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Assessing the Genetic Variability of Basil *(Ocimum)* Cultivars Using ISSR Markers

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Contents

1. ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
BDM	Basil Downy Mildew
Bp	Base Pair
BSA	Bovine Serum Albumin
CAPS	Cleaved Amplified Polymorphic Sequences
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide triphosphates
EMR	Effective multiplex ratio
IRAP	Inter-Retrotransposon Amplified Polymorphism
ISSR	Inter simple sequence repeats
MAS	Marker assisted selection
Nei's GST	Nei`s genetic statistic describing differentiation of populations
NGS	Next Generation Sequencing
PCoA	Principal coordinate analysis
QTLs	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism
RFLP	Restriction fragment length polymorphism.
Rp	Resolving power
SCAR	Sequence Characterized amplified region
SCoT	Start Codon Targeted
SNP	Single Nucleotide polymorphism
SSR	Simple Sequence Repeats
STS	Sequence tagged sites
UPGMA	Unweighted pair group method with arithmetic mean.

2. INTRODUCTION

The economic importance of basil and its use in the food industry cannot be overstated, *Ocimum sp.* whose use dates back to ages ago has been exploited for different purposes. It has been used as an aromatic and medicinal plant for years. The species that make up the genus *Ocimum* differ greatly from one another in terms of appearance, growth habits, flower, leaf, and stem color, chemical content, and more (Švecová & Neugebauerová, 2010).

Avetisyan et al. (2017) estimates that there are about 50 to 150 species of basil. Most culinary basils are cultivars of *O. basilicum*, sometimes known as sweet basil. Some are cultivars of other basil species, and others are hybrids. It is particularly challenging to determine which species a basil plant belongs to. This is because basil cross-breeds easily, and drawing boundaries between species is often difficult.

There is therefore concreted need to understand the genetic polymorphism of this important plant. The conservation of genetic diversity is crucial for any species' long-term survival in ever-changing habitats. Genetic diversity is a fundamental component of biodiversity (Mills & Schwartz, 2005). This is crucial because genetic drift and inbreeding depression put dispersed populations at a risk due to the loss of allelic richness and increasing population divergence, in other words, as Frankham (2005) puts it, there could be an increase in homozygosity within populations. Therefore, for efficient management and conservation, it is crucial to understand the genetic variability and diversity among and within various populations.

Research on basil has seen an increase in many spectrums, medicinal use and essential oils being among the top researched areas. This has been achieved by different methods including but not limited to chemical, morphological, karyological, and molecular. This study will focus on the latter. Considerable discrepancies in this genus has been reported by different researchers in previous publications as will be seen in the literature review. Different varieties, cultivars all of which belong to different species in this genus have been bred for customized uses thus providing an open ground for breeders to explore (Gupta et al., 2018).

Looking into the future, the traditional role of plant breeding in the production of feed, food, fiber, and ornamental plants will continue to be important. Remembering that plants are gradually developing new functions like the ability to use plants as bioreactors to produce medications will advance. Research is ongoing to improve methods for employing plants to produce therapeutic antibodies, create antibody-mediated disease resistance, and modify plant phenotypes through immunomodulation. With the advent of NGS and the recent razzmatazz of artificial intelligence, plant breeders are learning new skills, especially in the fields of biotechnology and bioinformatics

in applications to plant breeding. Older molecular markers are advancing as new markers like SNP markers continue to be improved by technology. Tools that help plant breeders control quantitative traits more effectively will be enhanced, Lal et al., (2018) and therefore this research looks to contribute to this journey.

Working on this research was not without a challenge; extracting DNA and doing it while maintaining purity was a challenge considering the speed needed and also the quantity presented. The other pertinent issue was loading the DNAs in the gel in the quickest time possible to facilitate proper visualization, but all the challenges I learned better ways to handle them through the help of my supervisor and other PhD students.

3. OBJECTIVE

The main objective of this study was to assess the genetic variation within 26 *Ocimum* cultivars using 19 ISSR primers. The study also sought out to understand if there could be any relationship between the species clusters and their morphological characteristics. The overall hypothesis was that there was a clear delimitation between the studied cultivars either based on the species or morphological traits.

4. LITERATURE REVIEW

4.1. Taxonomy and Distribution

The center of origin of basil is in the tropical and subtropical regions of Africa, Asia and South America (Khosla, 1995). Different chemotypes and morphotypes have been reported in this species. While information about the origin of basil may be inconsistent, some facts remain. Basil belongs to the *Lamiaceae* family, *Ocimum* genus (Carović-Stanko et al., 2010). Basil has several species estimated at about 60-65 with some sources and others at around 160 species (Patel et al., 2015). The existence of several botanical variations, cultivars, and chemotypes within the species that do not notably differ in morphology complicates the taxonomy of *O. basilicum* (Vieira et al., 2003).

4.1.1. Taxonomy of Genus Ocimum

Different from other economically important herbs like *Thymus, Rosmarinus and Salvia,* in the Labiatae family, *Ocimum* comes from the *Ocimeae* tribe whose distinct attribute is the declinate stamen; in other words, rather than rising beneath the upper lip, the stamens are positioned above the corolla's lower lip The Labiatae family is typically characterized by opposite and bisect leaves that are subtly spotted and also have square stems (Paton, 1999).

According to Briquet (1897), the Ocimeae tribe has three sub-tribes, Ocimum belongs to the sub-tribe Ociminae. The Ociminae sub-tribe was later subdivided into three colloquial groups by Ryding (1992), placing Ocimum in the Ocimum group.

In this study the focus will be mostly on the cultivars of *O. basiclicum* species which is otherwise known as the sweet basil whose cultivar examples include but are not limited to; 'Large leaf', 'Osmin basil', 'Mammoth basil', 'Dark opal', 'Red rubin', 'Genovese', 'Lettuce leaf' and 'Purple ruffles'. Of all the many cultivars and hybrids of basil, sweet basil is cultivated nearly all over the world (Abbas, 2014).

4.2. Morphology

According to Prakash (1990), *Ocimum basilicum L*. is an annual aromatic herb that is dicotyledonous and diploid with 48 chromosomes. There`s a significant variation morphologically within *Ocimum* (Aghaei et al., 2012).

Basil has generally white, pink, or purple flowers that are grouped in spikes or racemes. They have a corolla with two lips, a bottom lip with three lobes, and higher lips with two lobes each. The flowers have both male and female reproductive organs, making them hermaphrodites. Bees, butterflies, and other insects pollinate them (Putievsky and Galambosi, 1999).

Their opposing, simple, ovate to lanceolate-shaped leaves have serrated edges. Normally they measure 2 to 6 cm long and 1 to 3 cm wide, and they are glossy green or purple in color. The basil fruit is a tiny, dry capsule with four chambers that contains tiny, dark-colored seeds. The leaf coloration of basil is significantly affected by the cultivar, ranging from purple, green, and even red foliage. The cultivar can also affect the plant's size, stature, and shape; some cultivars grow more upright and compact while others are larger and bushier (Hiltunen, 1999).

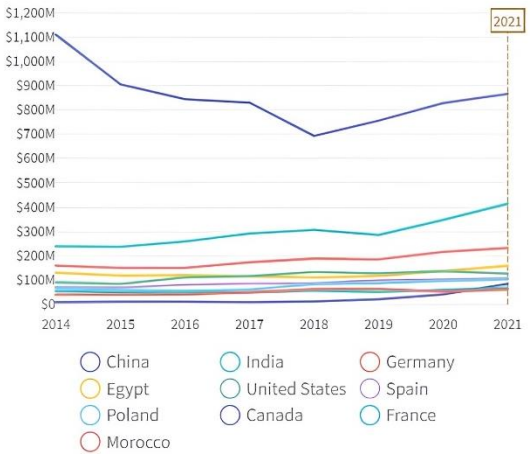
Normally, there are four stamens in the androecium, two of which connect at the corolla mouth and the other two near the base of the corolla. With the exception of *O. drdnatum,* which possesses infertile posterior stamens, all stamens are viable. Normally, the anterior stamens are free, however *Hemizygia* and *Syncolostemon* have a united anterior pair. Distinctly, the corollas of *O. campechianum* and *O. tenuiflorum* are incredibly tiny, making it challenging to compare their location of attachment to that of other species (Paton, 1999).

Looking at the gynoecium: each species' ovary is separated into four sections. These components grow into mericarps, which are single-seeded nuts. The nutlets can be elliptic or spherical, as in *O. basilicum* or *O. gratissimum* respectively. They are glabrous, however, they occasionally have pubescence, such as in *O. cufodontii.* The nutlets can create a lot of mucilage when wet, as in the case of *O. basilicum*, a little mucilage, as in the case of *O.gratissimum,* or no mucilage at all, as in the case of *O. lamiifolium* (Paton, 1999). *Hemizygia* and *Syncolostemon's* nutlets have a noticeable vein on the side that faces the calyx (Ryding, 1992).

It is laborious and unreliable to prove *Ocimum* differences based only on morphology and therefore for scientific research a proper understanding of the cultivars' genetic variation is necessary.

4.3. Economic importance of basil

With an export value of USD 3.52 billion and an import value of USD 3.39 billion as at 2021, the leading exporter of basil was China while the top importer was the United States. With an export value of USD 177.89 M, China had the highest export volume in the year 2021 to Japan as shown in the graphs in Figure 1 and 2 (Al Foah Date Company, 2023). Basil is a valuable crop that is used extensively for culinary, perfumery, ornamental, pharmacy, insecticidal purposes whether fresh or dried, cut or not, crushed or powdered (Makri & Kintzios, 2008).



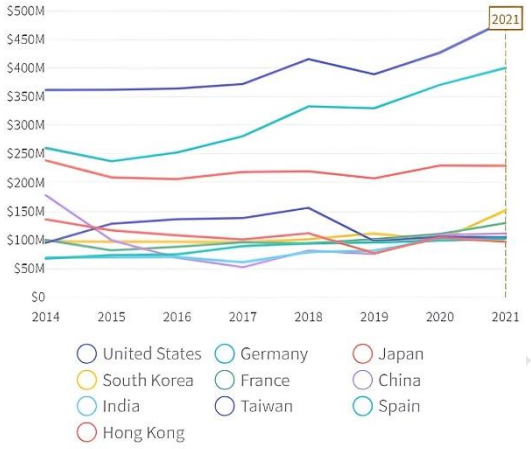


Figure 1. Top 10 basil exporters 2014-2021. **Figure 2.** ***Top 10 basil importers 2014-2021.***

(Al Foah Date Company, 2023)

4.4. Cultivation

Cultivation of basil is a vastly researched topic ranging from organic cultivation, nutrients optimization and mostly on the different factors affecting basil development in different climatic regions (Putievsky and Galambosi, 1999).

Basil grown for commercial purposes are generally propagated by seed either directly into the field or as in most cases; planted in a nursery before later transplanting. According to reports by Putievsky, (1983) basil germination in the laboratory took 4 days at the ideal day/night temperature when the humidity was over 80%. Outfield the shooting took place in Central Europe after 7–14 days and in India after 4–7 days.

Fundamentally the basil family prefer tropical climate for growth and development into horticultural maturity. It is adversely affected by frost and therefore should be grown in in frost-free conditions. Sowing and transplanting should be done when there is no risk of night frost, for open field cultivation in temperate regions, this is around early summer. In controlled environments like greenhouses, the sowing can be scheduled to align with the market (Putievsky, 1983).

Nutrient supply is a key input affecting the quality and quantity of basil. The ratio of fertilization is directly affected by the nutrient level in the soil. Experiments done on fertilizer application focused on the optimum balance of the three macro nutrients; NPK. Weichman (1948), a german researcher concluded that NPK at a ratio of 104:12:73 kg/ha gave an optimum yield of basil. Of the three macro nutrients, Nitrogen has proven to be the most significant in affecting the

yields of basil, this was later confirmed by Wahab and Hornok (1981), increasing N doses gave a considerable rise in the yield of both fresh and dry basil (Hälvä & Puukka, 1987).

According to Czabajski (1978), in an experiment done in Poland for three years N fertilization increased the yield of dry yield basil by 44%, the research also highlighted that increasing phosphorus and potassium had no significant effect on the basil yield. Interestingly, in both experiments, increasing N only increased yields to a certain level before the yield plateaued (’Shahram & ’Omid, 2011).Besides being the king of herbs, cultivating basil has its challenges, Diseases and pests being the major ones.

4.5. Diseases

There are a number of diseases that can influence basil development, yield, and desired quality. Some of these diseases include: Cercospora leaf spot; which is caused by *Cercospora ocimicola*, is characterized by circular to irregular, dark spots on leaves with bright centers. Gray Mold; is caused by *Botrytis cinereal*, dense, brown to gray fuzzy growth occurs on stems, leaves and fallen plant debris; leaves dying and dropping from plant; severe lesions on stem may cause plant death. Leaf spot caused by *Pseudomonas cichorii* and root rot caused by *Rhizoctonia solani* are also some of the common diseases of basil. Using drip irrigation and preventing water splashes on the foliage can control fungal diseases but also spraying a fungicide containing potassium bicarbonate on a weekly basis can also be done(Garibaldi et al., 1997).Basil Downy Mildew; Just like other downy mildew infections, BDM sporulates nearly exclusively on the abaxial surface of diseased leaves and characterized by dark purplish-brown sporangia (Djalali Farahani-Kofoet et al., 2014). BDM is caused by *Peronospora belbarhii*, and it is the most important basil disease in the united states. It spreads faster since the ideal cultivation conditions (warm and humid) are coincidentally also the best for air distribution of the spores. The earliest siting of the disease was in Uganda in 1932 from which it spread across the world. In Europe, it was first reported in Switzerland in the year 2001 later followed by spread into countries like Italy and Hungary like shown chronologically in Figure 3 (Wyenandt et al., 2015).

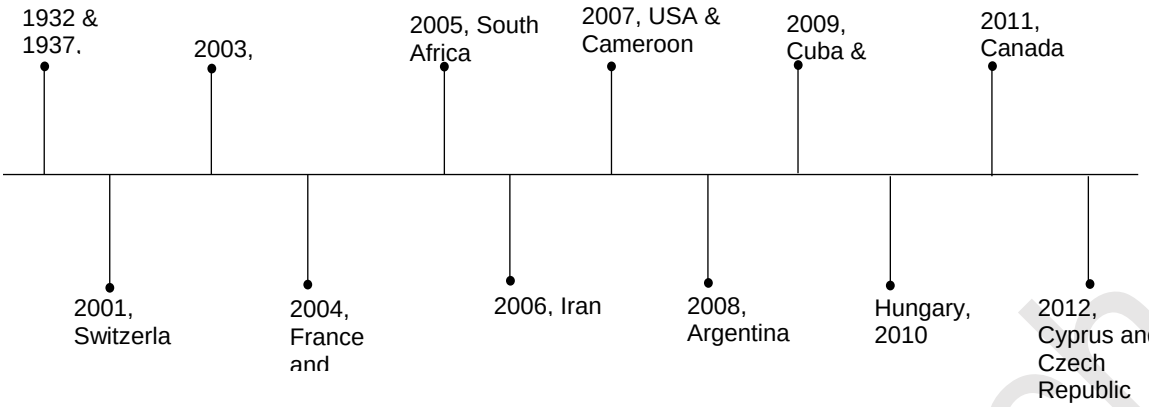


Figure 2 Chronological spread of BDM historically

4.6. Breeding of basil

Since basil has been cultivated and utilized for food and herbal purposes for thousands of years, it is challenging to pinpoint the exact date of the first breeding. However, there is proof that selective breeding of basil may have started in prehistoric societies like Egypt and India, where the herb was highly prized for its curative qualities. Through natural selection and human intervention, various basil cultivars with distinctive physical and chemical traits arose over time. Basil was first brought to Europe in the sixteenth century when it quickly gained traction in both cooking and medicine(Jailawi et al., 2021), in an effort to enhance the basil plant's flavor, scent, and therapeutic qualities, selective breeding of the plant became more organized during this time. Research on basil breeding is still ongoing today with the aim of creating new cultivars with enhanced characteristics like pest and disease resistance, yield, and drought tolerance. Modern basil breeding programs use a variety of techniques, including conventional breeding, mutant breeding, and genetic engineering.

4.6.1. Chilling tolerance

Basil's ability to withstand cold temperatures is a genetically heritable trait that can be objectively used in breeding programs (Ribeiro & Simon, 2007). After a selection program from 2002 to 2006, Ribeiro and Simon, (2007) picked two cultivars of sweet basil; 'Italian Large Leaf' (Battistini seeds, Cesena, Italy) and 'Sweet Basil' (Meyer Seeds, Baltimore, Maryland). Their mode of evaluation was water content (DW). The highest chilling tolerant lines had higher leaf water content than low tolerant lines. To achieve commercial acceptance, it was crucial to maintain the parent populations' original phenotype.

4.6.2. Disease resistance.

Caused by the fungus *Peronospora belbahrii*, Basil Downy mildew (BDM) is one of the most important diseases for crop breeders (Cohen et al., 2017). The selection criteria for BDM breeding include plant architecture, leaves morphology, and secondary metabolites. There is currently a considerable demand for genetic resistance in commercial sweet basil (*O. basilicum*), considering the ineffectiveness of available chemical remedies and how fresh basil is used.

BDM resistance was first observed in three commercial species; *O. basilicum* and *O.* × *citriodorum* which both demonstrated low levels of sporulation in response to Basil downy mildew and O. *americanum* which showed no sporulation (Wyenandt et al., 2015). However, the most promising resistance was observed from none-sweet basil which have no economic focus currently based on their phenotypic and aromatic traits (Djalali Farahani-Kofoet et al., 2012).

4.7. Basil essential oils

The perfume, pharmacy, and food industries use aromatic essential oils, extracted from the leaves and flowers of basil. Several aroma compounds can be found in chemotypes of basil such as citral, eugenol, linalool, methyl chavicol, and methyl cinnamate and are traded in the international essential oil market (Quinn et al., 1990).Research has been conducted on basil essential oil to determine its chemical make-up, biological activity and prospective applications. The following are some of the main conclusions of such studies:

Antioxidant activity; One of the aspects of essential oils that has received the most research is their antioxidant activity. A number of biochemical methods could be used to evaluate the antioxidant activity of basil however according to a study conducted by food chemists in Brazil on 24 basil genotypes. They used assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ferric reducing antioxidant power (FRAP) and ABTS+ radical scavenging. Substances like linalool and eugenol are present in basil, these are well known for scavenging free radicals mostly reactive oxygen species (ROS), and guarding against oxidative stress. Comparing eugenol to the other chemicals statistically, exhibited stronger antioxidant activity and higher mean values. All studied combinations with eugenol in them, as well as all essential oils, displayed enhanced antioxidant activity. This finding implies that eugenol's presence may have an impact on the antioxidant activities. However, this could be taken skeptically since an analysis of the synergistic and antagonistic interaction of the compounds also posed a statistically significant variation in the quantity of antioxidants (Araújo Coutoet al., 2019).

Anti-inflammatory activity: Taking a particular focus on the flavonoids and phenolic compounds as anti-inflammatories, it is evident in a number of studies that these compounds are present in basil. According to Khare (2008), the basil plant includes phenolic and flavonoid chemicals, and its green leaves are rich in essential fatty acids, vitamins, and minerals. The flavonoids, protein, phenolic compounds, glycoside, tannins, gums, saponin amino acids, steroids, mucilage, flavones, and triterpenes were all present in the aerial sections of *Ocimum basilicum* in a study conducted by Akoto et al., (2020). To understand how these compounds, act as anti-inflammatories, in vitro anti-inflammatory assay (Egg Albumen Denaturation Method) is mainly used. Inflammation and rheumatoid arthritis have been linked to the denaturation of proteins, which is widely studied. The ability of some anti-inflammatory medications to prevent thermally induced protein denaturation has been demonstrated to vary on concentration and dose. The anti-inflammatory action of a plant extract is demonstrated by its capacity to prevent the heat denaturation of a protein (egg albumin). Based on this, flavonoids and phenolic compounds found in basil have conferred anti-inflammatory activities (Mohammed et al., 2014).

Antimicrobial activity: According to research done in Thailand by Rattanachaikunsopon & Phumkhachorn, (2010), the oil exhibits potent antibacterial activity against a wide range of pathogens, including fungi like *Candida albicans* and bacteria like *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. This shows that basil essential oil may have applications as a natural antibacterial agent for the treatment of illnesses and food preservation. The research also discovered that all nine essential oils tested (the major constituents being linalool with 64.35%, 1,8-cineole with 12.28%, eugenol with 3.21%, germacrene D with 2.07%, α-terpineol with 1.64% and ρ-cymene with 1.03%) suppressed the bacterial strains to varying degrees using the swab paper disc method to test their antibacterial activity against reference and clinical strains of *Salmonella* enteritidis (which is the most important causes of foodborne gastroenteritis globally). Basil oil showed the highest antibacterial activity of any examined bacterial strain, measured by the widths of the inhibition zone.

Chemical composition; Several biotic and abiotic variables, including seasonality, temperature, water, nutrient availability, luminosity, pest attacks, illnesses, and genetic makeup, affect the content of essential oils generated by plants. Synergistic, additive, or antagonistic effects between key chemicals may also result to differences in these contents (Araújo Couto et al., 2019).

4.8. Basil Cultivars

There are various cultivars of basil, each with special qualities and applications. The most widely cultivated species of basil is sweet basil (*Ocimum basilicum*), which is used extensively in Italian cooking for its sweet, aromatic flavor. Southeast Asian cuisine uses Thai basil (*Ocimum basilicum var. thyrsiflora*), which has a licorice-like flavor. The herb tulsi, also known as holy basil or *Ocimum sanctum*, has a pungent, clove-like flavor and is utilized in traditional Indian medicine (Cohen, 2014).Other basil varieties include purple basil (*Ocimum basilicum* 'Purpurascens'), which has deep purple leaves and is used both as an ornamental plant and in cooking, and lemon basil (*Ocimum x citriodorum*), which has a powerful lemon flavor and is used in teas and sweets. If we group the basil cultivars broadly based on their characteristics we have: Sweet basil; 'Sweet', 'Genovese', 'Large leaf',' Lettuce Leaf', 'Mammoth' 'Purple basil'; 'Dark opal', 'Purple ruffles', 'Red rubin' and 'Osmin'. Cultivars with different fragrances; 'Lemon scented', 'Cinnamon', 'Anise', 'Licorice', 'Camphor', 'Spicy'. According to Growables, (2023) (which is a Florida based garden) some of the common cultivars of basil are as listed in Table 1. with their description, image and names.

Table 1. Some Basil cultivars and their characteristics (Growables, 2023)***.***

Name	Image	Description
O. basilicum 'Genoverse'		Oval, glossy, wide leaves have a thin toothed lining. Over time, the leaf's edge curls inward and takes on a cupped appearance. Between the veins, the leaf blade protrudes, giving the surface a wrinkled appearance.
Ocimum basilicum var. purpurescens 'Amethyst'		Older leaves are purplish near the base and yellowish green around the leaf margin with dark purple veins while younger leaves are predominantly glossy, dark purple. The leaves' toothed edges bend downward to make what resembles Sweet Basil's umbrella-shaped leaves.

O. basilicum 'Christmas'		It`s a hybrid created by crossing Genoese basil with Thai basil. It has the Genovese's brilliant shining green leaves, it features stems that are dark purple and blooms that are paler purple that resemble Thai basil.
O. basilicum 'Lettuce leaf'		Grows to a height of about 24". The big leaves smell like cinnamon and licorice to most nostrils. Uses for the superb flavor include salads, pestos, and sliced tomatoes.
O. basilicum 'Mrs. Burns' Lemon'		Mrs. Burns' Lemon' Grown since the 1920s and introduced by Mrs. Burns in 1939, 'Mrs. Burns' Lemon' is a large basil growing to 3' with a strong lemon aroma. Great in vinaigrettes, beverages and desserts.
O. basilicum 'Cinnamon'		Leaves have a spicy aroma as well as leaving a warm feeling on the tongue when tasted alone. Good in teas and baked goods
O. basilicum var. purpurescens 'Dark opal'		Has deep purple glossy leaves that give a liquorice type flavour, and produces cerise pink flowers.

O. basilicum 'Purple delight'		An excellent purple basil with no green showing and a strong flavor and scent. It has medium sized leaves and has stronger, more upright growth
O. basilicum 'Purple ruffles'		Is a strong, flamboyant basil grown for aesthetic purposes. Despite having a pleasant sweet cinnamon or spicy scent, the food is quite bland in flavor. This basil sometimes loses its deep purple hue and turns an ugly bronzy purple in some growing conditions.
O. basilicum 'Siam queen' or 'True Thai'		Having a pleasant spicy scent, it`s nice for gardening and cooking. This basil has purple blossoms that form a cone, rather than a spike of blossoms. Originated from Thailand.
O. basilicum 'Spice'		Popular for pestos and salads, grows with huge leaves that have crinkled-texture.
O. basilicum 'Sweet'		This common basil has wavy leaves, white blooms, and can reach a height of approximately (60cm). These basils are widely consumed because of their well-balanced cinnamon, clove, and anise smell.

4.9. Molecular markers

A molecular marker is a distinctive DNA fragment that is connected to a specific region of the genome and can be recognized using a straightforward assay that contrasts the polymorphism found in the nucleotide sequences of various specimens. The polymorphisms are often the result

of different forms of mutations like deletions, insertions, duplication, translocation(Mondini et al., 2009).

Through marker-assisted selection (MAS), DNA markers offer a huge potential to increase the effectiveness and accuracy of conventional plant breeding. Numerous recent studies done on Quantitative trait loci (QTLs) have provided plentiful marker-trait associations. (Collard & Mackill, 2008).

Figure 4. shows an overview of the most popular DNA molecular markers technologies; the markers are broadly divided as either PCR based or RFLP, ISSR is PCR based.

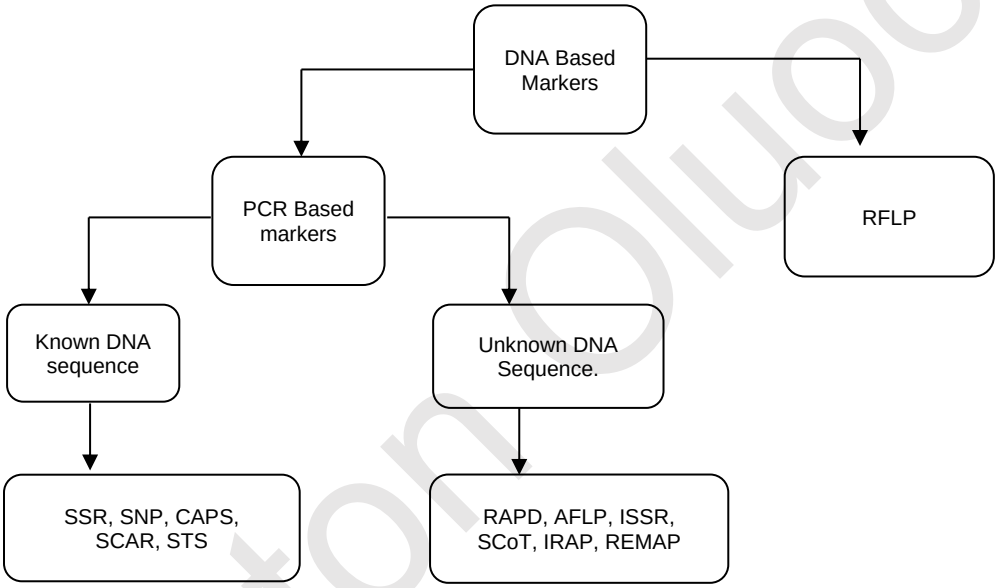


Figure 3 Classification Molecular Markers (György, 2022)

4.9.1. Restriction Fragment Length Polymorphism (RFLP) Markers

First used by Grodzikcer et al. in 1974, for 10 years, RFLP was the only DNA-based marker as it was also the first of a kind. It is also the only marker system based on hybridization. The technique entails the cutting of the DNA at the restriction sites with restriction enzymes obtained from bacteria like *E. coli.* The DNA products are then denatured and transferred using southern blotting where probe-labelling is done using either a radioactive or fluorescent compound. The products are hybridized to the blotting membrane and the results visualized. RFLP was however phased out with the introduction of PCR in 1985 which provided scientists with better alternatives. RFLP also required comparatively large amount of DNA, it needed radioactive labeling which is harmful to humans, and was also labor intensive (György, 2022).

4.9.2. Random Amplified polymorphic DNA (RAPD) Markers

Random Amplified Polymorphic DNA was developed in 1990 by William et al. RAPD works by amplifying genomic DNA in PCR using a single random primer of around 8-12 nucleotide long. The reliability of the PCR amplification and it`s product is dependent on different parameters such as the length and size of the hybridizing primer and genome, the GC content of the selected primer; i.e. 40% GC is ideal because any less GC content will have the primer melt during extension which has the temperature at 72°C. The PCR products are subsequently separated in an agarose gel and stained with ethidium bromide for visibility. By examining the presence or lack of certain bands, polymorphism at or between primer binding sites can be identified in the electrophoresis (Nadeem et al., 2017).

The advantages of using RAPD are that it doesn`t need prior knowledge of the sequence, works on any species, it`s inexpensive, quick and easy and there is no need for labeled probes. However, its drawbacks include low reproducibility, low annealing temperatures due to the short primers which makes the annealing less specific and also the PCR conditions, it is a dominant marker, and quality of DNA greatly influence the results (György, 2022).

4.9.3. Amplified Fragment Length Polymorphism (AFLP) Markers

Amplified Fragment Length Polymorphism is a highly polymorphic marker with good reproducibility and it doesn`t require any prior knowledge of the DNA sequence. AFLP is basically a combination of RAPD and RFLP where the total DNA is digested with two restriction enzymes (a frequent cutter and a rare cutter) and then the restriction half-site-specific adaptors (oligonucleotides) are ligated to all restriction fragments. This is then followed by PCR where selective amplification of some of these fragments with 2 PCR primers that have corresponding adaptor and restriction site specific sequence. AFLP results in a higher number of fragments and for a clear and reliable conclusion, visualization is mostly done with Polyacrylamide Gel Electrophoresis (PAGE) which is rather a complex process and this stands as one of the limitations of using AFLP (Nadeem et al., 2017).

4.9.4. Inter simple sequence repeats (ISSR) marker

Around 10 years after the unveiling of the polymerase chain reaction (PCR), ISSR also known as Inter Simple Sequence Repeats was reported by Zietkiewitz et al. (1994). Today ISSR has grown into an important marker that is used across different studies like genetic mapping, cultivar identification, gene tagging, genetic diversity, evolution and molecular ecology. The

technique is based on the variation of the regions between microsatellites (György, 2022).As shown in figure 5, the primers work towards the region in between the microsatellites.

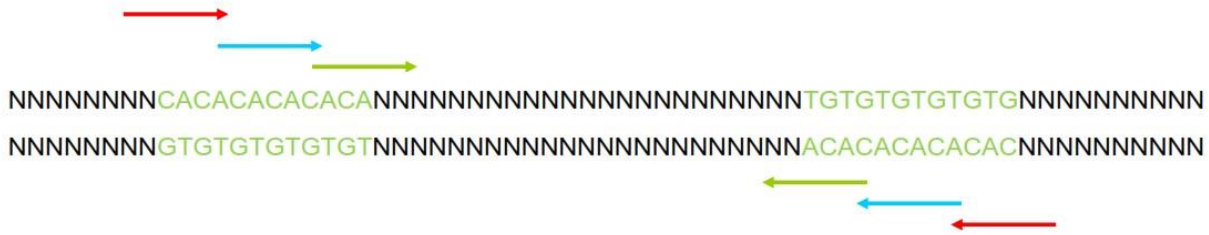


Figure 4; ISSR primers and how they act from both 5` and 3` ends

It entails using a single primer or a pair of primers based on simple sequence repeats that might be unanchored or anchored at 3' or 5' with 1-3 nucleotides to amplify genomic stretches flanked by inversely orientated and closely spaced microsatellite sequences (Wang, 2002 ,Amiteye, 2021). However according to Amiteye (2021), usually the anchored primers are ideal for PCR of ISSR because the unanchored primers may induce irregular amplification along the microsatellite region which would regress the reproducibility of the markers.

The amplification of ISSR is mostly a single-primer reaction. In contrast to many other markers reverse primers are copies of the same primer that are oriented and anneal to the template DNA in the opposite direction from forward primers, which are some of the primer's molecules (Sharafi et al., 2017).

ISSR is a dominant, multilocus marker, which in a single reaction generates multiple DNA fragments each of which is considered a locus (Wei & Tang, 2015). It is widely used in genetic diversity research because they are highly reproducible, they do not require prior knowledge of the DNA sequence they are comparatively cheap to develop (Yousefi *et al*., 2002). They target microsatellites which are universally distributed in the genome (Wei & Tan, 2015). With high annealing temperatures of ISSR, they are more reliable because this produces more specific PCR products (György, 2022).

It is imperative to understand that for a good ISSR marker analysis, high quality DNA is a prerequisite. Standardizing the amount of template DNA used in each PCR reaction is also crucial. To get reliable concentrations of amplification products, homogenous, and reproducible band intensities across samples, a consistent amount of DNA must be applied to each PCR. Following ISSR-PCR, PCR products are electrophoresed on an agarose or polyacrylamide gel and visualized. Scoring of the ISSR bands comes next, followed by data analysis (Amiteye, 2021).

4.9.5. SCoT (Start Codon Targeted marker technique)

Developed in 2009 by Collard and MacKill, the Start Codon Targeted marker technique is a novel method that was designed specifically to identify variations in the DNA of plants. It is designed based on a conserved region flanking the ATG start codon in plant genes, according to (Joshi et al.,1997) this flanking region has been precisely defined in prior research. SCoT is a dominant marker which means it only identifies a single dominant allele in a locus and cannot distinguish between homozygous and heterozygous genotypes.

It uses single 18-mer primers in the PCR which makes it have a higher annealing temperature of about 50°C. The long primers allow for more specificity of the attachment of SCoT primers compared to other random primers like RAPD. This marker is reproducible, not laborious, simple, requires no prior sequence knowledge and it`s also not time-consuming. This technique is suited for the great majority of plant research facilities with normal equipment since markers are detected by conventional gel electrophoresis with agarose gels and staining. Its application spans phylogenetics, DNA fingerprinting in different species, cultivar identification, and quantitative trait loci (QTL) mapping in important plants like sugarcane, grapes, potatoes, peanuts, and mangoes.

4.9.6. Simple Sequence Repeats (SSR)

SSR are co-dominant markers which gives a good advantage in population genetics studies (Kumar 2021). Discribed in 1989 by Litt and Lutty (Mishra et al., 2022), the technique is based on the microsatellite region of the genomes. Microsatellites are short tandem repeats of 1-6 bp long present in eukaryotic genome. The number of these tandem repeats vary and this makes SSR very polymorphic and thus ideal for individual differentiation. Serendipitously, the flanking regions are highly conserved and thus facilitating locus specific primer design. The primers used for PCR are sequence-specific. Visualization is mainly done with PAGE or capillary electrophoresis for a clear resolution because the difference of the sample might only be a few nucleotides (György, 2022). The advantages of SSR are; small quantities of DNA can be used (10-100 ng), it is abundant in the genome, High polymorphism, amenable to automation and wide range of application.

4.9.7. Single Nucleotide Polymorphism (SNP)

Being the most abundant form of genetic variation, SNPs are ideal for polymorphism studies within populations. Compared to mutation, genetic variation is classified as SNPs only if it occurs in at least 1% of the population. Knowledge of the whole genome sequence is the principle prerequisite for building these markers. The identification of SNPs can be done *in vitro*

or as is mostly the case *in silico* from NGS generated transcripts which are clustered and assembled for analysis. A comparative analysis of *de novo* sequencing of holy basil and Sweet basil revealed that there are 6,565 SNPs with 66.16% being transitions and 33.84% transversions (Rastogi et al., 2014). The *in-silico* method is cheaper as it makes use of already existing data mined from open-source EST databases like dbEST of NCBI or HarvEST. It is amenable to technology, co-dominant and bi-allelic (György, 2022).

Numerous allelic discrimination and detection platforms have been used to produce a variety of SNP genotyping approaches. The simplest and most straightforward approach among them is RLFP (SNP-RFLP), while the CAPS marker technique can also be used for SNP discovery (Nadeem et al., 2017).

4.9.8. Cleaved Amplified Polymorphic Sequences (CAPs)

Also known as “PCR-RFLP” because it uses endonuclease just like in RFLP however it differs from RFLP in the sense that the endonuclease is used to cleave only a DNA fragment but in RFLP it`s used in the whole genome. One of the main advantages of CAPs is high reproducibility and co-dominance (György, 2022). CAPs were first used to map genes to the Arabidopsis genome in 1993 by Ausubel and Konieczny. CAPs primers are built using DNA sequence obtained from genomics databases, cloned cDNA or cloned RAPD bands. Being a versatile marker, CAPs efficiency to identify polymorphism can be augmented by using it together with SCAR, AFLP or RAPD (Nadeem et al., 2017).

4.9.9. Application of Different Markers

In the Study of Ocimum basilicum Labra et al. (2004) did an integrated comparative analysis of the variation in Ocimum basillicum L. cultivars using AFLP assay, essential oil composition and morphological characterization. They used 9 basil cultivars which are mainly used for ornamental and culinary purposes in the Mediterranean area. The AFLP assay used was as described by (Vos et al., 1995) with a minor customization of the DNA content. The biggest finding of this research was that genomic congruence does not always mean same or different products such as oil composition and agronomic importance. A good example was between ‘Basilico a foglia fine’ and ‘Basilico a foglia lattuga’ cultivars which are similar genetically but have different essential oil composition. Therefore, AFLP derived UPGMA dendrogram which was the primary statistics tool used in this analysis could only be used for genera taxonomic studies. The study also concluded that using DNA genotyping, accessions can be categorized regardless of environmental factors and plant growth stages. Essential oil characterization and morphological descriptions may only provide primary classification but not hints for varietal classification.

In a study done by Giachino et al. (2014) genetic variation among 14 basil accessions in Turkey was expansively inspected with RAPD markers and molecular characterization. The accessions were selected based on methods used in pharmaceutical, perfumery and spice industry. The study also objectively evaluated the correlation between the genetic variability and essential oil of the cultivars. The RAPD-PCR assay was performed as instructed by (Williams et al., 1990) In the RAPD marker analysis they evaluated nearly one-third of the 71 primers used. The study believed this was consistent with previous studies done like De Masi et al. (2006) whom out of 20 RAPD primers used, he evaluated 7. The analysis took into consideration both the monomorphic and polymorphic band ratios with more emphasis on the latter. Euclidian distance and UPGMA methods were used in this study as in other literatures that compared genetic variation and essential oils in evaluating basil. The genetic and Euclidian distance matrices had a low correlation coefficient ($r = 0.40$), but it was still significant ($p = 0.0015$). According to this study, there was no clear evidence of a match between the RAPD analysis-obtained clusters and the essential oil chemotypes, and genotypes located in the same molecular cluster are usually always associated with the same chemotype. Literature produced by a DNA fingerprinting study done in India by Gupta et al. (2021) using ISSR and SCoT markers on 36 genotypes of *Ocimum* revealed that there were no significant relationship observed between the genetic distribution and geographical origin of the *Ocimum* accessions. The analysis was done by PCA analysis that showed a huge variation within the population. The ISSR analysis factored in the percentage polymorphic band (PPB) which was 0.8. PIC which ranged between 0.21-0.095, resolving power (Rp) which was between 4.6 to 10.8, the EMR and the marker index.For diversity analysis UPGMA approach was used to generate a dendrogram which clustered the accessions in two clusters. To assess the clustering pattern efficiency as depicted by UPGMA, PCoA and Mantel test were used. A substantial positive association between the genetic distance matrices generated by the SCoT and ISSR markers method was validated by the Mantel test.

5. MATERIALS AND METHODS

5.1. Material

5.1.1. Plant Material

Leaves of 26 cultivars of *Ocimum spp*. were used as a source of DNA.

Table 2. The accession used in course of this study.

Sample code	Accession	Species
V1	'Purple Ruffles'	*Ocimum basilicum*
V2	'Red Rubin'	*Ocimum basilicum*
V3	'Rubin Kaira' (Stavia)	*Ocimum basilicum*
V4	'Fine Verde Compacto'	*Ocimum basilicum*
V20	'Penang Lemon' (Pinang)	*O. africanum*
17	'Tulsi Temperate'	*O. sanctum/tenuiflorum/americanum*
18	'Rama Tulsi'	*O. sanctum/tenuiflorum*
V11	'K/Cardinal'	*Ocimum basilicum*
V13	'Feleny'	*Ocimum basilicum*
V14	'Red Boza'	*Ocimum basilicum*
V16	'Christmas'	*Ocimum basilicum*
21	'Pepper scented'	*O. selloi??*
23	'Barhat'	*Ocimum basilicum*
25	'Tulsi Vana'	*O. gratissimum*
S8	'Vietnamise'	*Ocimum basilicum*
V7	'Aromatto'	*Ocimum basilicum*
V10	'Corsican'	*Ocimum basilicum*
45	'Anisse'	*Ocimum basilicum*
46	'O.b. mrihani'	*Ocimum basilicum*
47	'Clove scent'	*Ocimum basilicum*
48	O.b. 'licorice scent'	*Ocimum basilicum*
49	O. citronum 'lemon basil'	*O. americanum*
15	'Mrihani'	*Ocimum basilicum*
16	'African'	*O. gratissimum??/africanum???*
V29	'A Palla verde Seuza'	*Ocimum basilicum*
54	'Vana Tulsi'	*Ocimum Sanctum*

The Fresh plant material as shown in Table 2, were derived from the gene banks of MATE, Institute of Horticulture, Department of Medicinal and Aromatic Plants and Center for Ecological Research, Lendület Seed Ecology Research Group.

5.1.2. DNA Extraction

The fresh basil leaves were ground using pestle and motor in liquid nitrogen ready for further processing. For a refined DNA extract, the NucleoSpin Plant II DNA kit (Macherey Nagel, Germany) was used for DNA Extraction, this was then followed by assessing the DNA concentration quality using the Nano Drop ND-1000 spectrophotometer (Bioscience, Budapest, Hungary). The isolated DNA was stored in a refrigerator at -20°C at the Buda Campus, Hungarian University of Agriculture and Life Sciences, institute of genetics and Biotechnology.

5.1.3. ISSR primers

The primers set used were from the University of British Columbia. A total of 19 ISSR primers were used to evaluate the genetic variability in *Ocimum spp* cultivars as shown in Table 3. There were variable selective nucleotides in the primers like in the case of primer 807, 809, 817, 818, 823, 825 and 826 which had 1 selective nucleotide. Primers 835, 840 and 841 had 2 selective nucleotides. Some of the primers also had consensus nucleotides like in the case of 840, 888 and 889. There was also a unique pentanucleotide microsatellite in ISSR 881.

Table 3. Sequence of the ISSR primers used in this study

ISSR	Sequence	ISSR	Sequence
807	AGA GAG AGA GAG AGA GT	841	GAG AGA GAG AGA GAG AYC
809	AGA GAG AGA GAG AGA GG	857	ACA CAC ACA CAC ACA CYG
817	CAC ACA CAC ACA CAC AA	861	ACC ACC ACC ACC ACC ACC
818	CAC ACA CAC ACA CAC AG	873	GAC AGA CAG ACA GAC A
823	TCT CTC TCT CTC TCT CC	881	GGG TGG GGT GGG GTG
825	ACA CAC ACA CAC ACA CT	885	BHB GAG AGA GAG AGA GA
826	ACA CAC ACA CAC ACA CC	887	DVD TCT CTC TCT CTC CT
827	ACA CAC ACA CAC ACA CG	888	BDB CAC ACA CAC ACA CA
835	AGA GAG AGA GAG AGA GYC	889	DBD ACA CAC ACA CAC AC
840	GAG AGA GAG AGA GAG AYT		

5.2. Methods

5.2.1. Polymerase Chain Reaction (PCR)

The samples were filled to a total volume of 20 µl for PCR thermocycling where a Swift MaxPro thermocycler (Esco Healthcare Pte, Singapore) was used. The following ingredients were used in the PCR mixture: 10x PCR buffer, 0.2 mM dNTP mixture, 0.2 units of DreamTaq polymerase, 1 mM of primer, 1% BSA, 2% DMSO, and sterile distilled water. 20–80 ng of template DNA was used in each reaction. A standard PCR cycle was employed, consisting of a 3 min initial denaturation step at 94 °C, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, and a final extension step at 72 °C for 5 min.

5.2.2. Gel Electrophoresis

For separation, all PCR amplification products were electrophoresed on 1% agarose gels that were stained using 1% ethidium bromide (the staining was done in a laminar flow due to the hazardous nature of ethidium bromide). This was run in Tris-borate extract (TBE) solution for 30-60 minutes at 120 volts.

Using a 1 kb bp DNA ladder as a reference, we evaluated the quality and size of the DNA fragments. We visualized the DNA bands under UV light thanks to the Gel documentation approach, each fragment was documented as either present (1) or absent (0) in a binary matrix table ready for data analysis.

5.2.3. Statistical analysis

Data cleaning and analysis was done in MS office Excel 2019 and the results were prepared for further processing. This MS Excel software was used to calculate the Polymorphic information content (PIC) using the formula $PIC_i = 2f_i(1 - f_i)$; where PIC_i is the polymorphic information content of marker "$_i$", f_i is frequency of the amplified allele (band present), and $(1 - f_i)$ = frequency of the null allele (band absent) according to (Roldán-Ruiz et al., 2000). It was important to note that the maximum accepted value of PIC for dominant markers is 0.5. Allelic frequency and the percentage polymorphism were also calculated. The Scientific data analysis software; PAST version 4.03 was used for cluster analysis to illustrate the dendrogram.

6. RESULTS

6.1. The amplification of ISSR markers

Of the 26 samples, 18 showed a good amplification using 11 primers out of the 19 that were used. Only clear distinguishable bands were scored and evaluated as either present or absent and the results summarized in the Table 4.

Table 4. ISSR amplification results of amplification of Ocimum spp.

Primer	Total amplified	Polymorphic	%Polymorphic	PIC
823	10	10	100	0.285185
827	6	5	83.33333	0.347737
840	6	6	100	0.419753
807	10	10	100	0.346914
818	5	5	100	0.285185
825	4	4	100	0.285185
809	3	3	100	0.285185
873	6	6	100	0.285185
881	5	3	60	0.285185
887	5	5	100	0.352066
888	11	11	100	0.376674
Average	**6.454545**	**6.181818**	**94.84848**	**0.323114**

In summary, the ISSR primers produced DNA fingerprints in *Ocimum* cultivars with an average polymorphism of 94.85% where only two of the evaluated primers did not show polymorphism. ISSR 888 generated the highest number of bands with the average count of bands per primer being 6. The average PIC value was 0.32 and this ranged from 0.285 to 0.419. The typical range of the band sizes was between 250 bp to 2500 bp measured against the standard ladder which ranged from 250 bp to 10000 bp shown in Figure 6.

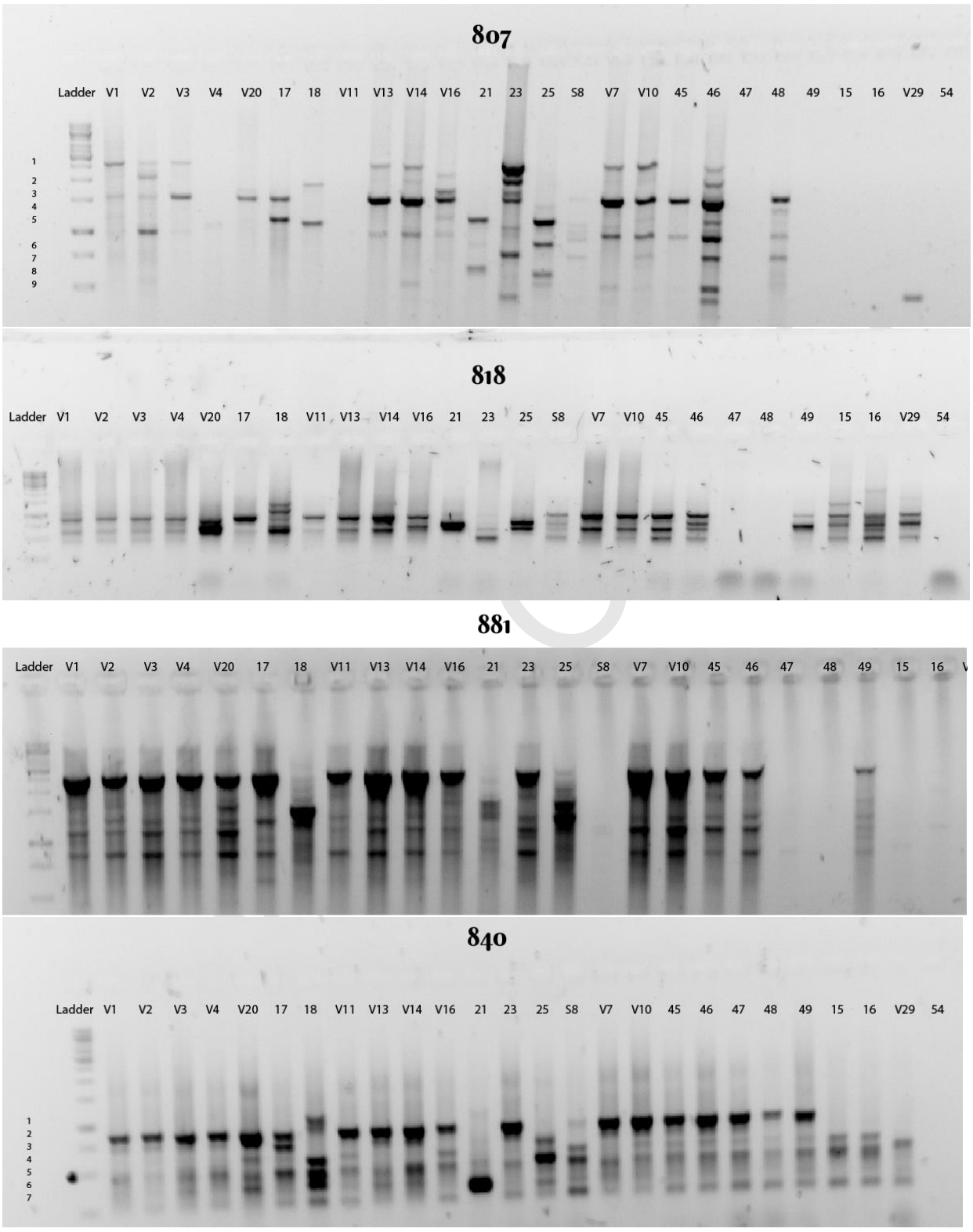


Figure 5. ISSR profiles produced with different primers in gel photos. To the left is 1k bp DNA ladder.

ISSR 807 showed a comparatively high polymorphism and DNA samples of 'Vanna tulsi' (54) and 'Mrihani' (15) did not attach for amplification in most of the primers.

6.2. Cluster analysis

Hierarchical clustering was produced by calculating the Jaccard similarity index between the accessions using the binary scoring data from the ISSR primers. There were two rounds of analysis because the first analysis was stretched with outliers as shown in Figure 7. and therefore, prompting cleaning of the data further to obtain a clear representation as shown in Figure 8.

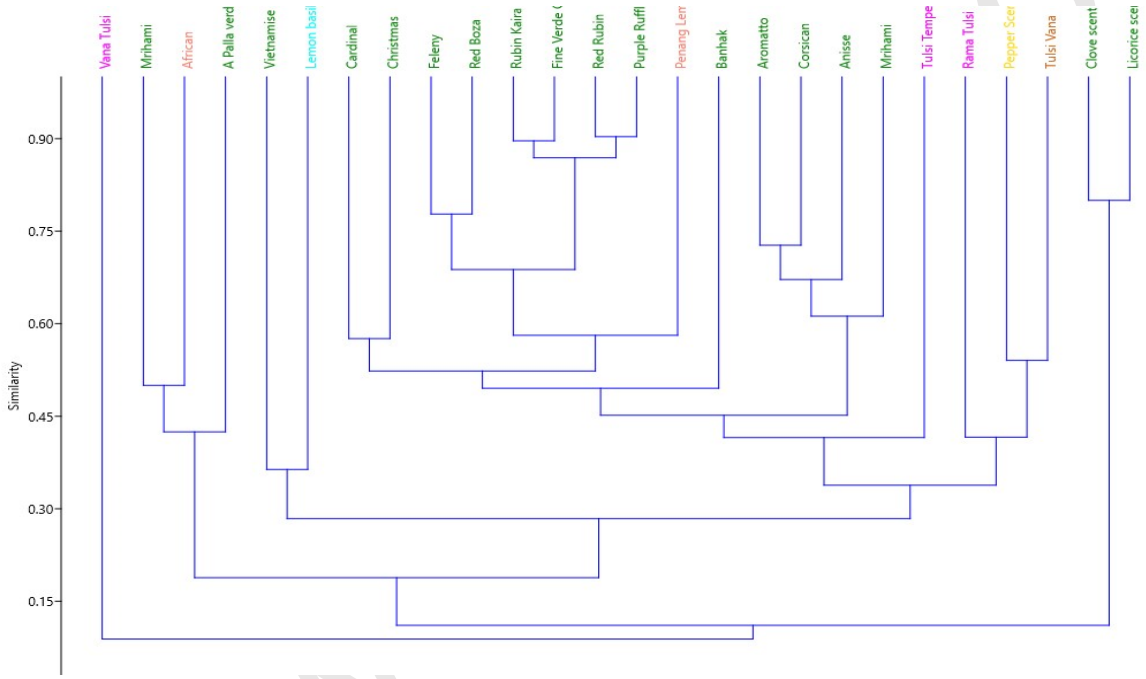


Figure 6. UPGMA Dendrogram of all samples of Ocimum spp. culivars colour coded based on species. Green: Ocimum basilicum, Purple: O. americanum Blue: O. selloi Red: O. gratissimum Pink: O. sanctum/tenuiflorum/americanum

According to the Jaccard similarity index, the genetic variation between the 18 accessions amplified by the ISSR primers aforesaid was high with the Jaccard similarity distance values ranging between 0.289 to 0.897 as shown in Annex 1. The maximum similarity index (0.897) was observed between 'Red Rubin' (*O. basiclicum)* and 'Purple Ruffles' (*O. basiclicum)* while 'Corsican' (*O. basiclicum)* and 'Rama Tulsi' (*O. sanctum/ tenuiflorum)* showed the smallest index (0.289). This then meant that 'Red Rubin' and 'Purple Ruffles' were comparatively closely related while 'Corsican' and 'Rama Tulsi' cultivars were far relatives.

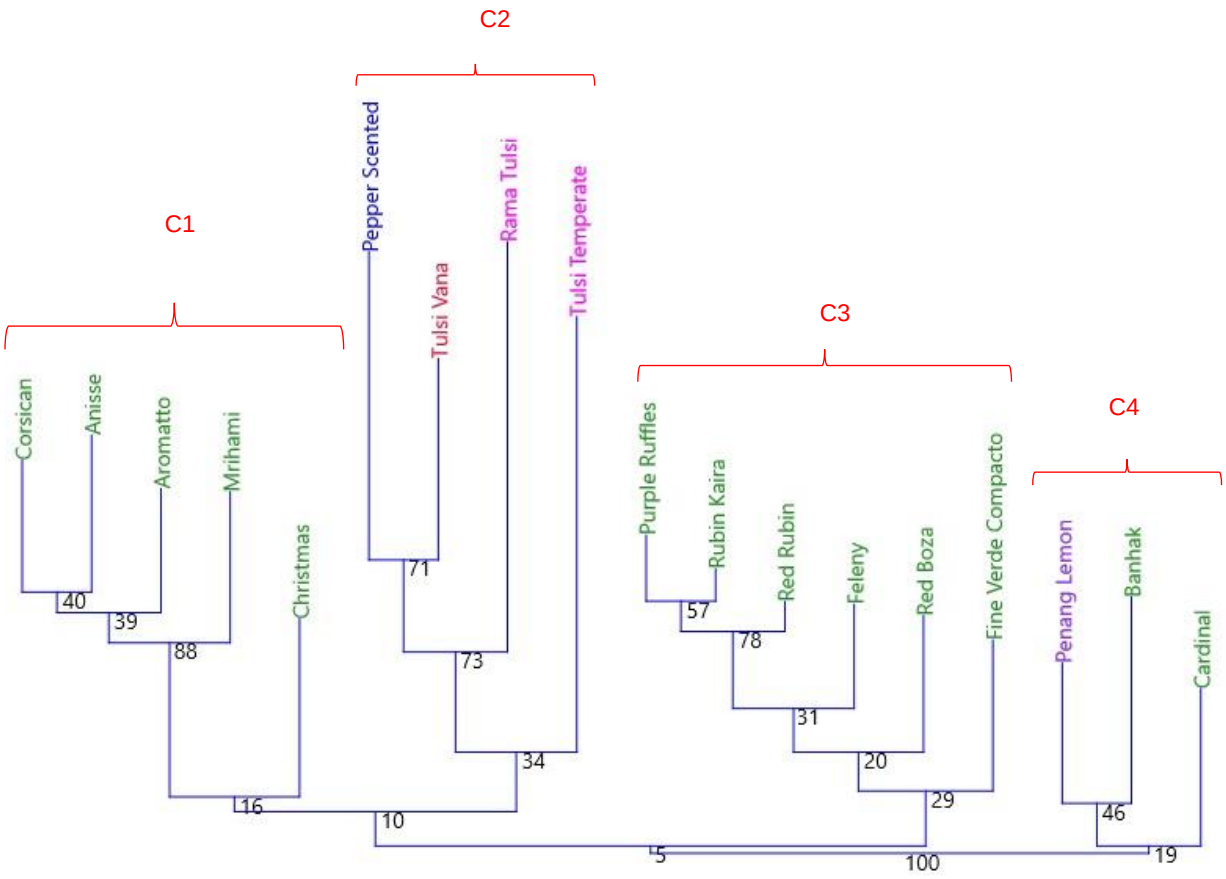


Figure 7. UPGMA Dendrogram of filtered Ocimum spp. culivars colour coded based on species. Green: Ocimum basilicum, Purple: O. americanum Blue: O. selloi Red: O. gratissimum Pink: O. sanctum/tenuiflorum/americanum. C stands for Cluster.

At an approximate similarity level of 0.425 the dendrogram was partitioned into 4 clusters; the 1st one had 'Corsican', 'Anisse', 'Aromatto', 'Mrihami', 'Christmas' all of which are *O. basilicum* the 2nd cluster had 'Pepper Scented' (*O. Selloi*) , 'Tulsi Vana' (*O. gratissium*), 'Rama tulsi', 'Tulsi temperate' *(Ocimum Sanctum)*, the 3rd cluster which was the largest, grouped purely *O. basilicum* i.e. 'Purple Ruffles', 'Rubin Kaira', 'Red rubin', 'Feleny', 'Red Boza', 'Fine Verde compacto' and the 4th Cluster had 'Penang lemon', 'Barhat' and 'Cardinal'.

Now it was clear contrasting both dendrograms, Figure 9 which had outliers was more haphazard compared to Figure 8 which had the outliers filtered, we could observe subtle clustering.

6.3. Principal coordinate analysis (PCoA)

The PCoA was also done in two rounds with the first being with all samples as shown in Figure 9. and a later refined analysis as in Figure 10. which was used for discussion. The PCoA analysis showed a largely congruent visualization of clusters as depicted in the earlier analysis on the UPGMA dendrogram and also the Jaccard similarity indices in Annex 1. The accessions were clustered in the same groupings as explained in the dendrogram save for 'Tulsi temperate' and 'Tulsi verna' which were clustered separately in PCoA but were under the same branch in the UPGMA dendrogram.

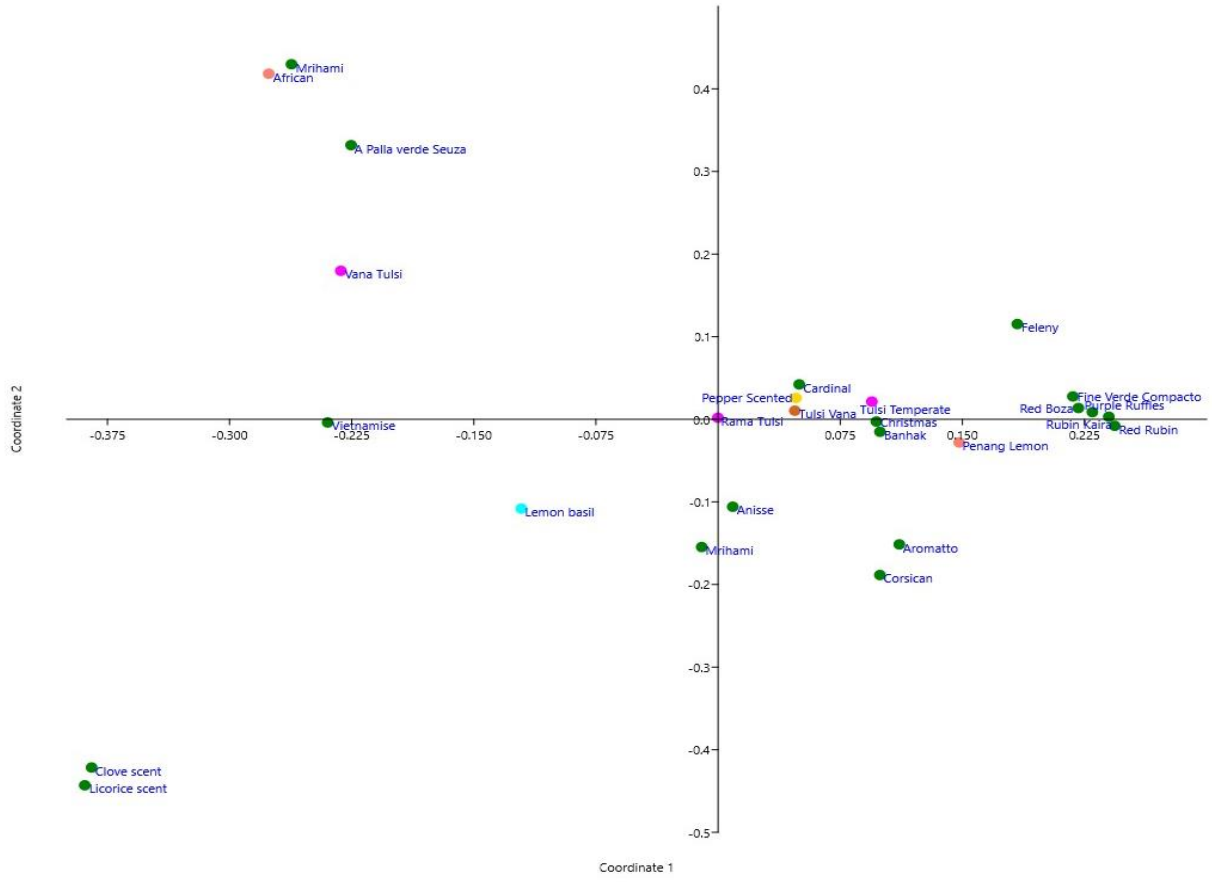


Figure 8. Clusters of all Ocimum spp. culivars colour coded based on species. Green: Ocimum basilicum, Purple: O. americanum Blue: O. selloi Red: O. gratissimum Pink: O. sanctum/tenuiflorum/americanum

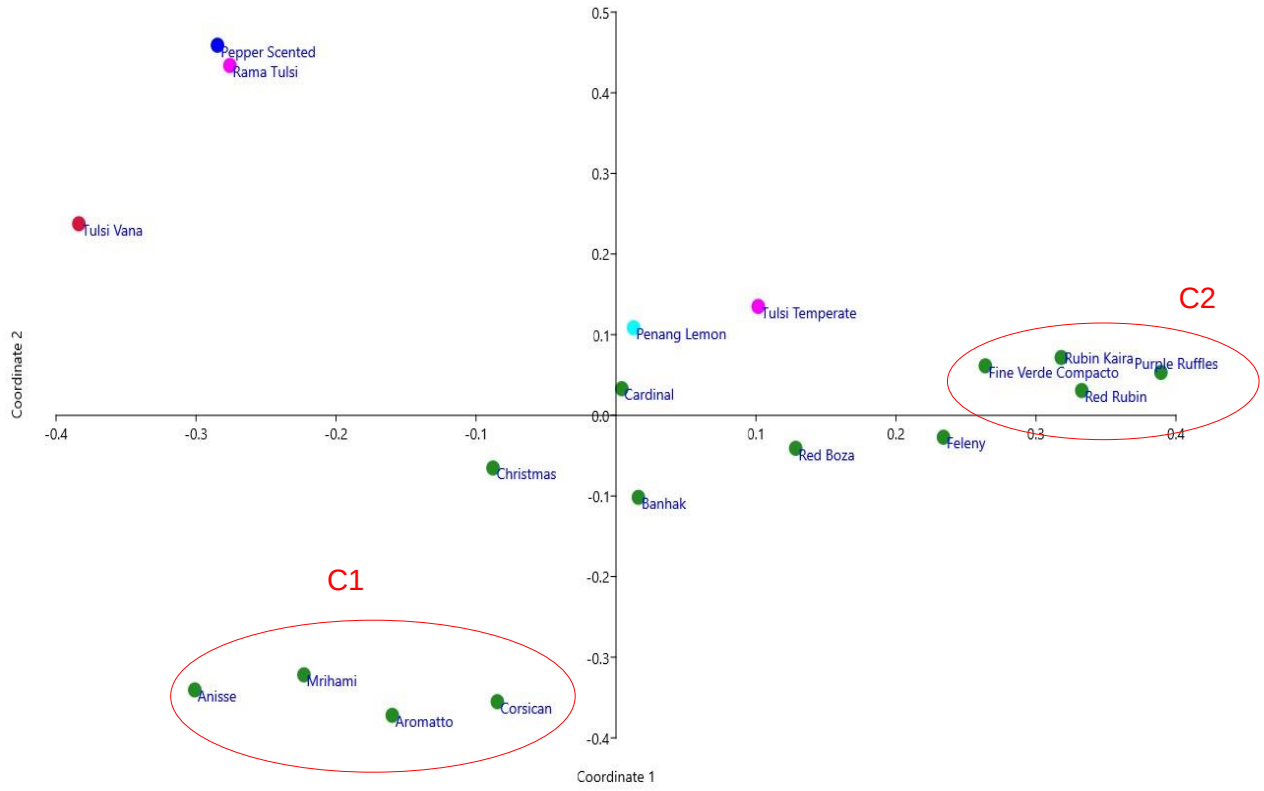


Figure 9 Clusters of selected Ocimum spp. culivars colour coded based on species. Green: Ocimum basilicum, Purple: O. americanum Blue: O. selloi Red: O. gratissimum Pink: O. sanctum/tenuiflorum/americanum. C1 is Cluster 1 which is made of O. basilicum and C2 is Cluster 2; made of O. basilicum too.

8. DISCUSSION

In all of the 3 deployed analysis; UPGMA dendrogram, Jaccard similarity index and PCoA clustering, the results were largely in harmony except for a few differences that were not really significant for this study.

There were 2 PCoA clusters of *Ocimum basilicum* between 0.25 and 0.4 (C1) and 0.3 and 0.4 (C2) of coordinate 1 (x) and coordinate 2(y) respectively. Other *Ocimum basilicum* cultivars were spread closely across the graph. 'Paper scented' (*Ocimum Selloi),* 'Penang lemon' (*Ocimum africanum*), 'Vana Tulsi' (*Ocimum sanctum*) and 'Rama tulsi' (*O. sanctum/tenuiflorum)* were distinctly apart but these were only individual species samples whose occurrence would not be averaged. It is important to note that for the *Ocimum sanctum* species, their occurrence were far apart and could not be clustered as one. It was clear that using the selected ISSR markers, the genetic relationship between the selected cultivars could be clustered however this needs a large sample of data and a high number of replicates just to be sure of the occurrence as there were slight overlaps between the species and also no distinct clustering of same species. This finding was in part consistent with other literatures like Chen et al. (2013) who used 36 ISSR markers plus RAPD and SRAP markers to fingerprint 37 *Ocimum* accessions.

The high polymorphism witnessed in these results are consistent with other previous studies like in *Ocimum kilimandscharicum* and *Ocimum basilicum* (Dhawan et al., 2016) and also in DNA fingerprinting of *Vetiveria zizanioides* (Dhawan et al., 2018)

The results obtained from molecular marker analysis of related cultivars is subject to many factors like the type of primers used, the subjectivity of gel scoring and the genetic differences within the DNA samples being examined (Saunders et al., 2001). Genetic variation between related cultivars and specifically in the case of *Ocimum* genus are difficult to attribute using only one method at a time. Previous publications have attested to this and in turn used combinations of different standardized descriptors. (Carović-Stanko et al., 2010b) used molecular markers (RAPDs and AFLPs), genome sizing, and chromosome number to study phylogeny in *Ocimum* taxa where they found more groups within the genus. In a paper done by Labra et al. (2004) they also concluded that using a combination of molecular markers, morphological traits and volatile oils presents the optimal solution for taxonomic classification with basil cultivars.

7. CONCLUSIONS

In this study, the 19 ISSR markers, out of which only 11 were selected for furthers analysis of the 18 *Ocimum* cultivars, the markers worked well, they had good amplification with 71 fragments and very high polymorphism averaging at 94.85%. The ISSR markers could isolate the different species though with a few overlaps that could be attributed to the subjectivity of gel scoring.

From this study it was also observed that a large sample size and high replication of both the DNA and the primers is needed for a clear analysis. It is also important to take care of amplification efficiency while running the experiment.

SUMMARY

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NEWTON ODUOR OLUOCH

Assessing the Genetic Variability of Basil *(Ocimum)* Cultivars Using ISSR Markers.

MSC. In Agricultural Biotechnology (Plant)

Basil *(Ocimum)* is a well-established plant whose economic importance, agronomic value and ecological impetus is deserving research. To understand *Ocimum* in an impactful approach, it`s clear that researchers have to define it`s taxonomic boundaries. Basil hails from the Lamiaceae family, it is documented to have about 50 to 150 species (Avetisyan et al. 2017). The taxonomical intricacies in basil are attributed to several factors like crossbreeding in basil species that allows gene flow (Avetisyan et al. 2017). Its uses span from pharmaceutical therapy, antimicrobial activity, culinary and also in the ornamental industry (Cohen, 2014). While there are numerous examples of basil species, the following are the most common; *Ocimum sanctum, Ocimum gratissimum, Ocimum viride, Ocimum baslicum, Ocimum americanum, O. kilimandscharicum Guerk.* Different molecular markers present a plethora of novel platforms to significantly study the variation in species and cultivars, in this study, ISSR primers were the choice, the technique is based on the variation of the regions between microsatellites (György, 2022). Our study had the following objectives; (i) to assess the genetic variation within 26 *Ocimum* cultivars using 19 ISSR primers. (ii) To understand if there could be any morphological resemblance between clustered species. The overall hypothesis was that there was a clear delimitation between the studied cultivars either based on the species or morphological traits. The plant materials used in this experiment were obtained from the MATE Institute of Horticulture gene bank. The ISSR primers produced DNA fingerprints in *Ocimum* cultivars with an average polymorphism of 94.85% with only 2 of the tested primers being monomorphic. ISSR 888 generated the highest number of bands with the average count of bands per primer being 6. The average PIC value was 0.32 and this ranged from 0.285 to 0.419. The typical range of the band sizes was between 250 bp to 2500 bp measured against a 1kb ladder. Detailed analysis was done as cluster analysis and PCoA. The hierarchical clustering was obtained by calculating the Jaccard similarity index between the cultivars whereby the genetic variation between the 18 selected accessions was high with the Jaccard similarity distance values ranging between 0.289 to 0.897. The maximum similarity index (0.897) was observed between 'Red Rubin' (*O. basiclicum)* and 'Purple Ruffles' (*O. basiclicum*) while 'Corsican' (*O. basiclicum*) and 'Rama Tulsi'

(*O. sanctum/ tenuiflorum*) showed the smallest index (0.289). This then meant that 'Red Rubin' and 'Purple Ruffles' were comparatively closely related while 'Corsican' and 'Rama Tulsi' cultivars were far relatives. The PCoA analysis showed a largely congruent visualization of clusters as depicted in the earlier analysis on the UPGMA dendrogram and also the Jaccard similarity indices. The accessions were clustered in the same groupings as explained in the dendrogram. Of the 26 samples, 18 showed a good amplification using 11 primers out of the 19. It was observed that; (i) the PCoA clustering had the *Ocimum basilicum* in 2 subtle clusters with other *O. basilicum* accessions spread throughout the plot. (ii) Despite the observed clustering there were no clear morphological traits that would point out commonality. The other species were clearly separated from the rest but their position could not be attributed to traits because they were individual species and no average result could be gotten from them. It was clear that using the selected ISSR markers, the genetic relationship between the selected cultivars could be clustered however this needs a large sample of data and a high number of replicates just to be sure of the occurrence as there were slight overlaps between the species and also no distinct clustering of same species. In this study, 11 markers worked well, they had good reproducibility with 71 fragments and very high polymorphism averaging at 94.85%. The ISSR markers could isolate the different species though with a few overlaps that could be attributed to the subjectivity of gel scoring. From this study it was also observed that a large sample size and high replication of both the DNA and the primers is needed for a clear analysis. It is also important to take care of amplification efficiency while running the experiment.

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BIBLIOGRAPHY

1. Abbas, M. S. (2014). 'Assessment of density and cultivation type on growth and yield of two cultivars of basil (*Ocimum basilicum* L.).' *International Journal of Agronomy and Agricultural Research (IJAAR)*, *5*(1), pp. 74–79. http://www.innspub.net
2. Aghaei, M., Darvishzadeh, R., & Hassani, A. (2012). 'Molecular characterisation and similarity relationships among iranian basil (Ocimum basilicum L.) accessions using inter simple sequence repeat markers.' *Revista Ciência Agronômica*, *43*(2), pp.312–320. https://doi.org/10.1590/S1806-66902012000200014
3. Akoto, C. O., Acheampong, A., Duah Boakye, Y., Naazo, A. A., & Adomah, D. H. (2020). 'Anti-Inflammatory, Antioxidant, and Anthelmintic Activities of Ocimum basilicum (Sweet Basil) Fruits.' *Journal of Chemistry.* pp. 2-3. https://doi.org/10.1155/2020/2153534
4. Amiteye, S. (2021). 'Basic concepts and methodologies of DNA marker systems in plant molecular breeding.' *Heliyon*, *7*(10), e08093. https://doi.org/10.1016/j.heliyon.2021.e08093
5. Araújo Couto, H. G. S. de, Blank, A. F., Oliveira e Silva, A. M. de, Nogueira, P. C. de L., Arrigoni-Blank, M. de F., Nizio, D. A. de C., & Pinto, J. A. de O. (2019). 'Essential oils of basil chemotypes: Major compounds, binary mixtures, and antioxidant activity.' *Food Chemistry*, *293*, pp. 446–454. https://doi.org/10.1016/j.foodchem.2019.04.078
6. Avetisyan, A., Markosian, A., Petrosyan, M., Sahakyan, N., Babayan, A., Aloyan, S., & Trchounian, A. (2017). 'Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars.' *BMC Complementary and Alternative Medicine*, *17*(1), p. 60. https://doi.org/10.1186/s12906-017-1587-5
7. Carović-Stanko, K., Liber, Z., Besendorfer, V., Javornik, B., Bohanec, B., Kolak, I., & Satovic, Z. (2010a, b). 'Genetic relations among basil taxa (Ocimum L.) based on molecular markers, nuclear DNA content, and chromosome number.' *Plant Systematics and Evolution*, *285*(1), pp. 13–22. 13–22. https://doi.org/10.1007/s00606-009-0251-z
8. Chen, S.-Y., Dai, T.-X., Chang, Y.-T., Wang, S.-S., Ou, S.-L., Chuang, W.-L., Cheng, C.-Y., Lin, Y.-H., Lin, L.-Y., & Ku, H.-M. (2013). 'Genetic diversity among Ocimum species based on ISSR, RAPD and SRAP markers.' *AJCS*, *7*(10), pp. 1463–1471.
9. Cohen, M. (2014). 'Tulsi - Ocimum sanctum: A herb for all reasons.' *Journal of Ayurveda and Integrative Medicine*, *5*(4), p. 251. https://doi.org/10.4103/0975-9476.146554
10. Collard, B. C. Y., & Mackill, D. J. (2008). 'Marker-assisted selection: an approach for precision plant breeding in the twenty-first century.' *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), p. 557. https://doi.org/10.1098/RSTB.2007.2170

11. De Masi, L., Siviero, P., Esposito, C., Castaldo, D., Siano, F., & Laratta, B. (2006). 'Assessment of agronomic, chemical and genetic variability in common basil (Ocimum basilicum L.).' *European Food Research and Technology*, *223*(2), pp. 273–281. https://doi.org/10.1007/s00217-005-0201-0
12. Dhawan, S. S., Gupta, P., Mishra, A., Singh, S. K., & Chauhan, H. S. (2018). 'Genetic differentiation within *Vetiveria zizanioides* : selection and breeding of commercial perfumery grass Vetiver.' *Journal of Herbs, Spices & Medicinal Plants*, *24*(4), pp. 323–331. https://doi.org/10.1080/10496475.2018.1493015
13. Dhawan, S. S., Shukla, P., Gupta, P., & Lal, R. K. (2016). 'A cold-tolerant evergreen interspecific hybrid of *Ocimum kilimandscharicum* and *Ocimum basilicum*: analyzing trichomes and molecular variations.' *Protoplasma*, *253*(3), pp. 845–855. https://doi.org/10.1007/s00709-015-0847-9
14. Djalali Farahani-Kofoet, R., Römer, P., & Grosch, R. (2012). 'Systemic spread of downy mildew in basil plants and detection of the pathogen in seed and plant samples.' *Mycological Progress*, *11*(4), pp. 961–966. https://doi.org/10.1007/s11557-012-0816-z
15. Frankham, R. (2005). 'Genetics and extinction.' *Biological Conservation*, *126*(2), pp. 131–140. https://doi.org/10.1016/j.biocon.2005.05.002
16. Garibaldi, A., Gullino, M. L., & Minuto, G. (1997). 'Diseases of Basil and Their Management.' *The American Phytopathological Society*, *81*(2). https://apsjournals.apsnet.org/doi/pdf/10.1094/pdis.1997.81.2.124
17. Giachino, R. R. A., Sönmez, Ç., Tonk, F. A., Bayram, E., Yüce, S., Telci, I., & Furan, M. A. (2014). 'RAPD and essential oil characterization of Turkish basil (Ocimum basilicum L.).' *Plant Systematics and Evolution*, *300*(8), pp. 1779–1791. https://doi.org/10.1007/s00606-014-1005-0
18. Gupta, P., Mishra, A., Lal, R. K., & Dhawan, S. S. (2021). 'DNA Fingerprinting and Genetic Relationships Similarities Among the Accessions/Species of Ocimum Using SCoT and ISSR Markers System.' *Molecular Biotechnology*, *63*(5), pp. 446–457. https://doi.org/10.1007/s12033-021-00316-9
19. Gupta, S., Srivastava, A., Shasany, A. K., & Gupta, A. K. (2018). 'Genetics, Cytogenetics, and Genetic Diversity in the Genus Ocimum' (pp. 73–87). https://doi.org/10.1007/978-3-319-97430-9_6
20. György, Z. (2022). 'Molecular marker technologies in Molecular Plant Breeding.' *Magyar Agrár és Élettudományi Egyetem, Budapest.* (Pp. 22-39).

21. Hälvä, S., & Puukka, L. (1987). 'Studies on fertilization of dill (Anethum graveolens L.) and basil (Ocimum basilicum L.): I Herb yield of dill and basil affected by fertilization.' *Agricultural and Food Science*, *59*(1), pp. 11–17. https://doi.org/10.23986/afsci.72240
22. Jailawi, A. A., Ovidiu, J. I., & Maria, D. E. (2021). 'Study on the cultivation of 'Genovese' and 'Aromat de Buzău' basil varieties in the NFT system.' J*ournal of Horticulture, Forestry and Biotechnology*, *Volume 25*(2), pp. 27–31.
23. Khosla, M. K. (1995). 'Study of inter-relationship, phylogeny and evolutionary tendencies in genus dcimum. Indian journal of genetics and plant breeding'. *Indian Journal of Genetics and Plant Breeding*, *55*(1), pp. 71–83.
24. Labra, M., Miele, M., Ledda, B., Grassi, F., Mazzei, M., & Sala, F. (2004). 'Morphological characterization, essential oil composition and DNA genotyping of Ocimum basilicum L. cultivars.' *Plant Science*, *167*(4), pp. 725–731. https://doi.org/10.1016/j.plantsci.2004.04.026
25. Lal, R. K., Gupta, P., Chanotiya, C. S., & Sarkar, S. (2018). 'Traditional Plant Breeding in Ocimum' (pp. 89–98). https://doi.org/10.1007/978-3-319-97430-9_7
26. Makri, O., & Kintzios, S. (2008). *Ocimum* sp. (Basil): 'Botany, Cultivation, Pharmaceutical Properties, and Biotechnology.' *Journal of Herbs, Spices & Medicinal Plants*, *13*(3), 123–150. https://doi.org/10.1300/J044v13n03_10
27. Mills, M. H., & Schwartz, M. W. (2005). 'Rare plants at the extremes of distribution: broadly and narrowly distributed rare species.' *Biodiversity and Conservation*, *14*, pp. 1401–1420. https://doi.org/10.1007/s10531-004-9666-6
28. Mishra, A., Singh, P. K., Bhandawat, A., Sharma, V., Sharma, V., Singh, P., Roy, J., & Sharma, H. (2022). 'Analysis of SSR and SNP markers.' In *Bioinformatics.* Elsevier. (pp. 131–144). https://doi.org/10.1016/B978-0-323-89775-4.00017-1
29. Mohammed, M. S., Osman, W. J. A., Garelnabi, E. A. E., Osman, Z., Osman, B., Khalid, H. S., Mohamed, M. A., & Osman, J. A. (2014). 'Secondary metabolites as anti-inflammatory agents.' *The Journal of Phytopharmacology*, *3*(4), pp. 275–285. www.phytopharmajournal.com
30. Mondini, L., Noorani, A., & Pagnotta, M. A. (2009). 'Assessing Plant Genetic Diversity by Molecular Tools.' *Diversity*, *1*, pp. 19–35. https://doi.org/10.3390/d1010019
31. Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., & Baloch, F. S. (2017). 'DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing.' *Http://Mc.Manuscriptcentral.Com/Tbeq*, *32*(2), pp. 261–285. https://doi.org/10.1080/13102818.2017.1400401

32. Patel, R. P., Kumar, R. R., Singh, R., Singh, R. R., Rao, B. R. R., Singh, V. R., Gupta, P., Lahri, R., & Lal, R. K. (2015). 'Study of genetic variability pattern and their possibility of exploitation in Ocimum germplasm.' *Industrial Crops and Products*, *66*, pp. 119–122. https://doi.org/10.1016/j.indcrop.2014.12.043
33. Paton Alan, R. M. H. M. M. H. (1999a). 'Ocimum: an overview of classification and relationships.' in *Basil*. Taylor and Francis, United Kingdom. pp. 1-14. https://doi.org/10.1201/9780203303771.
34. Putievsky Eli, Galambosi, B. (1999). 'Production systems of sweet basil. In Basil.' Taylor and Francis Group. (pp. 59–59).
35. Putievsky, E. (1983). 'Temperature and daylength influences on the growth and germination of sweet basil and oregano.' *Journal of Horticultural Science*, *58*(4), pp. 583–587. https://doi.org/10.1080/00221589.1983.11515160
36. Quinn, J., Murray, R., Simon, J. E., Quinn, J., & Murray, R. G. (1990). '*Basil: A source of essential oils*.' pp. 484–489. https://hort.purdue.edu/newcrop/proceedings1990/V1-484.html
37. Raimo Hiltunen, Y. H. (1999). '*Basil*.' CRC Press. pp. 7-8 https://doi.org/10.1201/9780203303771
38. Rastogi, S., Meena, S., Bhattacharya, A., Ghosh, S., Shukla, R. K., Sangwan, N. S., Lal, R. K., Gupta, M. M., Lavania, U. C., Gupta, V., Nagegowda, D. A., & Shasany, A. K. (2014). 'De novo sequencing and comparative analysis of holy and sweet basil transcriptomes.' *BMC Genomics*, *15*(1), 1–18. https://doi.org/10.1186/1471-2164-15-588/TABLES/9
39. Rattanachaikunsopon, P., & Phumkhachorn, P. (2010). 'Antimicrobial activity of basil (Ocimum basilicum) oil against Salmonella enteritidis in Vitro and in food.' *Bioscience, Biotechnology and Biochemistry*, *74*(6), 1200–1204. https://doi.org/10.1271/BBB.90939
40. Ribeiro, P., & Simon, J. E. (2007). 'Breeding Sweet Basil for Chilling Tolerance.' *American Society for Horticultural Sciences.* (pp. 302-305)
41. Roldán-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., & De Loose, M. (2000). 'AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.).' *Molecular Breeding*, *6*, pp. 125–134.
42. Saunders, J. A., Pedroni, M. J., Penrose, L. D. J., Fist, A. J., Saunders, J. A., & Pedroni, M. J. (2001). 'AFLP Analysis of Opium Poppy DNA Extraction. *Crop Science*, *41*.' https://doi.org/https://doi.org/10.2135/cropsci2001.4151596x
43. 'Shahram, S., & 'Omid, A. (2011). 'Nutrient Supply and Fertilization of Basil.' *Advances in Environmental Biology*, *5*(5), pp. 956–960.

44. Sharafi, A. A., Abkenar, A. A., & Sharafi, A. (2017). 'Molecular genetic diversity assessment of Citrus species grown in Iran revealed by SSR, ISSR and CAPS molecular markers.' *Journal of Science and Research: Revista Ciencia e Investigación*, *2*(8), p. 22-27 https://doi.org/10.26910/issn.2528-8083vol2iss8.2017
45. Švecová, E., & Neugebauerová, J. (2010). '*A study of 34 cultivars of basil (Ocimum L.) and their* morphological, economic and biochemical characteristics, using standardized descriptors.' *Acta Universitatis Sapientiae: Alimentaria, 3*, pp. 118-125.
46. Vieira, R. F., Goldsbrough, P., & Simon, J. E. (2003). 'Genetic Diversity of Basil (Ocimum spp.) Based on RAPD Markers.' *Journal of the American Society for Horticultural Science*, *128*(1), pp. 94–99. https://doi.org/10.21273/JASHS.128.1.0094
47. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. van de, Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., & Zabeau, M. (1995). AFLP: 'A new technique for DNA fingerprinting.' *Nucleic Acids Research*, *23*(21), pp. 4407–4414. https://doi.org/10.1093/nar/23.21.4407
48. Wang, J. (2002). 'ISSR markers and their applications in plant genetics'. *Yi Chuan = Hereditas*, *24*(5), p. 613.
49. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). 'DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.' *Nucleic Acids Research*, *18*(22), pp. 6531–6535. https://doi.org/10.1093/nar/18.22.6531
50. Wyenandt, C. A., Simon, J. E., Pyne, R. M., Homa, K., McGrath, M. T., Zhang, S., Raid, R. N., Ma, L. J., Wick, R., Guo, L., & Madeiras, A. (2015). 'Basil downy mildew (Peronospora belbahrii): Discoveries and challenges relative to its control'. *Phytopathology*, *105*(7), pp. 885–894. https://doi.org/10.1094/phyto-02-15-0032-fi/asset/images/large/phyto-02-15-0032-fi_f4.jpeg

SOURCES FROM INTERNET

1. Al Foah Date Company. (2023). *Tridge Exhibition*. Accessed on 30/March/2023 https://www.tridge.com/intelligences/basil/export
2. Growables. (2023). *Basil Cultivars*. Growables.Org. Accessed on 30/March/2023 https://www.growables.org/informationVeg/BasilCultivars.html

ANNEX

Annex 1

Table 4; Jaccard similarity and distance indices

	'Purple Ruffles'	'Red Rubin'	'Rubin Kaira' (Stavia)	'Fine Verde Compacto'	'Penang Lemon' (Pinang)	'Tulsi Temperate'	'Rama Tulsi'	'K/Cardinal'	'Feleny'	'Red Boza'	'Christmas'	'Pepper scented'	'Barhat'	'Tulsi Vana'	'Aromatto'	'Corsican'	'Anisse'	'O.b. mrihani'
'Purple Ruffles'	1	0.896552	0.892857	0.714286	0.5	0.515152	0.3	0.487805	0.735294	0.611111	0.461538	0.351351	0.5	0.292683	0.435897	0.53125	0.378378	0.380952
'Red Rubin'	0.896552	1	0.866667	0.8	0.575	0.472222	0.341463	0.560976	0.771429	0.694444	0.538462	0.358974	0.538462	0.333333	0.475	0.529412	0.421053	0.452381
'Rubin Kaira' (Stavia)	0.892857	0.866667	1	0.742857	0.525	0.457143	0.358974	0.512195	0.764706	0.638889	0.487179	0.378378	0.526316	0.35	0.5	0.515152	0.405405	0.404762
'Fine Verde Compacto'	0.714286	0.8	0.742857	1	0.7	0.414634	0.333333	0.682927	0.634146	0.65	0.547619	0.348837	0.547619	0.386364	0.454545	0.461538	0.372093	0.534884
'Penang Lemon' (Pinang)	0.5	0.575	0.525	0.7	1	0.414634	0.428571	0.682927	0.488889	0.571429	0.585366	0.380952	0.666667	0.452381	0.454545	0.461538	0.404762	0.534884
'Tulsi Temperate'	0.515152	0.472222	0.457143	0.414634	0.414634	1	0.315789	0.404762	0.461538	0.365854	0.410256	0.297297	0.375	0.307692	0.35	0.382353	0.4	0.333333
'Rama Tulsi'	0.3	0.341463	0.358974	0.333333	0.428571	0.315789	1	0.355556	0.311111	0.348837	0.425	0.388889	0.295455	0.394737	0.302326	0.289474	0.307692	0.348837
'K/Cardinal'	0.487805	0.560976	0.512195	0.682927	0.682927	0.404762	0.355556	1	0.619048	0.595238	0.65	0.372093	0.609756	0.512195	0.511628	0.45	0.428571	0.55814
'Feleny'	0.735294	0.771429	0.764706	0.634146	0.488889	0.461538	0.311111	0.619048	1	0.756757	0.6	0.357143	0.560976	0.428571	0.575	0.513514	0.45	0.444444
'Red Boza'	0.611111	0.694444	0.638889	0.65	0.571429	0.365854	0.348837	0.595238	0.756757	1	0.702703	0.333333	0.536585	0.475	0.55	0.486486	0.461538	0.52381
'Christmas'	0.461538	0.538462	0.487179	0.547619	0.585366	0.410256	0.425	0.65	0.6	0.702703	1	0.341463	0.55	0.487179	0.525	0.542857	0.555556	0.575
'Pepper scented'	0.351351	0.358974	0.378378	0.348837	0.380952	0.297297	0.388889	0.372093	0.357143	0.333333	0.341463	1	0.341463	0.545455	0.317073	0.305556	0.361111	0.302326
'Barhat'	0.5	0.538462	0.526316	0.547619	0.666667	0.375	0.295455	0.609756	0.560976	0.536585	0.55	0.341463	1	0.414634	0.525	0.5	0.513514	0.5
'Tulsi Vana'	0.292683	0.333333	0.35	0.386364	0.452381	0.307692	0.394737	0.512195	0.428571	0.475	0.487179	0.545455	0.414634	1	0.425	0.388889	0.444444	0.404762
'Aromatto'	0.435897	0.475	0.5	0.454545	0.454545	0.35	0.302326	0.511628	0.575	0.55	0.525	0.317073	0.525	0.425	1	0.709677	0.71875	0.722222
'Corsican'	0.53125	0.529412	0.515152	0.461538	0.461538	0.382353	0.289474	0.45	0.513514	0.486486	0.542857	0.305556	0.5	0.388889	0.709677	1	0.714286	0.617647
'Anisse'	0.378378	0.421053	0.405405	0.372093	0.404762	0.4	0.307692	0.428571	0.45	0.461538	0.555556	0.361111	0.513514	0.444444	0.71875	0.714286	1	0.676471
'O.b. mrihani'	0.380952	0.452381	0.404762	0.534884	0.534884	0.333333	0.348837	0.55814	0.444444	0.52381	0.575	0.302326	0.5	0.404762	0.722222	0.617647	0.676471	1

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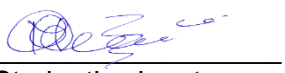

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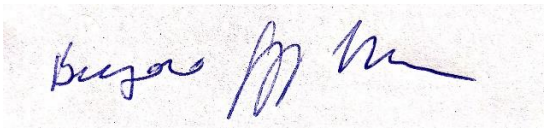

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