followed by 20 minutes of electrophoresis at 115 V for the EST SSR markers and for the SCoT primers 60 min approx. The 1 kb GeneRulerTM DNA ladder was used to determine the size of the amplified DNA fragments. The banding patterns were photographed under UV light with a gel documentation system (Bio-Rad).

3.6. Data analysis

In the cases of the PCR products of 'Budakalászi' and 'Munstead' Lavender the concentration of the DNA was week, so they were not taken into account in the data analysis.

The DNA fragments amplified with EST SSR were forwarded to BIOMI laboratory (outside of MATE university) for capillary electrophoresis analysis. Following that, the electropherograms were examined using the Peak Scanner Software 1.0, which recognized peaks and assessed the size of fragments from the samples sent.

For DNA amplified using SCoT markers, one electrophoretic band at the migration point was documented as 1 (presence), while no band was recorded as 0 (absence), and a binary matrix was formed in MS office Excel for analysis of data.

3.7. Statistical analysis of Scot primers

UPGMA (Unweighted Pair Group Method with Arithmetic Averages) cluster analysis using PAST version 4.03 software was used to investigate the genetic relationship among the genotypes, this allows to obtain a UPGMA Dendrogram. By carefully analyzing the electrophoresis gel image obtained from the SCoT primers, the total number of amplified bands and the percentage of polymorphism were determined. PIC (Polymorphic Information Content) was calculated using MS office Excel, using the formula provided by Ghislain et al., (1999) where $PIC = 1 – p^2 – q^2$, where p is the band frequency, and q is no band frequency.

4. RESULTS

4.1. Electrograms for EST SSR.

The analysis allowed to notice that some EST-SSR primers did not work properly since in most cases they amplified more than one fragment, making it difficult to identify what the correct size of the allele should be. The electropherogram was saturated at the start of the analysis, the remainder of the analysis was ambiguous, and the alleles were of low quality, as illustrated in Figures 13 and 14. The only solution available for most samples where this type of primer was used is to repeat the PCR. The y-axis of EST-SSR electrograms is the peak intensity measured in relative fluorescence units (rfu), and the x-axis is the number of base pairs.

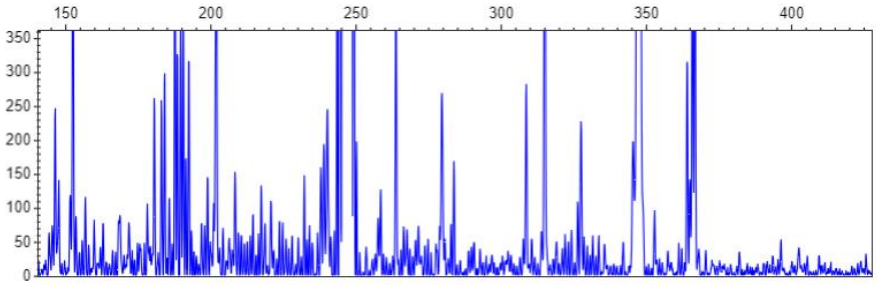

Figure 13. Electropherograms of the sample GPP using the primer LAB008.

The polymorphisms in the lavender population analyzed were identified by studying the electropherograms and detecting the varied sizes of the alleles in the samples as is showed in Table 8. According to Wooten et al (2009) fragments should not be considered alleles if their length is less than 125 bp because there is a greater likelihood of homoplasy. In this study, a peak was accepted as an allele if it was within 100 bp, as the smallest length up to 400 bp, since easily identifiable peaks were observed in this range.

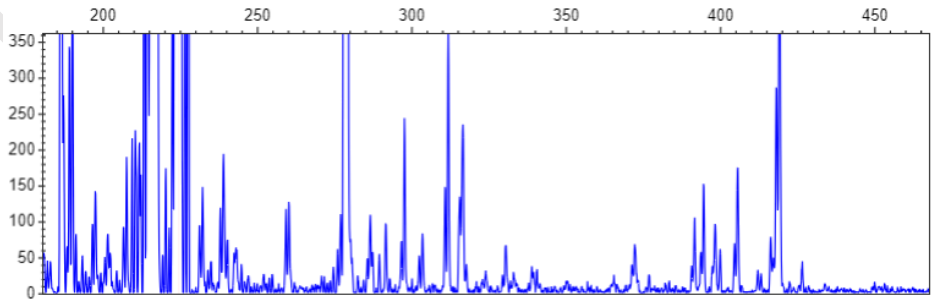

Figure 14. Electropherograms of the DNA sample 1/4 using the primer LAB009.

For the rest of the primers, some identified alleles can be called peaks as shown in Figure 15, however many alleles will go unnoticed due to the structure of the electropherogram, and those that have been identified are reported in Table 8.

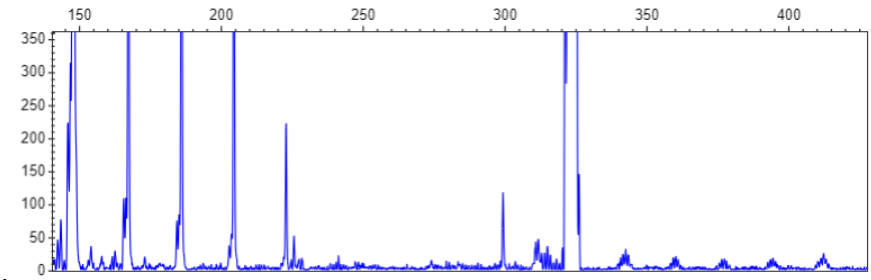

Figure 15. Fragment size of DNA sample GPP primer LAB041.

The electropherograms of the samples in which EST SSR primers got several alleles, was decided to use the information from the original research made by Karaca et al., (2013) where used this type of primer and described the original size of the alleles in that case.

The information obtained from the Peak Scanner of these microsatellites is unlikely, since several alleles were obtained, so we proceeded to select those that are of the reported size or are close. In the Table 8 it is showed that for the first primer LAB 008 the reported size of the alleles is 134 bp so the samples which reported similar values are 'Maillette', 'Grappenhall', 'Judit', 1/1, 1/4 and 2/2 which reported alleles of 130 bp. In the case of the primer LAB009, only two samples (GRS;1/1) showed alleles of 342 pp, that is close to the reported value of 347 bp. The primer LAB041 got MAI and 1/4 (245 bp) close to the reported 249 bp. The first LAB 042 showed two alleles; 240 bp and 251 bp in the samples MAI, GRS, JD, 1/1, 1/4, and the reported value is 246 bp so the alleles could be found between this range. In the case of the primer LAB046 in the sample GRS registered the sample size allele as in the other research 245 bp. The primer LAB051 on the other hand, showed up 2 alleles 122 bp and 146 bp in MAI, HID, JD, 1/1 this last one showed an extra value which is the reported in previous research 225 bp. For the primer LAB075 the original size is 165 bp and the samples that got values close to it (157 bp, 164 bp) were GRS, JD, 1/1, 1/4 and 2/2. For the last primer LAL4 got alleles of 188 bp (MAI, HID, JD, 1/4 and 2/2 and the original size is 186 bp.

The EST SSR primers amplified several alleles which are believed to be false products since the correct number should be between 1 and 2 alleles, due to this it was decided to select those that were in accordance with those previously reported by Karaca et al. (2013). However, although in some cases the results in the Table 8 could be reduced from the actual sizes, in the majority this was not possible. The data obtained with this primer were unlikely, so it was decided to work with the SCoT primers.

Table 8. Alleles found in each sample with the applied primers.

Primer / Sample	LAB008	LAB009	LAB041	LAB042	LAB046	LAB051	LAB075	LAL 4
Spy	152;156;191;220;228	176;255;278	147;157;322	106;141;143;164;171;293;307			109	
Mai	120;**130**;143;152;156;163;191;221;228;327	154;171;186;250;278	147;**245**;322	164;**240**;273;293		**122;146**	109;230	**188**;199
GPP	119;**130**;143;152;156;162;191;221;275;327	154;176;255	147;323	157;293			108;128	
HID						**122;**		114;**188**
GRS	119;143;152;156;191;221;228;275;327	150;154;176;255;278; **342**		106;118;157;164;168;**240;252**;293	100;107;110;122;137;148;160;173;178;186;208;213;**245**;267;281;294;345		109;128;152;**157**	199
JD	119;**129**;143;152;155;164;190;221;228;275;326	150;180;255;278;		141;143;118;157;164;171;**240;251**;273;291		**146;**	109;128;**157**;194	114;**188**;192;199
BUDA		150;171;186						199
MSD	163;							193
1/1	119;**130**;143;153;157;164;191;221;229;276;327	;150;154;171;186;255;278;**342**	157	105;119;126;141;148;156;164;172;187;**239;251**;275;306		**122;146;224**	128;140;152;**157;164**;194;230	
1/4	119;**129**;143;153;156;163;192;220;228;275;327	154;171;186;278	157;**245**;323	105;119;126;141;148;154;163;168;187;**239;251**;275;306			109;131;140;152;**157;164**;229	114;**188**;193
2/2	120;**130**;143;152;157;163;191;221;228;275;327	150;171;186;250:278;357					131;140;153;**165**;229	114;**188**;193
original SIZE	134	347	249	246	245	225	165	186

4.2. SCoT Gel Electrophoresis analysis

Most of the DNA samples have a high polymorphism and work with the primers tested, however samples such as BUDA or MSD were not amplified as expected during the PCR as is show in Figure 16, so their polymorphism was not confirmed with any primer.

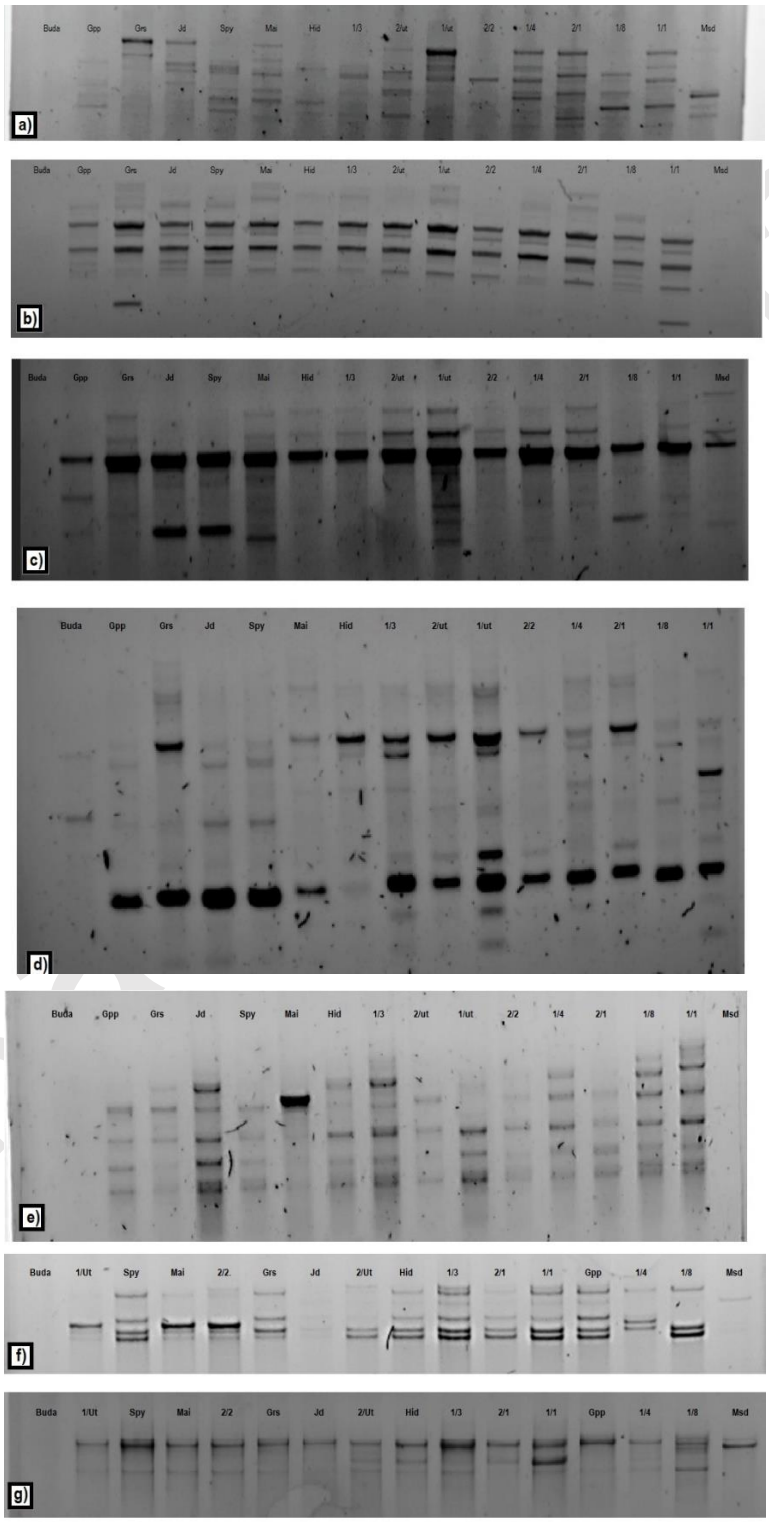

Figure 16. SCoT electrophoresis picture created in gel with several primers.

Figure 16 represent the gel images obtained with each SCoT primer a) Primer SCoT 23 b) Primer SCoT 24 c) Primer SCoT 26 d) Primer SCoT 27 e) Primer SCoT 29 f) Primer SCoT 32 g) Primer SCoT 34.

4.3. Amplification results with EST-SSR markers

14 of the 16 samples exhibited good amplification with the 7 primers utilized. Only distinct bands were considered and rated as present or absent, with the results reported in Table 9.

Table 9. SCoT amplification results of *Lavandula*

Primer	Total number of amplified bands	Number of polymorphic bands	Percentage of polymorphism (%)	Polymorphism information content (PIC)
Sc23	9	9	100%	0.46
Sc24	12	9	75%	0.29
Sc26	8	7	87.5%	0.34
Sc27	7	6	85.7%	0.31
Sc29	9	8	88.8%	0.31
Sc32	14	12	85.7%	0.39
Sc34	12	11	91.6%	0.34
Average	10.14	8.85	87.75%	0.35

SCoT primers provided DNA fingerprints with an average polymorphism of 87.75% in Lavandula cultivars. The primer SCoT 32 produced the highest number of amplified bands, 14 in total, 12 of which were polymorphic. The primers SCoT 23 and SCoT 34, on the other hand, have the highest percentages of polymorphism, with 100% and 91.6%, respectively. The average PIC value was 0.35, with 0.31 being the lowest and 0.46 being the highest.

4.4. Cluster analysis

SCoT markers were used due to their dominant nature and as each allele was recorded in a binary matrix as present (1) or absent (0) for each band that existed or not, it allowed to analyze the similarity among species using PAST version 4.03 software. All the sample tested were grouped in 4 clusters as is showed in Figure 17. Initially, each primer was used to generate the dendrogram separately. Findings were consistent across the markers. the 7 sets of data were integrated to build the dendrogram to examine the genetic diversity among the samples with the aim to use the information of all the markers to generate a better supported dendrogram.

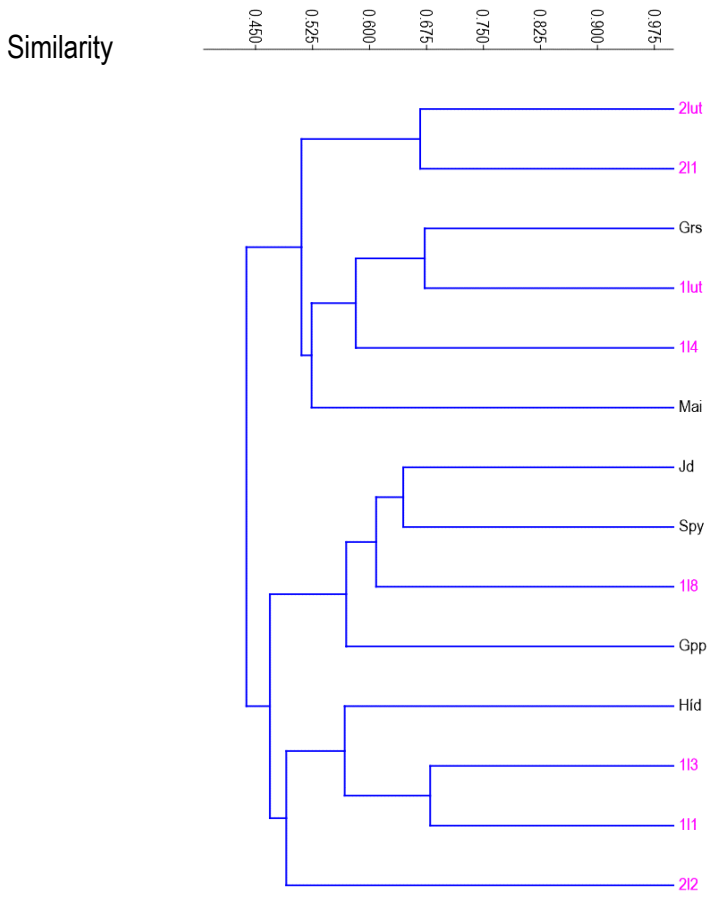

Figure 17. Hierarchical clustering in the Jaccard index of lavender samples using the SCoT primer.

This dendrogram was prepared using the Jaccard similarity index using binary score data from the SCoT primers. In Figure 17. we can see the hierarchical clustering. According to the Jaccard similarity index, genetic variability among the 14 accessions amplified using the previously mentioned SCoT primers was substantial, with Jaccard similarity distance values measuring 0.68 as the highest point. In Table 10 we can observe that the most significant similarity index was observed in the first cluster made up of 'Hidcote', 2/2, 1/3 and 1/1, between the two last samples with 68% of similarity. 'Grappenhall' Lavender, 1/8 'Super', 'Judit' form the second cluster in which the highest percentage was 64% between 'Judit' and 'Super'. In the third cluster are the samples 'Maillette' Lavender, 1/4, 1/ut and 'Grosso' Lavender. The last two samples got a 67% of similarity. Finally, the fourth cluster was between 2/1 and 2/ut with 67% of similarity.

Table 10. Hierarchical clustering in the Jaccard index of lavender samples using the SCoT primer.

	Gpp	Grs	Jd	Spy	Mai	Híd	1l3	2lut	1lut	2l2	1l4	2l1	1l8	1l1
Gpp	100%	44%	58%	58%	38%	45%	44%	41%	31%	46%	38%	44%	55%	42%
Grs	44%	100%	53%	50%	54%	48%	52%	47%	67%	41%	57%	57%	47%	53%
Jd	58%	53%	100%	64%	34%	46%	53%	34%	46%	40%	42%	37%	61%	51%
Spy	58%	50%	64%	100%	44%	52%	50%	39%	46%	47%	37%	42%	61%	48%
Mai	38%	54%	34%	44%	100%	42%	39%	50%	51%	46%	52%	49%	44%	45%
Híd	45%	48%	46%	52%	42%	100%	61%	49%	42%	51%	42%	51%	49%	52%
1l3	44%	52%	53%	50%	39%	61%	100%	44%	43%	46%	44%	49%	44%	68%
2lut	41%	47%	34%	39%	50%	49%	44%	100%	44%	47%	50%	67%	45%	40%
1lut	31%	67%	46%	46%	51%	42%	43%	44%	100%	40%	59%	54%	46%	47%
2l2	46%	41%	40%	47%	46%	51%	46%	47%	40%	100%	43%	37%	47%	50%
1l4	38%	57%	42%	37%	52%	42%	44%	50%	59%	43%	100%	58%	47%	50%
2l1	44%	57%	37%	42%	49%	51%	49%	67%	54%	37%	58%	100%	44%	53%
1l8	55%	47%	61%	61%	44%	49%	44%	45%	46%	47%	47%	44%	100%	45%
1l1	42%	53%	51%	48%	45%	52%	68%	40%	47%	50%	50%	53%	45%	100%

The principal coordinate analysis (PCoA) of SCoT markers data in Figure 18 revealed a similar representation of clusters that is consistent with the genotyping pattern in UPGMA dendrogram showed in Figure 17 and with the percentage of similarity demonstrated in Table 10. The accessions were grouped in the same 4 clusters as was explained before in the dendrogram.

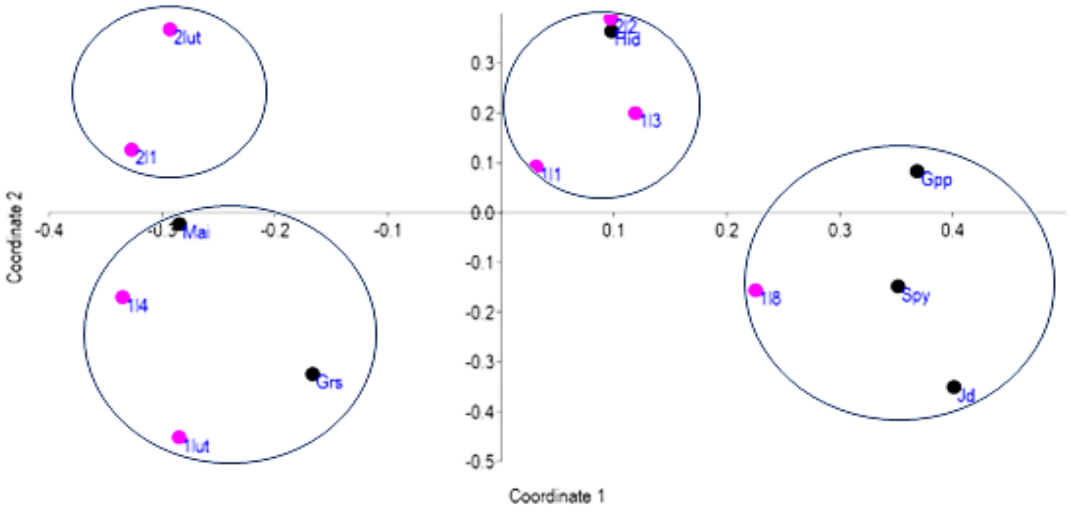

Figure 18. Principal coordinate analysis (PCoA) of all Lavender cultivars color coded based on the origin of the samples.

5. DISCUSSION

DNA-based molecular markers have been found to be the best for determining genetic variation at the DNA level, genetic diversity analysis, verify plant compounds used for medicines, and for marker-assisted breeding (Agarwal et al., 2019). However, numerous genetic markers, including as SNP, RFLP, and SCAR, have not been developed for lavender because of a scarcity of genomic and standard genome resources (Ashraf et al., 2019).

In this study two molecular markers EST-SSR and SCoT were used to study the origin of lavender cultivated in Tihany, Hungary. The quantity and pattern of polymorphic bands in a set of cultivars can be influenced not only by the primers used for PCR-amplification of DNA, but also by the genetic variety of the plant material employed for research (Saunders et al., 2001).

Seven EST-SSR cross-transferable (LAB) primers previously tested in genetic studies for the Lamiaceae family were selected. These markers were previously tested with species of *Salvia*, *Origanum* and *Thymus,* members of the Lamiaceae family, in amplification studies with successful results (Karaca et al., 2013). However, in the genus *Lavandula* it was not like that. The results obtained with these primers were not satisfactory since they amplified more than one DNA fragment, making it difficult to interpret them. Karaca et al. (2013) reported that as the evolutionary gap between species grows, the transferability of e-microsatellite markers decreases. This could have affected the results obtained here since markers derived from *Stenogyne rugosa* and *Salvia ssp* were used. As a result, it was decided to work with SCoT Primer.

SCoT markers are simple, dominant, inexpensive, and repeatable markers that have previously been utilized successfully in other genetic diversity investigations of other genera such as *Echinacea*. (Jedrzejczyk, I. 2020). It was considered appropriate to work with them and analyze the polymorphisms in the *Lavandula* samples. Because there is currently no research regarding lavender grown in Hungary or with analyzes related to lavender using SCoT primers, the percentage of lavender polymorphisms is compared with studies carried out with different molecular markers, accessions, and varieties.

With the SCoT markers, an average PIC value of 0.35 was found, which is significant than the maximum permitted 0.5 for dominant markers, also Adal et al. (2015) working with EST-SSR reported PIC values of 0.83 and 0.84 for *L. angustifolia* and *L. x intermediate*, respectively (codominant marker). This number implies that SCoT markers may supply useful information for distinguishing and characterizing the lavender types under consideration.

Using SCoT markers, genetic diversity was established across all Lavandula samples evaluated by polymorphism in a small and conserved area adjacent the ATG start codon. These

primers proved quite beneficial in assessing genetic connections and diversity among the lavender samples tested. The obtained polymorphism was 87.75% on average. Adal et al. (2015) reported 85% of polymorphism in 201 alleles studies from *L. angustifolia* and *L. x intermedia* using EST-SSRs. This was consistent with another study of molecular diversity of *Lavandula x intermedia* cultivated from the Croatian islands of Hvar and Vis, the greatest percentage of polymorphism obtained among the species studied was 76.34% using AFLP molecular analysis (Jug-Dujaković et al.,2022). And finally agrees with Gadouche et al. (2019) who assessed the genetic polymorphism in *L. dentate* lavender from littoral Algerian in which the highest percentage obtained by a RAPD molecular marker was 88.88%.

All these investigations corroborate the results obtained in this study where the percentage of polymorphism of the genus *Lavandula* is high, which could mean that there are genetic differences in specific regions of the genome between the lavender samples studied. The results demonstrate that the population of lavender grown in Tihany, Hungary is genetically diverse in relation to the SCOT markers analyzed, in other words it could mean that there is not a single genotype but several genotypes originating from several places.

In this investigation, its biggest percentage of similarity obtained between two cultivars was 68% between 1/3 and 1/1, samples which belong to the species *L. angustifolia*. Adal et al. (2015) on the other hand, found a 74% similarity between 'Folgate' and 'Sachet' two varieties of *L. angustifolia*. The two *L. angustifolia* samples exhibit a significant Jaccard index, indicating that the DNA sequences tested by SCOT primers are very similar as well. The other samples have lower index that indicate that there are notable genetic differences in a set of markers since most belong to different species or are varieties of them. The dendrogram obtained for the 8 cultivars and 6 species of *Lavandula* through the UPGMA analysis consisted of four groups where the cultivars are not grouped in one cluster, but they are mixed with the other species.

6. CONCLUSION

In the present study, eight EST-SSR molecular markers were used, seven of which were cross-genera transferable and seven SCoT markers, to investigate 16 *Lavandula* varieties cultivated in Tihany. Because the performance of the EST-SSR markers was unsatisfactory it was decided to employ exclusively SCoT markers. The SCoT markers yielded very high percentages of polymorphism with an average of 87.75%, proving that there is variation in specific regions of the genome among the lavender samples examined. This demonstrates that the lavender population cultivated in Tihany, Hungary, is genetically diverse, implying that it is not a single genotype but numerous genotypes from different locations.

7. SUMMARY

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Studying the origin of lavender cultivated in Tihany by molecular markers.

MSC. In Agricultural Biotechnology (Plant)

Lavender belongs to the Lamiaceae family, it is an aromatic plant with medicinal uses that is cultivated in arid habitats with calcareous sedimentary rocks, supports drought and has a great capacity for adaptation. It is endemic to the Mediterranean. For many centuries, lavender oil has been used to wash wounds and in their recovery. Lavender was used by Greek and Roman armies to rinse and bandage their wounds. There are about 39 species of the genus *Lavandula* around the world, several hybrids and at least 400 registered cultivars; *Lavandula angustifolia* and *Lavandula* × *intermedia* are the best-known species of this genus for its use in the extraction of essential oils due to its chemical properties. The market value of lavender oils depends directly on the species or cultivars that have been used for its extraction. Due to this, different approaches have been considered in the development of lavender genomic resources, molecular markers, comparative analysis of flower volatiles, and specific breeding and marker-assisted selection. To improve yield productivity, the application of molecular approaches and metabolic engineering is becoming increasingly popular, which has made it possible to improve other genetic qualities such as oil quality, resistance to adverse growing conditions, protein content. linalyl acetate and diseases. The objective of this research is to study the origin of lavender that has been grown in the Tihany region in Hungary, through the use of two different molecular markers such as EST-SSR and SCoT for the analysis of genetic variability and polymorphism. The DNA was extracted from the leaves of eight cultivars of lavender 'Budakalászi', 'Hidcote Blue','Grosso', 'Grappenhall', 'Maillette', 'Super', 'Judit', 'Munstead' Lavender and eight genotypes of *L. angustifolia* that grown in Tihany, Hungary. The samples were obtained from MATE's gene bank. In this seven cross-genera transferable EST-SSRs markers for Lamiaceae and the one selected EST-SSRs marker designed for *L. angustifolia* and seven SCoT primers were used on *Lavandula* accessions. In the cases of the PCR products of 'Budakalászi' and 'Munstead' the concentration of the DNA was week, so they were not taken into account in the data analysis. The EST SSR primers amplified several alleles which are believed to be false products since the correct should be maximum of two alleles,

due to this it was decided to select those allele sizes that were in accordance with those previously reported by Karaca et al. (2013). Even though in some cases the allele numbers could be reduced from the actual sizes, in the majority of the cases this was not possible. The data obtained with these markers were unreliable, so it was decided to work with the SCoT primers instead. 14 of the 16 samples exhibited good amplification with the 7 SCoT primers utilized. Only distinct bands were considered and rated as present or absent. After the data analysis the SCoT primers provided DNA fingerprints with an average polymorphism of 87.75% in lavandula cultivars. This was consistent with previous study done by Adal et al., (2015) who reported 85% of polymorphism in 201 alleles studies from *L. angustifolia* and *L. x intermedia* using EST-SSRs. And another research done by Gadouche et al., (2019) 76.34% using AFLP molecular and Jug-Dujaković et al., (2022) with RAPD molecular marker 88.88%. Two of the cultivars which belong to *L. angustifolia* samples exhibit a significant Jaccard index 0.68, indicating that the DNA sequences tested by SCOT primers are very similar as well. The other samples have lower index that indicate that there are notable genetic differences in a set of markers since most belong to different species or are varieties of them. The dendrogram obtained for the 14 genotypes of *Lavandula* through the UPGMA analysis consisted of four groups where the Tihany genotypes were not grouped in one cluster, but they were mixed with the cultivars. The average PIC value was 0.35, with 0.31 being the lowest and 0.46 being the highest. In concordance with Adal et al. (2015) who worked with EST-SSR reported PIC values of 0.83 and 0.84 for *L. angustifolia* and *L. x intermediate*, respectively (codominant marker). This number implies that SCoT markers may supply useful information for distinguishing and characterizing the lavender types under consideration. All these investigations corroborate the results obtained in this study where the percentage of polymorphism of the genus *Lavandula* is high, which could mean that there are genetic differences in specific regions of the genome between the lavender samples studied. Which demonstrates that the population of lavender grown in Tihany, Hungary is genetically diverse in relation to the SCOT markers analyzed, in other words it could mean that there is not a single genotype but several genotypes originating from several places.

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9. REFERENCES

1 Adal, A. M., Demissie, Z. A., & Mahmoud, S. S. (2015). Identification, validation, and cross-species transferability of novel Lavandula EST-SSRs. Planta, 241, 987-1004.

2 Agarwal, A., Gupta, V., Haq, S. U., Jatav, P. K., Kothari, S. L., & Kachhwaha, S. (2019). Assessment of genetic diversity in 29 rose germplasms using SCoT marker. Journal of King Saud University-Science, 31(4), 780-788.

3 Andriolli, B. V. (2014). Micropropagation of lavender (Lavandula x intermedia Emeric ex Loisel., Lamiaceae) Grosso variety for essential oil extraction.

4 Aprotosoaie, A. C., Gille, E., Trifan, A., Luca, V. S., & Miron, A. (2017). Essential oils of Lavandula genus: a systematic review of their chemistry. Phytochemistry Reviews, 16, 761-799.

5 Ashraf, A., Sultan, P., Qazi, P., & Rasool, S. (2019). Approaches for the genetic improvement of Lavender: A short review. Journal of Pharmacognosy and Phytochemistry, 8(2), 736-740.

6 Bardakci, F. (2001). Random amplified polymorphic DNA (RAPD) markers. Turkish Journal of Biology, 25(2), 185-196.

7 Benabdelkader, T., Zitouni, A., Guitton, Y., Jullien, F., Maitre, D., Casabianca, H., ... & Kameli, A. (2011). Essential oils from wild populations of Algerian Lavandula stoechas L.: composition, chemical variability, and in vitro biological properties. Chemistry & biodiversity, 8(5), 937-953.

8 Bombarda, I., Dupuy, N. J. P. D., Le Van Da, J. P., & Gaydou, E. M. (2008). Comparative chemometric analyses of geographic origins and compositions of lavandin var. Grosso essential oils by mid infrared spectroscopy and gas chromatography. Analytica Chimica Acta, 613(1), 31-39.

9 Cavanagh, H. M. A., & Wilkinson, J. M. (2002). Biological activities of lavender essential oil. Phytotherapy research, 16(4), 301-308.

10 Cantino, P. D. (1992). Evidence for a Polyphyletic Origin of the Labiatae. Annals of the Missouri Botanical Garden, 79(2), 361–379. https://doi.org/10.2307/2399774

11 Cavanagh, H. M. A., & Wilkinson, J. M. (2002). Biological activities of lavender essential oil. Phytotherapy research, 16(4), 301-308.

12 Chioca, L. R., Ferro, M. M., Baretta, I. P., Oliveira, S. M., Silva, C. R., Ferreira, J., ... & Andreatini, R. (2013). Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. Journal of ethnopharmacology, 147(2), 412-418.

13 Collard, B. C., & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant molecular biology reporter, 27, 86-93.

14 Crișan, I., Ona, A., Vârban, D., Muntean, L., Vârban, R., Stoie, A., ... & Morea, A. (2023). Current trends for lavender (lavandula angustifolia Mill.) crops and products with emphasis on essential oil quality. Plants, 12(2), 357.

15 Despinasse, Y., Moja, S., Soler, C., Jullien, F., Pasquier, B., Bessière, J. M., ... & Nicolè, F. (2020). Structure of the chemical and genetic diversity of the true lavender over its natural range. Plants, 9(12), 1640.

16 Détár, E., Németh, É. Z., Gosztola, B., Demján, I., & Pluhár, Z. (2020). Effects of variety and growth year on the essential oil properties of lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula x intermedia Emeric* ex Loisel.). Biochemical Systematics and Ecology, 90, 104020.

17 Détár, E., Németh, É. Z., & Pluhár, Z. (2020). Antioxidant capacity and total polyphenol content of Lavandula cultivars at different growing areas in Hungary. International Journal of Horticultural Science, 26, 65-69.

18 Downderry Nursery. (2018) Parts of a Lavender Available at: https://downderry-nursery.co.uk/parts-of-a-lavender/ (Accessed: 14 October 2023).

19 Erland, L. A. E., & Mahmoud, S. S. (2016). Lavender (Lavandula angustifolia) Oils. Essential Oils in Food Preservation, Flavor and Safety, 501–508.

20 Fanlo, M., Melero, R., Moré, E., & Cristóbal, R. (2009). Cultivation of aromatic, medicinal and condiment plants in Catalonia. Forest Technology Center of Catalonia, E-25280 Solsona (Spain). Pages, 22-31.

21 Gadouche, L., Saadi, A. y Zidane, A. (2019). Polimorfismo molecular en lavanda dentada del litoral argelino. Revista de Genética y Biodiversidad, 3(2), 40-48.

22 Ghislain, M., Zhang, D., Fajardo, D., Huamán, Z., & Hijmans, R. J. (1999). Marker-assisted sampling of the cultivated Andean potato Solanum phureja collection using RAPD markers. Genetic resources and crop evolution, 46, 547-555.

23 Godwin, I. D., Aitken, E. A., & Smith, L. W. (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis, 18(9), 1524-1528.

24 Grbeša, A. (2016). MORFOLOŠKA OBILJEŽJA I LJEKOVITA SVOJSTVA LAVANDE, TE MOGUĆNOSTI NJEZINA UZGOJA (Doctoral dissertation, Josip Juraj Strossmayer University of Osijek. Faculty of agriculture. DEPARTMENT FOR PLANT PROTECTION).

25 Jedrzejczyk, I. (2020). Genome size and SCoT markers as tools for identification and genetic diversity assessment in Echinacea genus. Industrial crops and products, 144, 112055.

26 JLBG. "Anouk Spanish Lavender." Juniper Level Botanic Garden, Carolina del Norte. Año de la imagen (n.d). Plant Delights Nursery

27 Jug-Dujaković, M., Ninčević Runjić, T., Grdiša, M., Liber, Z., & Šatović, Z. (2022). Intra-and Inter-Cultivar Variability of Lavandin (Lavandula× intermedia Emeric ex Loisel.) Landraces from the Island of Hvar, Croatia. Agronomy, 12(8), 1864.

28 Karaca, M., Ince, A. G., Aydin, A., & Ay, S. T. (2013). Cross-genera transferable e-microsatellite markers for 12 genera of the Lamiaceae family. Journal of the Science of Food and Agriculture, 93(8), 1869-1879.

29 Konzen, E. R., Peron, R., Ito, M. A., Brondani, G. E., & Tsai, S. M. (2017). Molecular identification of bamboo genera and species based on RAPD-RFLP markers. Silva Fennica, 51(4).

30 Lafhal, S., Vanloot, P., Bombarda, I., Kister, J., & Dupuy, N. (2016). Chemometric analysis of French lavender and lavandin essential oils by near infrared spectroscopy. Industrial Crops and Products, 80, 156-164.

31 Lafhal, S., Vanloot, P., Bombarda, I., Kister, J., & Dupuy, N. (2016). Identification of metabolomic markers of lavender and lavandin essential oils using mid-infrared spectroscopy. Vibrational Spectroscopy, 85, 79-90.

32 Lesage-Meessen, L., Bou, M., Sigoillot, J. C., Faulds, C. B., & Lomascolo, A. (2015). Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology. Applied microbiology and biotechnology, 99, 3375-3385

33 Lis-Balchin, M., & Hart, S. (1999). Studies on the mode of action of the essential oil of LavenderLavandula angustifolia P. Miller. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 13(6), 540-542.

34 Lis-Balchin, M. (Ed.). (2002). Lavender: the genus Lavandula. CRC press.

35 López, V., Nielsen, B., Solas, M., Ramírez, M. J., & Jäger, A. K. (2017). Exploring pharmacological mechanisms of lavender (Lavandula angustifolia) essential oil on central nervous system targets. Frontiers in pharmacology, 8, 280.

36 Minami, M., Kita, M., Nakaya, T., Yamamoto, T., Kuriyama, H., & Imanishi, J. (2003). The inhibitory effect of essential oils on herpes simplex virus type-1 replication in vitro. Microbiology and immunology, 47(9), 681-684.

37 Mori, H. M., Kawanami, H., Kawahata, H., & Aoki, M. (2016). Wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model. BMC complementary and alternative medicine, 16, 144.

38 Mueller, U. G., & Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. Trends in ecology & evolution, 14(10), 389-394.

39 Müller, W. E., Sillani, G., Schuwald, A., & Friedland, K. (2021). Pharmacological basis of the anxiolytic and antidepressant properties of Silexan®, an essential oil from the flowers of lavender. Neurochemistry International, 143, 104899.

40 Pistelli, L., Najar, B., Giovanelli, S., Lorenzini, L., Tavarini, S., & Angelini, L. G. (2017). Agronomic and phytochemical evaluation of lavandin and lavender cultivars cultivated in the Tyrrhenian area of Tuscany (Italy). Industrial Crops and Products, 109, 37-44.

41 Pokajewicz, K., Białoń, M., Svydenko, L., Fedin, R., & Hudz, N. (2021). Chemical composition of the essential oil of the new cultivars of Lavandula angustifolia Mill. Bred in Ukraine. Molecules, 26(18), 5681.

42 Pradeep Reddy, M., Sarla, N., & Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. euphytica, 128, 9-17.

43 Prashar, A., Locke, I. C., & Evans, C. S. (2004). Cytotoxicity of lavender oil and its major components to human skin cells. Cell proliferation, 37(3), 221-229.

44 Renaud, E. N., Charles, D. J., & Simon, J. E. (2001). Essential oil quantity and composition from 10 cultivars of organically grown lavender and lavandin. Journal of essential oil research, 13(4), 269-273.

45 Roller, S., Ernest, N., & Buckle, J. (2009). The antimicrobial activity of high-necrodane and other lavender oils on methicillin-sensitive and-resistant Staphylococcus aureus (MSSA and MRSA). The journal of alternative and complementary medicine, 15(3), 275-279.

46 Santoni, S., Faivre-Rampant, P., Prado, E., & Prat, D. (2000). Molecular markers for analysis of genetic resources and plant breeding. Cahiers Agricultures, 9(4), 311-327.

47 Salahudin, H., Kamal, S., Akhter, N., Bibi, I., Rehman, K., Akash, M. S. H., & Gohar, U. F. (2023). Lavender. In Essentials of Medicinal and Aromatic Crops (pp. 965-982). Cham: Springer International Publishing.

48 Saunders, J. A., Pedroni, M. J., Penrose, L. D., & Fist, A. J. (2001). AFLP analysis of opium poppy. Crop Science, 41(5), 1596-1601.

49 Senan, S., Kizhakayil, D., SASIKUMAR, B., & SHEEJA, T. E. (2014). Methods for development of microsatellite markers: an overview. Notulae Scientia Biologicae, 6(1), 1-13.

50 Sienkiewicz, M., Lysakowska, M., Ciecwierz, J., Denys, P., & Kowalczyk, E. (2011). Antibacterial activity of thyme and lavender essential oils. Medicinal chemistry, 7(6), 674-689.

51 Singh, A. K., Singh, J., & Sharma, S. (1989). Multivariate analysis in relation to genetic improvement in lavender, Lavandula officinalis Chaix. Plant breeding, 102(4), 302-305.

52 Stanković, I. M., Vrandečić, K., Ćosić, J., Milojević, K. N., Bulajić, A. R., & Krstić, B. B. (2014). The spreading of Alfalfa mosaic virus in lavandin in Croatia. Pesticides and Phytomedicine/Pesticidi i fitomedicina, 29(2).

53 Stanev, S. (2010). Evaluation of the stability and adaptability of the Bulgarian lavender (Lavandula angustifolia Mill.) sorts yield. Agricultural science and Technology, 2(3), 121-123.

54 Stanev, S., Zagorcheva, T., & Atanassov, I. (2016). Lavender cultivation in Bulgaria-21st century developments, breeding challenges and opportunities. Bulg. J. Agric. Sci, 22(4), 584-590.

55 Talebi, S. M., Askary, M., Amiri, R., Sangi, M. R., & Matsyura, A. (2022). Effects of nanoparticles treatments and salinity stress on the genetic structure and physiological characteristics of Lavandula angustifolia Mill. Brazilian Journal of Biology, 82.

56 Upson, T. (2002). The taxonomy of the genus Lavandula L. Lavender: the genus Lavandula. Taylor & Francis, London, 2-34.

57 Upson, T., & Andrews, S. (2004). The genus lavandula. (No Title).

58 USDA, Agricultural Research Service, National Plant Germplasm System. (2023). Germplasm Resources Information Network (GRIN Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland.

59 Van Oost, E., Leus, L., De Rybel, B., & Van Laere, K. (2021). Determination of genetic distance, genome size and chromosome numbers to support breeding in ornamental Lavandula species. Agronomy, 11(11), 2173.

60 Wells, R., Truong, F., Adal, A. M., Sarker, L. S., & Mahmoud, S. S. (2018). Lavandula essential oils: a current review of applications in medicinal, food, and cosmetic industries of lavender. Natural Product Communications, 13(10), 1934578X1801301038.

61 Wińska, K., Mączka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A., & Szumny, A. (2019). Essential oils as antimicrobial agents—myth or real alternative?. Molecules, 24(11), 2130.

62 Wooten, J. A., & Tolley-Jordan, L. R. (2009). Validation of phylogenetic signals in amplified fragment length data: testing the utility and reliability in closely related taxa. BMC Research Notes, 2(1), 1-12.

63 Yassine, E. Z., Dalila, B., Latifa, E. M., Smahan, B., Lebtar, S., Sanae, A., & Abdellah, F. (2016). Phytochemical screening, anti-inflammatory activity and acute toxicity of hydro-

ethanolic, flavonoid, tannin and mucilage extracts of Lavandula stoechas L. from Morocco. Int J Pharm Phytochem Res, 8(1), 31-37.

SOURCES FROM INTERNET

1. BIO LEVENDULA ILLÓOLAJ JUDIT. (n.d.). Retrieved from https://prezentbudapest.hu/products/bio-levendula-illoolaj-judit
2. Budakalasz.hu (2020). Egy kis Provence Budakalász közelében. Retrieved from https://www.budakalasz.hu/egy-kis-provence-budakalasz-kozeleben/
3. CityGreen.hu. (2023). Angol levendula (Lavandula x intermedia) gondozása. Retrieved from https://citygreen.hu/az-angol-levendula-lavandula-x-intermedia-gondozasa/
4. HelloVidék. (2020). Óriási biznisz a levendula: ezért érdemes belevágni egy ültetvény telepítésébe. Retrieved from https://www.hellovidek.hu/utazas/2020/06/26/oriasi-biznisz-a-levendula-ezert-erdemes-belevagni-egy-ultetveny-telepitesebe
5. Kultgarden (2023). A levendulák 8 varázslatos fajtája. Retrieved from https://kultgarden.hu/kertepites/a-levendulak-8-varazslatos-fajtaja/
6. Lab of Flowers. (2018). Lavender Mailette - Lavender Ang. var. Mailette, France, Organic. Lab of Flowers. https://labofflowers.com/store/ols/products/lavender-mailette-lavender-ang-var-mailette-france-organic
7. Legarre, P. (2020). What makes lavender “culinary”? And other questions you’ve always had about cooking with lavender. Retrieved from https://sagecreationsorganicfarm.com/what-is-culinary-lavender-cooking-with-lavender/
8. Megyeri Szabolcs Kertészete, (2013). Lavandula x intermedia Grappenhall halványkék provanszi levendula. Megyeri Szabolcs Kertészete. Retrieved from https://www.megyeriszabolcskerteszete.hu/lavandula_x_intermedia_grappenhall_halvanykek_provanszi_levendula
9. Megyeri Szabolcs Kertészete. (2023). Lavandula x intermedia ‘Judit’. Retrieved from "https://www.megyeriszabolcskerteszete.hu/nehany_tanacs_az_angol_levendula_ultetesere_gondozasara
10. Magister Products. (2012). Lavendel Dream Visage. Magister Products. Retrieved from https://www.magisterproducts.hu/lavendel-daytime-visage
11. Nichol's Garden Group Ltd. (2020). "Lavender Munstead Image". Nichols.https: Retrieved from //www.nichols.co.nz/Garden-Centre/Shop/Product-Details/Lavender/Lavender-Munstead-1L?ID=16624

12. Snowy River Lavender. (2015). Lavandin Cultivar Profile/ Super. Retrieved from http://snowyriverlavender.com.au/lavandin-cultivar-profile-super/
13. UPOV (2022) Lavandula, Lavender (Lavandula L.) (Revision); Document Code, TG/194/2(PROJ.2) ; Meeting Code, TWO/54 ; Publication Date, May 11, 2022. Retrieved 14 April 2023, from https://www.upov.int/edocs/mdocs/upov/e..

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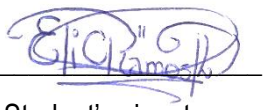

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