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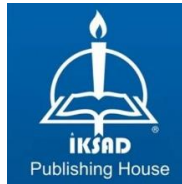Dr. Sibel KADIOĞLU
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PREFACE

The production and trade volume of the agricultural sector in the world is increasing day by day. The agricultural sector has indispensable and strategic importance compared to other sectors. For this reason, it has been seen that the interest of large and multinational enterprises in agriculture is increasing. Agricultural production should be carried out by expert agricultural enterprises using modern techniques. For this reason, it is important to know and disseminate innovative agricultural technologies. The purpose of creating this book is to ensure that agriculture is economically, ecologically and technologically sustainable in the light of new developments and to share the developments in the agricultural field with the scientific world. I hope that the book will add awareness and innovative approaches to studies in natural sciences and agriculture. I would like to thank all the authors who contributed to the preparation of this book titled "Regenerative Agriculture".

Dr. Veysel AYDIN

CHAPTER 1

THE USE OF ARBUSCULAR MYCORRHIZA IN CEREAL CULTIVATION

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INTRODUCTION

Cereals are a major source of nutrition and account for about 32% of world agricultural production of 9.3 billion tons (FAO, 2023). As in all agricultural crops, cereals also require maintenance operations such as fertilization and tillage in order to obtain quality products and high yields (Koday, 2014). Pesticides and fertilizers used unconsciously to increase agricultural production have led to the emergence of products of poor quality and threatening human health. Chemicals accumulated in the soil and mixed with groundwater adversely affect human and plant health and disrupt the ecological balance. For this reason, producers have turned to agricultural production that does not harm human health and nature (Çığ et al., 2022). Bacteria, endo and ecto mycorrhizae and many other beneficial microorganisms that increase the development of plants, nutrient uptake, resistance to stress conditions, and live together or free in the plant root zone are some of the environmentally friendly agricultural practices (Bozdoğan, 2019).

The first time the idea of mycorrhiza was introduced by the German phytopathologist Frank in 1885. The researcher found fungi that were densely present in some plant roots but did not cause any negative effect on the plant and named them "mycorrhiza", which is a combination of the Greek words for fungus and root (myko+rhiza) (Yıldız, 2009). The most common mycorrhizal fungi species are arbuscular mycorrhizae (AM), ectomycorrhizal (EcM) and ericoid mycorrhizae (ErM) (Bennett and Classen, 2020). AM fungi live symbiotically in the roots of 72% of plants (Bonfante 2018). Arbuscular mycorrhizal fungi (AMF) take carbon compounds from the host plant

and provide mineral nutrients to the plant in return. Fruit trees interact with garden and field crops, positively affecting them (Balestrini et al., 2020).

The Role of Arbuscular Mycorrhizae in Soil Nutrient Uptake and Plant Growth

Arbuscular mycorrhizal fungi have many roles on the plant during various developmental stages, especially in cereals. AMFs have an impact on nutrient availability and nutrient uptake in the soil. It also increases antioxidant activities, photosynthetic rate and tolerance to environmental stress in plants (Khan et al., 2022). Mycorrhizal fungi have developed several strategies that indirectly and directly facilitate the decomposition of soil organic matter. These are oxidation, enzymatic breakdown, carbon supply to the rhizosphere and stimulation of microorganisms (Frey, 2019). Mycorrhizae also provide the secretion of exudates and adhesive substances necessary for the improvement of soil structure (Rabot et al., 2018; Barbosa et al., 2019). Exudate production enhances phosphorus availability and mineralization by promoting the growth of phosphate solubilizing bacteria in the plant rhizosphere (Zhang et al. 2016; Frey, 2019). AMFs not only provide mineral compounds to plants, but also improve soil physicochemical properties (Riaz et al., 2021). It improves the quality of soil aggregation, regulates the structure of bacterial and plant communities and ensures balance in the ecosystem (Diagne et al., 2020). It also provides direct delivery of the products of plant photosynthesis to the rhizosphere. They act as conduits for the incorporation of plant photosynthesis product C into the soil organic matter structure (Frey, 2019).

Arbuscular mycorrhizal fungi (AMF) form symbioses with most plants, improving plant growth and nutrient assimilation (Thirkell et al., 2019). In plants, they increase nutrient uptake by expanding the root area (Diagne et al., 2020). In addition, AMF directly affects photosynthetic activities and leaf functions. (Masrahi et al., 2023). AMF fungi are mainly used in cereal cultivation to promote nutrient uptake in plants, increase growth and yield, and improve physiological properties (Khan et al., 2022). In cereals, primary and secondary metabolite synthesis, soluble protein and carbohydrates are increased (Masrahi et al., 2023). In a study of AMF, plant growth-promoting bacteria inoculation and control treatments, AMF increased the proportion of phosphorus (P), nitrogen (N) and potassium (K) in barley grain and straw and gave the highest results in P and K uptake compared to control and bacteria treatments (Masrahi et al., 2023). In another study, AM fungi were found to improve plant biomass, yield and quality of cereal crops (Khan et al., 2022). AMF-inoculated sorghum increased biomass, leaf number, plant height, total phosphorus, nitrogen and potassium uptake (Nakmee et al., 2016; Diagne et al., 2020). Barley inoculated with five AM fungi *Pacispora franciscana, F. mosseae, F. geosporum, R. irregularis* and *Glomus tenebrosum* increased yield and grain weight by 68% and 90%, respectively (Jerbi et al., 2022). All 53 wheat cultivars inoculated with AMFs of *Rhizophagus irregularis, Claroideoglomus claroideum, Funneliformis mosseae, Funneliformis geosporum, Claroideoglomus etunicatum* and *Glomus microaggregatum* showed increased yields. The effect of AMF on phenotypic criteria such as root and shoot biomass and quality criteria such as grain quality was found to be different in wheat varieties (Essiane-Ondo et al., 2019). In a study investigating the effect

of Funneliformis mosseae and Rhizophagus intraradices AMF species on wheat grain yield and Zn concentrations, mycorrhizal inoculation increased P uptake and grain yield (Ma et al., 2019). Another study reported that AMF has the potential to reduce Zn deficiency in maize (Saboor et al., 2021).

The Role of Arbuscular Mycorrhizae in Improving Plant Resistance Systems

AMF increases plant resistance to many stresses such as drought, salinity, heavy metals, extreme temperatures, pathogens and diseases. The adaptation mechanisms in abiotic stresses are generally ion selectivity, increased hydromineral nutrition, osmolyte production, gene regulation, antioxidant and phytohormone synthesis. The mechanism of AMF in biotic stresses is improvement of plant defense system, competition and resistance to pathogens (Khan et al., 2022). AMF also increases plant tolerance to environmental challenges, including salinity and drought, by increasing dry matter content and water uptake (Masrahi et al., 2023).

Arbuscular mycorrhizal fungi have a remarkable ability to control drought-induced oxidative damage (Zou et al., 2020). AMF increases the ability of roots to take up water from the soil, keeping leaf stomata open and increasing dry matter production. It also accumulates substances such as soluble sugars, proline, glycine betaine, organic acids, potassium and calcium in plants to facilitate water uptake (Tani et al. 2019). AMF under drought stress eliminates ROS accumulation by increasing antioxidant defense systems in plants. This reduces the negative effects of drought on DNA, proteins and lipids and enables the plant to fulfill its

functions under stress (Acuna-Rodrıguez et al. 2020). Flavonoids, which are important antioxidants, have been shown to directly scavenge ROS and stimulate spore germination, arbuscle formation and hyphae growth in host roots of mycorrhizal fungi (Salloum et al. 2018; Mirjani et al. 2019). In a study, it was found that total phenol content was increased by *Glomus versiforme* fungus in maize under drought stress. This explains that AMF act as active scavengers of ROS, protecting cellular structures and the functioning of their hosts (Begum et al. 2019). *Triticum aestivum* treated with mycorrhizal *Glomus mosseae* was found to have higher anthocyanins, total phenolic compounds and flavonoid content compared to uninoculated (control) plants in a restricted water environment (Bitaraf et al. 2020). Similarly, under drought conditions, AMF treatments increased plant internode length, plant height, root dry weight and above-ground dry weight compared to the control. In another study, AMF inoculation was found to significantly increase chlorophyll content in durum wheat cultivars under drought conditions. These effects of AMF are thought to be due to the improvement of growth-related functions such as CO_2 plant assimilation, leaf water potential, stomatal conductance and relative water content (Amer et al., 2023). AM fungi have been found to increase physiological traits such as photosynthesis and yield in sorghum plants by increasing water use efficiency and providing more water under drought conditions (Hajiboland et al., 2019). Barley seeds inoculated with *Pacispora franciscana, Funneliformis geosporum, Funneliformis mosseae, Glomus tenebrosum, Rhizophagus irregularis* showed an increase in the amount of flavonoids and polyphenols in both severe and moderate drought conditions compared to the control. Barley grains inoculated with AMF showed an increase in

Cu, K, Ca, Fe and Zn. At the end of the study, it was suggested that AMF inoculation can increase water stress tolerance and can be used as biofertilizer in sustainable agricultural systems (Jerbi et al., 2022).

AMF inoculation has been reported to improve the salinity tolerance of host plants by maintaining carbon and light utilization efficiency in plants under stress (Eroğlu et al., 2020). AMF also promoted the accumulation of organic solutes such as proline responsible for salinity tolerance (Masrahi et al., 2023). AMF has been shown to maintain carbon and light utilization efficiency under stress to improve salinity tolerance (Eroglu et al., 2020). It has also been reported to promote salinity tolerance by increasing water status, photosynthetic activity, and K^+/Na^+ homeostasis in plants (Balestrini et al., 2020). In a study, it was reported that AMF improved plant N accumulation in durum wheat grown under saline conditions (Fileccia et al. 2017). In a study investigating the effects of AMF, plant growth promoting bacteria and phosphorus fertilizer applications on barley under salinity conditions, AMF inoculation was found to be more effective than bacteria in terms of improving yield and plant components (Masrahi et al., 2023). AMF is thought to increase the photosynthetic capacity of maize under salinity stress and reduce stress-induced growth limitations (Khan et al., 2022). In a study investigating the effects of *Glomus tortuosum* on corn chlorophyll fluorescence, photosynthetic pigments, photosynthetic capacity, rubisco activity and morphology under salt stress, AMF inoculation was found to improve dry mass and leaf area. It has also been reported to improve physiological mechanisms of maize

plants under salinity stress by increasing chlorophyll content, rubisco activity, and light energy utilization efficiency (Xu et al., 2018).

AMF inoculation has been reported to improve plant tolerance to soil-borne pathogens. In a study, AMF was found to provide protection against infection by *Magnaporthe oryzae* in all rice varieties (Campo et al., 2020). In the study investigating the effectiveness of Rhizophagus intraradices AMF as a biocontrol agent against *Fusarium pseudograminearum* in wheat plants, AMF inoculation reduced disease severity by 39% and *F. pseudograminearum* population density by 75.7%. In addition, a decrease in lipid peroxidation levels and a significant increase in the activity of antioxidant enzymes were observed in AMF-inoculated plants. At the end of the study*, R. intraradices* was found to confer tolerance to wheat infection by maintaining redox balance and colonizing root zones and competing with *F. pseudograminearum* (Spagnoletti et al., 2021).

Under metal stress, excessive amounts of ROS are produced, causing oxidative damage to plant cellular structures. AMF inoculation is one of the most effective ways to remove ROS. AMF adsorb heavy metals and store them in their vacuoles. They also support heavy metal phytoremediation by supporting an important role in the immobilization and mobilization of heavy metals (Dhalaria et al., 2020). AMF was found to improve mineral nutrient and water uptake, increase shoot biomass, cause changes in root morphology, and indirectly induce tolerance in plants by alleviating oxidative stress produced by heavy metals (Riaz et al., 2021). It also secretes a glycoprotein called glomalin, which improves soil quality and reduces metal uptake in plants. AM fungi

chelate heavy metals and promote genes for protein synthesis (Dhalaria et al., 2020).AMF reduced Cd uptake in bread wheat and Cd accumulation occurred in non-mycorrhizal plant roots (Baghaie et al., 2019). AM fungi are effective in reducing the effects of Zn stress (Saboor et al.). It was found that maize plants inoculated with Rhizophagus irregularis under Zn toxicity had higher biomass compared to the control (Bui and Franken, 2018). The effect of AM inoculation of Glomus intraradices species AM and biochar treatments on maize growth, Cd uptake and antioxidant enzymatic activities were investigated. AM inoculation alone was found to facilitate maize growth, alleviating Cd stress more than biochar application alone (Liu et al., 2018). Because of these effects, the use of mycorrhizal fungi is a universally accepted method to combat heavy metal pollution (Riaz et al., 2021).

Conclusion

Agricultural crop cultivation requires fertile soils, but unfortunately, the intensive use of chemical pesticides and fertilizers has led to the contamination of agricultural land and the generation of significant amounts of pollutants. Numerous studies have proven that AMFs regulate soil structure through their direct or indirect effects, help the uptake of water and nutrients with the help of their hyphae network, increase tolerance to pathogens, biotic and abiotic stresses by releasing primary, secondary metabolites and atioxidants, and provide heavy metal phytoremediation. In summary, it is thought that AMFs can be used as biofertilizers in sustainable agricultural systems in order to reduce the use of chemical fertilizers due to the above-mentioned properties.

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CHAPTER 2

MOLECULAR MARKERS FROM PAST TO PRESENT AND THEIR APPLICATION AREAS

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1. INTRODUCTION

The scientific assumption that genes solely transfer genetic information unidirectionally to gene extensions (peptides) was disputed in 1970 with the detection of reverse transcriptase by H. Temin and D. Baltimore. This rare enzyme complex exists in RNA viruses (retroviruses). The identification of this enzyme represents a noteworthy biological advancement as it enables the synthesis of complementary DNA (cDNA) for active gene coding regions. This technique facilitates the exploration of a gene without prior comprehension of the gene product, as long as it is expressed within the appropriate tissue. In 1969, credit was given to W. Arber for the discovery of restriction enzymes, also referred to as restriction endonucleases, which cleave DNA at specific sites. D. O. Smith later corroborated this finding in 1971. Today, the use of appropriate restriction enzymes permits exact cleavage of DNA into repeatable and clearly defined fragments, thereby enabling the identification of the regions of interest. Therefore, DNA fragments from various plant species can be easily combined and analyzed using methods that simplify the identification of the target region. As a result, this leads to a comprehensive examination of its characteristics. Techniques for producing multiple copies of DNA fragments and sequencing them to determine the sequence of their nucleotide bases were developed between 1977 and 1985, commonly referred to as recombinant DNA technology. Please refer to the Chronology at the end of this introduction In 1977, recombinant DNA analysis led to a groundbreaking and unexpected discovery concerning gene structure in higher organisms, yeast, and Drosophila. The discovery revealed that

genes are not continuous coding DNA segments, but are often interrupted by non-coding segments (Watson&Tooze,1981). The size and sequence of coding DNA segments, or exons, and non-coding segments, or introns, vary for each individual gene, forming the exon/intron structure of eukaryotic genes. The coding DNA segments, or exons, and non-coding segments, or introns, vary in size and sequence for each individual gene and make up the exon/intron structure of eukaryotic genes. Molecular genetic DNA analysis has enabled the mapping of DNA markers, which are heritable differences in nucleotide sequence that can be identified, to specific locations on chromosomes (physical map). This facilitates the identification (mapping) of the chromosomal position of a gene of interest by analyzing the relationship between the segregation of a disease locus and polymorphic DNA markers (linkage analysis). Once the chromosome location of the gene has been identified, it can be isolated and characterized through positional cloning. The term "positional cloning" was coined by F. Collins. This involves the direct analysis of the gene's structure without the need for prior knowledge of its products, except for its approximate location. Since the elucidation of DNA's structure by Watson and Crick in 1953, molecular biology has experienced considerable progress, particularly after 1990. The production of transgenic plants in 1996 was a significant milestone in the field's development. Nowadays, new molecular and biotechnological techniques are employed to enhance and support conventional plant breeding programmes. Molecular markers are typically used in selection processes, genetic and linkage mapping, identification and conservation of varieties, determination of genetic distance between different genotypes, and breeding of qualitative and

quantitative traits (Bilgin and Korkut, 2005). In addition, there is an increasing need to identify stable, high-yielding and high-quality varieties for registration and certification. This can be achieved by supplementing field trials and laboratory tests with molecular markers. (Yorgancılar et al.,2015).

2. Molecular Markers
2.1 Molecular Marker Description

Molecular markers are fragments of DNA that are linked to a particular genomic region. Molecular markers are DNA fragments linked to a specific genomic region and can be used as general tests to detect differences between individuals. Molecular markers originate from point mutations, insertions, deletions or replication errors of DNA bases and are neutral. Typically, they occur in non-coding DNA regions. DNA markers are theoretically infinite and, contrary to morphological and biochemical markers, are not impacted by environmental factors or plant development. The identification of polymorphic regions in DNA molecules is based on the principle that when more than one form of a gene or trait is present in a population, it is considered polymorphic. A gene or phenotype is deemed polymorphic if variations in DNA sequence, amino acid sequence, chromosome structure, or phenotype are observed at various levels. To obtain DNA for a thorough genomic analysis, a small tissue sample from any part of the body is sufficient (Botstein et al., 1980). Moreover, DNA markers are stable and present in all tissues. They may be codominant or dominant and adhere to fundamental laws of inheritance (Williams et al., 1990).

2.2 Molecular marker technology applications in plant breeding

Genetic Identification and Relationship AnalysisThe determination of the genotype of genetic resources can aid in present and future screening. Such screenings are crucial for breeding materials and for establishing the foundation of genetic studies. By using molecular markers at different stages, genetic identification of breeding programmes is possible. This approach enables the selection of plants with desirable genetic traits and provides guidance for the breeding programme. Marker Assisted Selection (MAS) is a process that chooses plants with distinct properties like pest and disease resistance, excellent seed production, among others, by utilizing markers associated with the production of specific substances.The technique reduces time and resource utilization and assists in the identification of plants with desired traits, which can be utilized as parents in future advancements in crops. Bulk Segregation Analysis (BSA) is a genome-specific technique developed by Michelmore et al. in 1991. Its objective is to identify markers in particular regions. The technique comprises a segregated cross with a single origin. The population's individuals were divided into two pooled populations based on a comparison of DNA samples. Each pool or aggregate group consists of individuals with the same gene or trait of interest, but they vary in other genetic traits. Evaluation of a trait in the two compared pools aims to identify indicators, such as resistance to a specific diseaseSeed breeding is a specialist technique that enhances plant traits. In this process, molecular markers play a vital role by being used to analyse and select specific traits, such as herbicide tolerance,

disease and insect resistance, crop yield increase, improvement of oil content, nutritional enhancement, and amplification of stress tolerance. Populations produce relevant trait seeds, from which double haploid seeds are selected. Every seed is analyzed for its traits. After reviewing the analysis results, one or more doubled haploid seeds are selected. These chosen plants or plant tissues are subsequently grown and cultivated. This method incorporates clear explanations of the technical terminologies used. Molecular markers are proven to be an efficient tool for the breeding of seeds in the development of plants with desired traits. (Yıldırım, A.,2007). In plants, numerous genes control quantitative traits of significant economic importance, such as yield, weight, and height. Except for viable selfed lines, investigations on hybrid varieties have mainly focused on maximizing yield. The optimal breeding strategy for generating F1 individuals with heterosis through crossing is still unclear. To determine the genetic transmission of selfed lines, conduct test crosses or analyze hybrids produced from all possible combinations. The maximum heterosis occurs in F1 hybrids when there is significant diversity in the gene structure responsible for yield between the mother and father (i.e., different alleles). The combination of dominant genes from different alleles in a heterozygous state and a recessive gene leads to heterosis. Molecular markers are increasingly used in F1 plant breeding to identify parents with outstanding general and specific abilities in producing superior F1 hybrids. These studies analyse the genetic distance between the required parental lines. Furthermore, molecular markers are utilised to ascertain essential genes that impact yield and furnish preliminary insights into parents that possess the capacity to produce exceptional F1 variations. When closely related

individuals are interbred, heterosis does not manifest, rendering breeding futile and the resulting hybrids pointless. Additionally, it is possible to predict the parents which will produce superior F1 hybrids. To carry out these analyses, initial inquiries must detect significant gene markers and evaluate genetic dissociation. (Beşer, N., 2017).

2.3 Which features should be present in molecular markers

When selecting molecular markers, several factors must be considered. It is crucial that the markers are highly polymorphic and able to differentiate among diverse genotypes. Additionally, the markers should be present in all tissues and exhibit codominant inheritance, which enables distinction between heterozygous and homozygous individuals. It is also crucial that the markers are distinguishable from dominant individuals. The selected marker needs to be uniformly dispersed throughout the genome and extensively prevalent. It should demonstrate impartial or selective behaviour, be easily accessed, and have low implementation costs. High reproducibility and compatibility for automated, swift evaluation are also of utmost significance. Consistency is vital, ensuring identical findings when analysing the same genetic material.

2.4 Types of Molecular Markers

Molecular markers can be categorised into two groups: Hybridisation-Based and Polymerase Chain Reaction (PCR)-Based markers. Hybridisation-based markers, like RFLP, are included in the former category, whilst SSR, RAPD, AFLP, and ISSR belong to the latter. The language used is objective, clear and concise without any

ornamental language or personal opinions. The structure is kept logical consisting of short sentences with causal connections between them. Finally, it adheres to the conventions of academic writing such as style guides, consistent citation, and footnote place and formatting. Various other methods, such as SRAP, SCAR, STS, CAPS, CAAT, SCoT, and SNP markers are employed to detect polymorphism. These are further complemented by MP-PCR, AP-PCR, AS-PCR, and DAF strategies. Technical abbreviations are explained when introduced. Monomorphic markers do not indicate differences between individuals, whereas polymorphic markers vary among individuals of the same or different species. Technical term abbreviations will be explained when first used. Polymorphic markers exhibit either dominant or co-dominant characteristics that are determined by their ability to differentiate between homozygotes and heterozygotes. The structure is clear, with a logical progression and causal connections between statements, and sentences and paragraphs create a clear flow of information. The language is formal, precise and objective, avoiding biased or emotional language and using passive tone and impersonal construction. Spelling and vocabulary align with British English, with correct use of grammar and punctuation. Style guides will be adhered to, with consistent citation and use of a consistent footnote style and formatting. Quotes are clearly marked and filler words are avoided. Dominant markers are categorized as either present or absent, while codominant markers vary in size. In such instances, the bands of DNA markers that display distinct patterns of size on the gel are classified as marker alleles. Codominant markers possess the capability to accommodate multiple alleles while dominant markers can only manifest two alleles. In this particular scenario,

codominant markers differentiate between heterozygotes and homozygotes, whereas dominant markers are unable to fulfil this function. (Figure 1).
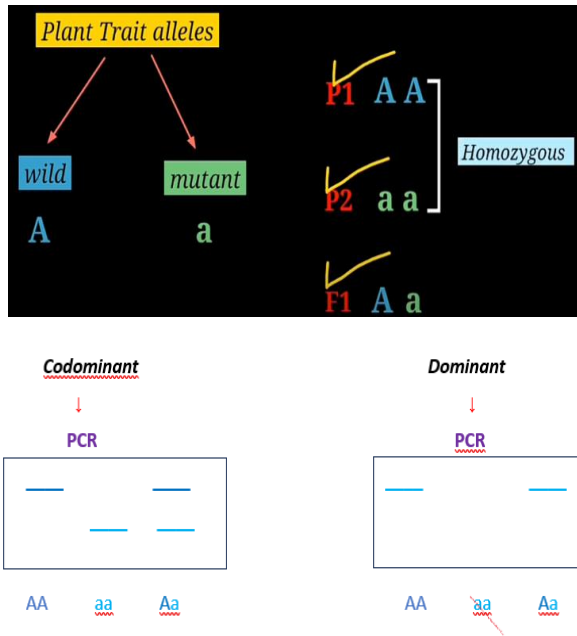
Figure1. Comparison of codominant and dominant markers

a. Non-PCR Based Techniques (Molecular Markers Based on Hybridisation)

Restriction Fragment Length Polymorphism (RFLP) is a frequently applied molecular marker technique to detect DNA polymorphism. The process entails the utilization of restriction enzymes to cleave DNA, running the resultant fragments on gel electrophoresis, transferring them to a nitrocellulose membrane, and hybridising them with labelled probes. It enables the identification of diverse DNA

fragments denoting the existence of an insertion, deletion, nucleotide substitution, or single nucleotide polymorphism (SNP). RFLP markers show substantial degrees of polymorphism, have codominant features, and exhibit high reproducibility. DNA blots are a standard method used in this technique, allowing for re-analysis using different probes. The probes used in RFLP are generally species-specific and obtained from single or cDNA for multiple loci or from genomic libraries. However, RFLP analysis has some limitations, such as the requirement for a suitable quantity and quality of DNA, the expense and toxicity of probes, and the time and labour-intensive nature of the technique. RFLP is beneficial for investigating genetic diversity within populations and species, phylogenetic studies, gene mapping of closely related taxa, and analysing gene flow and relationships (Young ve ark., 1992, Miller ve Tanksley, 1990; Desplanque ve ark., 1999).

b. PCR Based Techniques

After the discovery of PCR by Mullis and Faloona (1987), the pace and quantity of molecular biology research has increased, with numerous new approaches being added to PCR-based marker research. PCR is a DNA amplification process conducted with the aid of essential chemicals (dNTP, dream taq buffer solution, etc.), which uses tiny quantities of DNA and enzymes without the involvement of living organisms.

RAPD (Randomly Replicated PolymorphicDNA)

RAPD (Random Amplified Polymorphic DNA), synthesized using PCR techniques with the help of primers, is a method based on DNA replication. The primers act as both forward and reverse primers, and the

replicated particles typically range in size between 0.5-5 kb due to the diversity of regions with primer binding and differentlengths of DNA particles (Williams et al., 1990).

AFLP (Amplified Fragment Length Polymorphism)

The AFLP (Amplified Fragment Length Polymorphism) technique is a PCR-based method that employs restriction enzymes to enable efficient study of DNA polymorphisms. The methodology involves obtaining DNA fragments and ligating them with adapters, followed by selective amplification using PCR. The procedure can yield 50-100 fragments per reaction, resulting in a high level of polymorphism. It is also highly reproducible and can be used to determine polymorphisms in diverse genomes without requiring species-specific information. AFLP is favoured in plant genetic diversity studies and in determining kinship between individuals or cultivars, due to its efficiency and effectiveness. This technique is fast and reliable for assessing genetic variation and does not demand extensive preliminary sequence information (Althoff ve ark., 2007. Vos ve ark., 1995)

Minisatellite&Mirosatellite

VNTR markers are utilised toidentify different length repeat sequences. These markers, which range in size from 4-20 kb and are present at multiple loci, hybridise with probes. This differs from microsatellite and minisatellite markers (Jeffreys et al., 1985). Repeat units that are equal in number between the causes of variation in their numbers, non-crossing-over or gene returns (gene conversion), appear to be among the main causes of high mutation rates in minisatellite

regions.This results in increased polymorphism and contributes to the diversity of multi-locus profiles of individuals (Nakamura et al., 1987). The main advantages of minisatellites are their high polymorphism and reproducibility. Similar DNA particles obtained in this size may have disadvantages such as non-homologous and band profile variations in terms of loci or alleles and the lack of clear interpretation. Simple sequence repeats (SSRs), also referred to as microsatellites, are the smallest repeating motifs within DNA sequences, containing 1-6 base pairs. Technical term abbreviations should be clarified upon first use to aid reader understanding. If appropriate primers (usually 20-25 base pairs in length) have been designed for the flanking regions, the sequence can be replicated using PCR (Filiz et al.,2011). SSR primers can also be used across different living organisms. The DNA sequence may undergo replication errors, including skipped segments, incorrect base pairing and unequal distribution. It is crucial to address these issues with reliable and scientific solutions. The DNA sequence may undergo replication errors, including skipped segments, incorrect base pairing, and unequal distribution. According to Matsuoka et al. (2002), crossing-over events are the primary cause of microsatellite variability as identified through gelelectrophoresis. The use of a stable, codominant marker system with high polymorphism is required to create an informative marker system. This system should feature low DNA microsatellite markers that can be expanded. Furthermore, it is necessary to have an adequate availability of plentiful and dispersed markers throughout the genome to enable reproducibility and automation. The marker system has a disadvantage due to the high mutation rates of microsatellite regions, which is important in population genetics and gene mapping studies (Powell et

al., 1996). Being in the primary binding sites causes alteration, resulting in the development of alleles. Consequently, controversies may arise when genotypic and allelic frequencies are inaccurately interpreted (Freudenreich et al., 1997).

ISSR (Inter Simple Sequence Repeat)

ISSR is a dominant markerwith the advantage of allowing primer design without requiring sequence knowledge (Joshi et al., 2000). In the ISSR (Inter Simple Sequence Repeat) method, primers containing double, triple, quadruple, and quintuple repeated nucleotides are used to amplify the region between two microsatellites. The resulting PCR products are then subjected to agarose gel electrophoresis and ethidium bromide staining (Zietkiewicz et al., 1994). Please note that technical term abbreviations will be explained when first used. This process amplifies genomic loci in different band sizes. Primers typically contain one to four degenerate nucleotides that extend to microsatellite regions at either the 3' or 5' ends, with lengths ranging between 15 and 30 nucleotides. The elevated proportion of GC in the primers leads to a high binding temperature, ensuring secure binding and, consequently, the binding of each primer to DNA. The base content is determined by the binding temperature's composition. ISSR is a notable marker due to its ability to facilitate primer design without sequence information. Studies conducted by Gupta et al. (1994) and Zietkiewicz et al. (1994) utilized ISSR to study genetic similarity, gene mapping, and taxonomy. However, ISSR shares similar limitations with the RAPD marker system, including low reproducibility and homologous particles of similar size.

Moreover, KesawatveDas (2009) identifies a lack of specificity as an additional disadvantage of ISSR.

Single Nucleotide Polymorphisms (SNPs)

The genome of individuals in populations displays Single Nucleotide Polymorphisms (SNPs), which are variations occurring in sequences of nucleotides.SNP variation is prevalent in many species of living organisms. SNP formation is a result of changes in nucleotide sequences caused by splicing and insertions or deletions (InDels). Gene mapping, breeding with the aid of markers, and map-based cloning studies rely on SNPs as a vital tool. Usually, variations in non-coding DNA regions are frequently observed; whereas changes in coding amino acids occur at sites that could impact amino acids, potentially leading to a modification in the sequence. However, changing the amino acid sequence does not necessarily entail alterations in the gene product (Sunyaev et al., 1999).

Cleaved Amplified Polymorphic Sequences (CAPS)

Polymorphisms are variations in restriction fragment lengths caused by single nucleotide polymorphisms (SNPs) or insertions/deletions (INDELs) that generate or eliminate recognition sites for restriction endonucleases in polymerase chain reaction (PCR) amplicons generated from locus-specific oligonucleotide primers. The CAPS assay uses amplified DNA fragments that are digested with a restriction endonuclease to display RFLP. Unique primers are utilized to amplify a mapped DNA sequence from two closely related individuals (e.g. two distinct inbred ecotypes), A/A and B/B, and from the

heterozygous A/B. The fragments amplified from A/A and B/B contain two and three RE recognition sites, respectively. When it comes to heterozygous A/B, two separate PCR products will be obtained, one cleaved three times and the other cleaved twice. When fractionated by agarose or acrylamide gel electrophoresis, the PCR products digested by restriction enzymes will produce distinct patterns. Some groups may show up as binary patterns (Figure 2). Most CAPS markers are co-dominant and locus specific. Most CAPS genotypes are easy to score and interpret. CAPS markers are easy to exchange between laboratories. The CAPS assay does not require the use of radioactive isotopes.
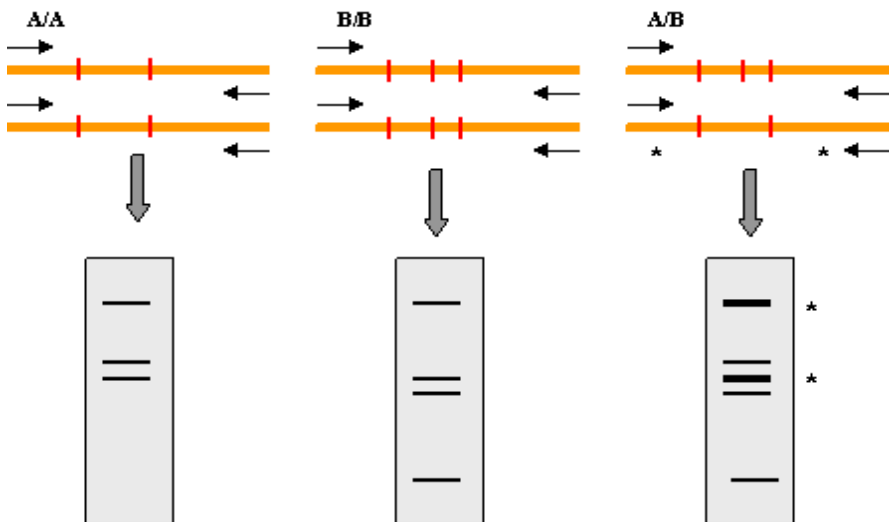
Figure 2. *Cleaved Amplified Polymorphic Sequences (CAPS)*
https://www.ncbi.nlm.nih.gov/probe/docs/techcaps

Retrotransposon (iPBS)

To date, numerous researchers have employed various molecular markers, including RAPD, AFLP, SSR, SRAP, SSAP, MASP, and ISSR, to ascertain genetic variation in wild germplasm and related species. It is recommended that newly developed DNA molecular marker techniques, such as transposon and retrotransposon markers, should be used to determine genetic variation in wild plant species. Moving DNA repeat sequences that can move within the genome are referred to as transposons, while the process of moving these sequences from one gene to another within the genome is known as transposition Retrotransposons are a type of transposable element (TE) encompassing mobile DNA sequences within the eukaryotic genome and are important components of plant genomes. Retrotransposons are mobile genetic elements and contribute to the physical size of the genome. For example, more than 75% of the maize genome is composed of retrotransposon sequences and long terminal repeat (LTR; Long Terminal Repeat) retrotransposons dominate the genome. These elements translate themselves into DNA using the enzyme reverse transcriptase and replicate using copy and paste (Figure3). The replication of retrotransposons generates genomic diversity, is distributed throughout the genome and shows a genetic inheritance that makes them highly useful as molecular markers. (Cakir et al., 2019). Phylogenetics, molecular ecology, biodiversity, linkage mapping and functional genomics have a wide range of applications for transposon-based marker techniques (Kwon et al., 2005; Soorni et al., 2013). Transposable elements are an important part of plant genomes and drive their evolution. They also provide a better understanding of

genome evolution, taking into account the mobility of a large number of TE families. In particular, molecular population genetics on individual TE replicates has recently been actively separating the silent families and highlighting the different evolutionary trajectories of retrotransposons between related species (Senerchia et al., 2013).
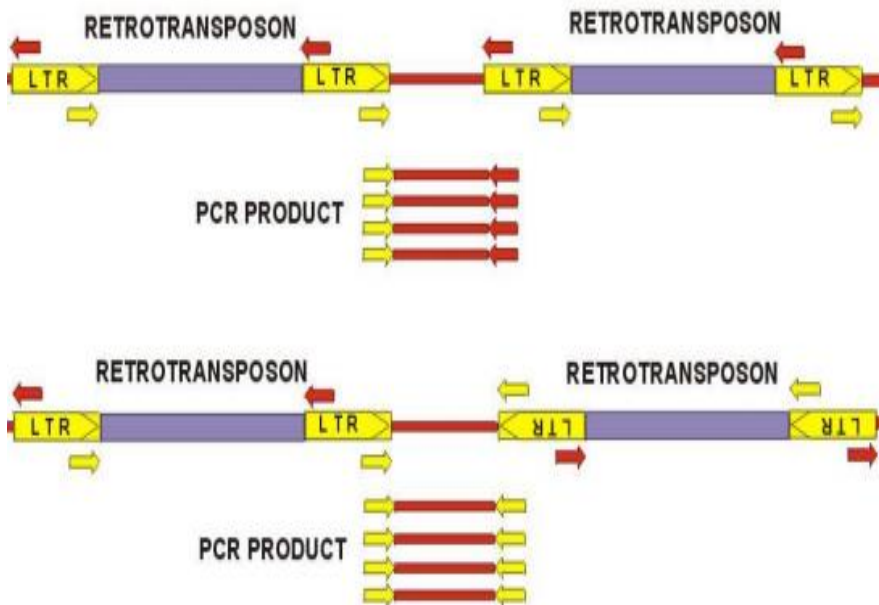
Figure3. Procedure of Retrotransposons (*iPBS*).
http://www.biocenter.helsinki.fi/bi/genomedynamics/markers .html)

Start Codon Targeted (SCoT) Polymorphism & CAAT Box-Derived Polymorphism (CBDP)

Start Codon Targeted (SCoT) Polymorphism The inverter start codon, which is a short conserved region in plant genes flanking the ATG in good condition, has been described in previous studies (Joshi and others 1997; Sawant et al. 1999). The ATG flanking the promoter initiation codon relative to the conserved region for SCoT was designed

as a single primer and used to amplify the genomic region (Figure 4). Recently, gene-targeted marker techniques have become an excellent and useful system for analysing genetic diversity. CAAT box-derived polymorphism (CBDP) is also known as a novel molecular marker based on the short conserved region of the CAAT box promoter (Figure 5). Singh et al, 2014 reported that eukaryotic genes share a conserved sequence in the CAAT box region upstream of the start codon (GGCCAATCT). Due to their many advantages, including reproducibility, high polymorphism and low cost, CAAT markers have been successfully used in genetics (Mahmood Aslan-Parvız,2020). CAAT markers have a characteristic nucleotide composition and are located approximately 80 base pairs upstream of the transcription start site, which has a significant effect on gene expression (Benoist ve ark.1980). In addition, by using longer primers with a higher annealing temperature, the CAAT method is expected to be more reliable and reproducible than random molecular markers (Collard and Mackill 2009). Consequently, this technique may offer advantages over random techniques in QTL mapping and DNA fingerprinting applications. (Andersen & Lubberstedt 2003, Etminan, A., 2018).

Figure 4. Procedure of SCoT (Collard, B.C., Mackill, D.J. 2009).
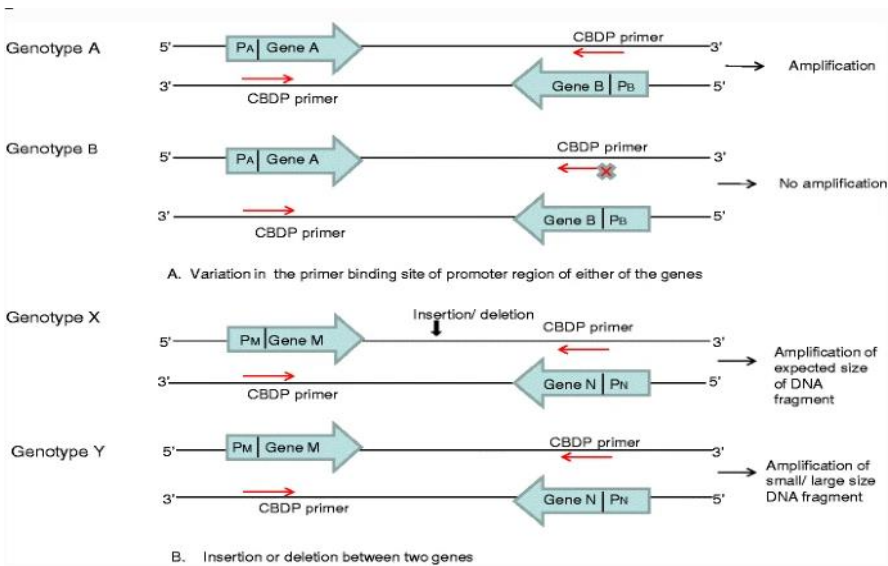
Figure5. Principle of CAAT box polymorphism (Singhet al.,2014).

Marker Assısted Selectıon (MAS)
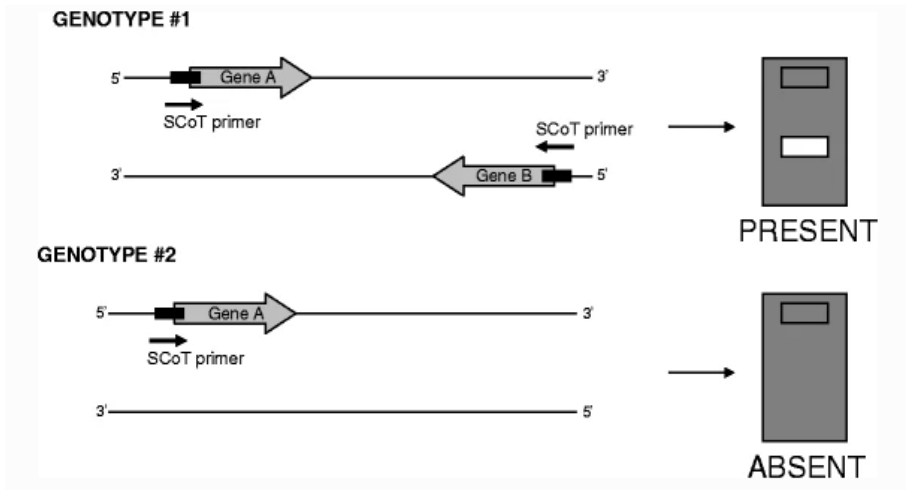
MAS is based on the use of easily identifiable molecular markers that are closely linked to genes controlling important agronomic traits. Plant breeding studies using MAS offer significant advances in

increasing the speed and efficiency of selection in classical breeding studies. Genetic linkage maps of most agronomic traits with molecular markers have been and are being established in many crops. The purpose of developing linkage maps is to determine the position of agronomic traits in the genome, molecular markers to identify closely linked markers for indirect selection in breeding. This means MAS, DNA markers closely linked to agronomically important genes, phenotypic selection or to assist phenotypic selection. A careful combination of MAS and phenotypic selection, based on a considered strategy, will guarantee positive results. Backcrossing allows efficient selection of the target area, linkage minimises scans and facilitates the detection of repeat parents. MAS can accurately identify the genetic resource in the F2 and F3 steps, providing significant advantages in the later stages of breeding programmes. The advantages of marker assisted selection studies are that it is a simpler method than phenotypic screening. Particularly difficult to identify, avoids wasting time and resources on traits. It facilitates the diagnosis of traits such as grain quality by allowing selection at the germination stage. It has high reliability as it is not affected by environmental factors. It allows single plant selection by segregating homozygous and heterozygous genotypes. It ensures complete identification of genetic resources and thus protection of genetic reserves. It helps to segregate varieties in case of confusion, thus protecting commercial rights. It allows genotypes with certain characteristics to be distinguished more strictly and accurately. Recent years have seen rapid advances in molecular biology and plant genetics. MAS has made great and important contributions to the use of genetic diversity conservation, production, preservation, breeding, etc. in plant

breeding studies. Over time, MAS applied plant breeding studies will make a more significant contribution to increasing the speed and efficiency of selection in classical studies (Yorgancılar et al.,2015).

3. Result

The use of molecular markers in plant breeding, backcrossing, gene-pyramiding, selection of recessive genes, selection of genes from wild gene sources increases the efficiency of classical breeding by providing benefits such as transfer and early selection, thus accelerating the development of new varieties. Molecular markers applications alone cannot replace classical breeding, but classical breeding is recognised as a complementary and supportive technique that increases success. The development of plant breeding using molecular marker technology and marker-assisted selection techniques can be carried out more effectively and in a much shorter time than classical breeding. It will be possible to achieve successful and reliable results in a short time. Each marker system has its advantages and disadvantages. Therefore, many studies have been have been carried out using combinations of different markers to overcome the disadvantages. In addition, development of molecular markers with advances in sequencing technologies has provided a technologies has provided a powerful tool in biotechnology research. It is important to knowledge of the techniques and applications of molecular markers to improve existing markers, and production of new markers.

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CHAPTER 3

MARKER STUDIES ON MAJOR *FABACEAE* FORAGES

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1. INTRODUCTION

Forage legumes offer particular benefits and drawbacks for ruminant production. Their primary advantages over grasses or cereals are often 1) reduced reliance on nitrogen fertiliser inputs, 2) high voluntary consumption and farm animal production under unrestricted feed supply, and 3) high protein contents. Forage legumes often have three main drawbacks: 1) less persistence under grazing compared grass, 2) higher risk of animal bloat, 3) difficulties in storing as silage or hay. Legume+grass combinations provide distinct advantages than legume or grass monocultures, such as well balanced feeding values, elevated resource usage efficiency, and imroved herbage production. On farms, it is extremely difficult to maintain the ideal legume content (40–60% of herbage dry matter), which is necessary to obtain given advantages. Legume forage based ruminant systems often have a low negative impact on environmental biodiversity, greenhouse gas emissions and nitrogen losses to water than ruminant systems based on cereals or grass supplemented with nitrogen fertilisers. The ability to reduce fertiliser nitrogen prices is the main economic advantage of legume forages over other forages, whereas the main disadvantage is typically a lower animal production intensity per hectar. Despite the fact that forage legumes for ruminant farming provide a number of advantages for both the farmer and society at large, their utilisation is generally characterised as being lower or declining compared to other forages. This is mainly a result of their perceived disadvantages at the farm level being greater than their perceived advantages. This could alter if the cost of fertiliser nitrogen to

product (meat/milk) rises happened in several countries over the past few years (Phelan et al., 2015).

In forage grasses and legumes, genomic selection is projected to primarily enhance selection accuracy, decrease cycle times, and decrease evaluation costs per genotype. Genomic selection application to forage breeding may have highest benefit under these conditions: 1) when individual plant phenotypic evaluations cannot accurately predict performance under sward conditions; 2) if it is hard or not possible to apply selection pressure within families; or 3) when time-consuming phenotypic evaluations require lengthy cycle times, such as 4-6 years (Simeao Resende et al., 2014).

Due to difficulties and confusions brought on by the wide variety of potential bred species, target environments, target traits, crop utilisations, seed markets, breeding approaches, as well as by conflicting demands from the seed industry, farmers, and society, forage crop breeding is vulnerable to the risks of lack of focus. Despite the great number of successful fodder crop breeding achievements, missteps brought on by lack of attention were not infrequent. Examples have included a variety of mismatches between selection and target environments, ignoring crucial qualities (such as seed yield), relatively ineffective multi-trait selection, and inadequate exploitation of genetic variation, among other factors. Delays in variety registration procedures and poor research on a number of important topics have also produced problems. High costs that can be reduced by marker-based methods prevent simultaneous selection for multiple features. The application of genomic selection and other marker-based selection strategies for

increased forage yield requires careful consideration of methodology, plant material, and phenotyping settings. As a general trend, it was forecast decreased breeding effort for minor forage crops and more breeding attention to legumes, as the adoption of ground-breaking technologies depends on seed market potential (Annicchiarico et al., 2016).

Variety development in a crop breeding programme is a complex process with cyclical procedures during long periods. A successful plant breeding programme depends on selecting the right breeding strategy (Hoyos-Villegas et al., 2019).

2. Marker studies on *Medicago sativa* L.

The most frequently planted forage legume in the world for hay and pasture is alfalfa (*Medicago sativa* L.) (Wang et al., 2013). It has high protein level, well feeding value, and favourable environmental impact which are the basis for its agronomical attraction (perenniality and biological nitrogen fixing ability). Allogamous, autotetraploid, and heterozygous describe the species of alfalfa. To improve the frequency of desirable individuals, breeders apply selection pressure for a few agronomic qualities in a breeding pool. Synthetic cultivars called "cultivated varieties" are produced via panmictic reproduction of a group of different numbers of parents across three or four generations. The parents may be full-sib families, half-sib families, or clones. Breeding initiatives typically concentrate on feed quality, lodging resistance, and pest or disease resistance. Because of the severe inbreeding depression, selection following inbreeding stages is avoided or limited. In order to develop new varieties, the breeders maintain a breeding pool by

extracting from plants or families. It is challenging to determine whether the original genetic variety existing in the breeding pool was preserved, expanded by introductions, or decreased through rigorous selection after numerous cycles of selection (Flajoulot et al., 2005).

Alfalfa cultivars are typically generated by phenotypic selection. Although phenotypic selection has effectively enhanced numerous features, including disease resistance, pest resistance, cold survival, etc., it takes time. For gain per unit cost and time, molecular breeding techniques like marker-assisted selection may improve crop development efficiency (Wang et al., 2013). For plant molecular breeding, effective and reliable molecular markers are crucial. In autotetraploid species like alfalfa, several alleles of SSR (simple sequence repeat) markers are more advantageous and effective for QTL mapping and genetic linkage mapping than dominant and bi-allelic markers (Wang et al., 2013). Numerous population-genetic investigations in this species are challenging due to severe tetrasomic inheritance and inbreeding depression. As a result, breeding strategies to produce new (synthetic) cultivars typically include crossing a number of outstanding individual plants. Through an assessment of genetic variety using molecular markers, one method of identifying the parental genotypes with the greatest genetic diversity can be used. In outcrossing fodder species like alfalfa, genetic diversity study is also crucial for seed purity analysis, cultivar identification, and germplasm management (Falahati-Anbaran et al., 2007).

Wang et al., (2013) was studied to directly enrich SSR markers from alfalfa ESTs (Expressed sequence tags). National Center for

Biotechnology provided a total of 12,370 alfalfa ESTs. A total of 774 SSR-containing ESTs were discovered. The most prevalent motif type was tri-nucleotide repeats (48.8%), followed by di-nucleotide (26%), tetra-nucleotide (11.5%), penta-nucleotide (9.7%), and hexanucleotide (3.9%). A total of 100 EST-SSR primer pairs were successfully developed, and among alfalfa accessions, 29 showed polymorphism. PIC values showed that the EST-SSR markers were highly polymorphic.

Mengoni et al., (2000) provided confirmation of the usefulness of chloroplastic SSR to detect polymorphism in populations of planted alfalfa. The mapping of 107 SSRs found in the Medicago truncatula EST database in *Medicago sativa* (Julier et al. 2003) allows for the investigation of genetic diversity. Using allele dosages or tetraploid genotypic data, some of these markers were easyly scored (Flajoulot et al., 2005).

Using eight SSR markers, Flajoulot et al. (2005) examined the degree of difference between seven cultivars resulting from a single breeding effort and between these cultivars and the breeding pool. These highly polymorphic and codominant markers provide methods for analysing the genetic diversity of alfalfa, along with current population genetic data extended to autotetraploids. There were between 3 and 24 alleles per locus. Gene diversity on average was very high. The parameter FST indicated a low significant diversity among cultivars. 15 of the 21 cultivar pairs have significant differences.

There is limited information on variation in diploid germplasm in the *M. sativa-falcata* complex. In the study of Şakiroğlu et al., (2010), genetic relationships of farmed germplasm was the focus of diversity

investigations in alfalfa. Using 89 polymorphic SSR loci, a collection of 374 genotypes derived from 120 diploid accessions including Medicago sativa ssp. caerulea, hemicycla and falcata were evaluated to estimate genetic diversity, infer the genetic underpinnings of the current morphologic taxonomy, and establish structure of population. A model-based clustering analysis of the genomic data revealed two clearly separate subpopulations that correspond to the morphologically characterised subspecies falcata and caerulea, providing confirmation of the subspecies hemicycla's hybrid character. Within each ssp. *caerulea* and ssp. *falcata*, there were two different subpopulations. Geographical distribution served as the basis for *caerulea* differentiation. Ecogeography was used to differentiate the two *falcata* groupings. The findings demonstrate that, with some exceptions, genetic marker data reflect taxonomic relationships based on morphology and that separate subspecies are readily distinguishable at the diploid level.

3. Marker studies on *Vicia faba* L.

Due to its high seed yield, high biomass, high seed protein content, crucial function in crop rotation, efficient nitrogen fixation, and ability to improve soil, the *Vicia faba* L. (faba bean) is a significant food and feed legume (Wang et al., 2012). *Vicia faba* marker-assisted genetic investigations are severely hampered by the large genome size. There are over 38,000 *Vicia faba* germplasm specimens preserved by 37 collections worldwide; the ICARDA in Syria is home to the largest collection. Following ICARDA with over 9 thousands accessions is CAAS in China with over 5,200 accessions (Duc et al., 2010). Significant genetic resources for *Vicia faba* breeding studies can be

found in local populations. Two *Vicia faba* germplasm pools exist in Europe: the Central European and North-west Europe gene pool, which mostly consists of small- and large-seeded *Vicia faba* major types, and the Mediterranean gene pool, which consists of a mixture of *Vicia faba* major, *Vicia faba* minor, and medium-seeded *Vicia faba* equina types (Terzopoulos & Bebeli, 2008).

One of the earliest crops cultivated by humans, faba beans provide high-protein seeds for both human and animal consumption. However, the ANFs (antinutritional factors) contained in *Vicia* spp. seeds provide a significant barrier to their increased use as grain legumes. The absence of vicine and convicine (v-c), which cause favism in humans and decreased animal performance or low egg production in laying hens, is a breeding goal for the *Vicia faba* L. (Gutierrez et al., 2006).

Using ISSR markers, the genetic diversity and connections of 802 landraces and varieties of *Vicia faba* L. from various geographical areas in China and abroad were studied by Wang et al., (2012). With 11 ISSR primers, 212 repeatable amplifed bands were produced, of which 209 were polymorphic. The most genetically diverse accessions came from North China, and the least diverse accessions came from Central China. Based on PCA (principal component analysis) and UPGMA clustering analysis, Chinese spring faba bean germplasm was clearly distinguished from Chinese winter faba bean. Winter influxes from the eastern Chinese provinces of Zhejiang, Jiangxi, Sichuan, and Guizhou were substantially different from those from other regions in China. Accessions from Europe were genetically more similar to those from North Africa as a continental geographic group. The grouping outcomes of accessions

from Asia, Africa and Europe were clearly related to their geographical origins based on ISSR data. The overall findings showed that the geographic origin and ecological habits of the faba bean germplasm were closely related to their genetic relationship.

Inbred line crosses from various Mediterranean-type faba bean genetic pools seem to hold promise to develop synthetic high yielding cultivars. ISSRs (Inter-Simple Sequence Repeats were used in the study of Terzopoulos & Bebeli, (2008) to 1) describe the genetic diversity of local Greek populations of *Vicia faba*, and 2) classify local populations and compare obtained agronomic/morphological data. On 57 bulked samples of twenty local populations of faba bean, 5 minor-type populations, and fifteen Mediterranean-type populations, four ISSR primers were applied. 192 DNA bands in total were discovered, of which 190 were polymorphic. High levels of genetic variation within populations were discovered by Analysis of molecular variance (AMOVA). Four minor-type populations were distinguished from the Mediterranean-type populations using cluster analysis utilising the Unweighted pair-group method with arithmetic mean (UPGMA) and Principal coordinate analysis (PCoA). The inhabitants of the Mediterranean region were further divided into two clusters, and the resulting groups were less diverse than the gene pool of the original Mediterranean region. In conclusion, the study provided evidence in favour of the hypothesis that there are at least two distinct germplasm pools for Mediterranean-type faba beans.

All legume crops suffer significant losses in both quality and quantity from storage insect pests. Mohamed & Abd-El Hameed, (2014)

identified genetic and biochemical markers for susceptibility and resistance to infestation by insects by examining some morphological characteristics and biochemical components in 8 faba bean genotypes. The findings demonstrated that the effect of insect infestation was significantly or non-significantly increased in the resistant genotypes of faba bean plants as compared to the susceptible genotypes by the leaf chlorophyll content, tannin, phenol, PPO (polyphenol oxidase), peroxidase (POD), and protein content. Wide variations across genotypes were shown by protein electrophoresis, which also identified several biochemical indicators (SDS-PAGE, sodium dodecyl sulphate poly acrylamide gel electrophoresis). In addition, Inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) investigations were used to identify molecular genetic markers for preserved insects' resistance.

Vicia faba L. (Faba bean) is a legume with high nutrition value and importance for soil protection and sustainable agriculture. Transcriptome sequencing was carried out by Carrillo-Perdomo et al., (2020) to decompose the *Vicia faba* genome complexity and to detect gene-based SNPs. To enable the generation of a high-density consensus genetic map encompassing 1,728 markers evenly dispersed in six linkage groups, a set of 1,819 gene-based SNP markers polymorphic across 3 recombinant line populations was chosen. Between *V. faba* and most closely related and sequenced legume species, such as barrel medic, pea, or chickpea, solid blocks of macrosynteny were found. In more diverse species like common bean or cowpea, several blocks could also be seen. The genetic tools generated in this work can be utilized for map-based cloning, genetic diversity, association mapping, linkage disequilibrium, or

comparative genomics. This will speed up marker-assisted selection and genomic-assisted breeding in the faba bean and increase the efficiency of candidate gene identification.

4. Marker studies on *Trifolium* spp.

Trifolieae is the tribe that includes 240 perennial and annual plant species that are both wild and farmed. Some are significant fodder plant used for grazing, silage production, temporary cover crops, and manure crops around the world (Istvanek et al., 2017). Both *Trifolium pratense* L. (red clover) and *Trifolium repens* L. (white clover) are perennial pasture legumes that are significant economically in many temperate regions in the world. For these species, a variety of molecular markers, mainly microsatellites or SSR, and genetic maps have been developed by Kölliker et al., (2001); Jones et al., (2003); Sato et al., (2005); Barrett et al., (2004); Wang et al., (2010); Isobe et al., (2003); Isobe et al., (2009).
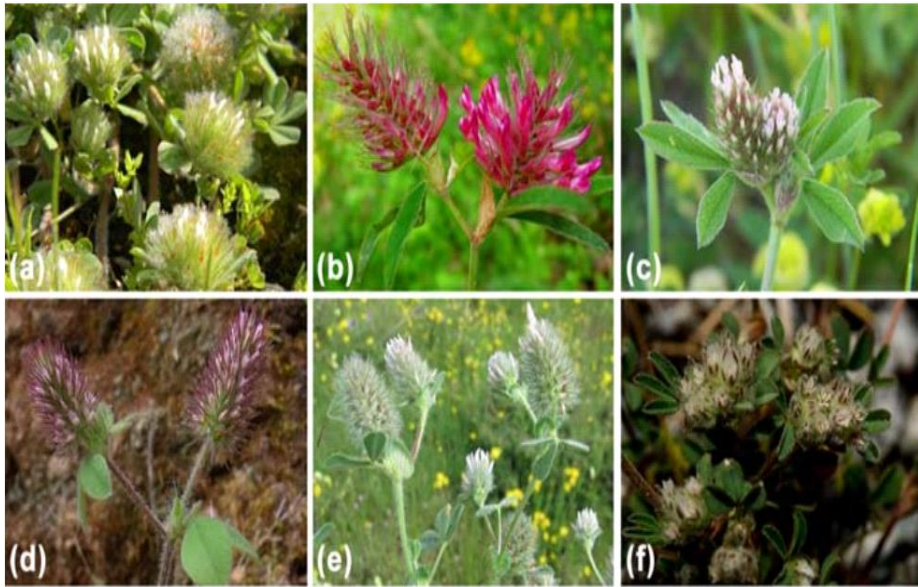
Fig. 1. a) *Trifolium cherleri* L., (F. M. Gutiérrez); b) *Trifolium patulum*, (L. Bernardo); c) *Trifolium striatum* L. subsp. *striatum*, (F. M. Gutiérrez); d) *Trifolium ligusticum*, (López Tirado); e) *Trifolium sylvaticum*, (L. Bernardo); f) *Trifolium saxatile* (F. Le Driant) (Scoppola et al., 2018).

Seven SSR markers and 21 morphological features gathered in the field and a greenhouse in Brazil were used by Dias et al. (2008) to characterise 57 Red Clover accessions from the U.S. core collection and 1 population grown in Brazil. For the majority of the 21 morphological features, there was significant variation between accessions. Five unique clusters were found after a cluster analysis based on morphological features, which divided the populations according to growth habit, persistency, and dry matter productivity in addition to flowering earliness, which has already been mentioned. According to an examination of molecular variance, the majority of variation (84%) occurred at the population level. Although molecular markers likewise divided the accessions into 5 clusters, the composition of the discovered

groups using morphological and molecular data was not the same. Utilizing genetic diversity in breeding programs necessitates selecting the most promising populations, combining advantageous features like persistency and forage productivity, and maybe utilizing within-population variation to find beneficial genotypes that mat serve as parents of the synthetic varieties.

By specifically reanalyzing previously published assembly, Istvanek et al. (2017) undertook a study to increase the present understanding of red clover's coding areas and improve its practical use. Following manual review of the Blast2GO annotation, approximately 5 thousand genes were characterized by Illumina paired-end sequencing. In response to biological activities, genes were categorized into biosynthetic and metabolic pathways, with 7,517 genes being assigned to particular pathways. In all routes, 17,727 enzymatic nodes were also described. Red clover coding sequences were used to identify 6,749 potential microsatellite loci and approximately 4 thousand potential SSR (simple sequence repeat) markers that may produce PPCR products. For the purpose of assessing polymorphism in specific plants, a set of 8,622 scattered across the genome SNPs was constructed. Targeted coding sequences and the validation of homozygosity or heterozygosity in 5 confirmed loci were successfully sequenced using a single marker using temperature switch PCR. To rapidly implement genome-based breeding strategies, find the genes responsible for important traits, and conduct genome-wide association studies, big sets of SSRs and SNPs that are predicted across the entire genome are essential. Breeders can also benefit from a thorough understanding of the genetic links among

breeding stock when planning crosses or for the protection of plant varieties. Diagnostic applications can benefit from single-marker testing.

One of the most common cool-season legume forage worldwide is *Trifolium repens* L. (white clover). With the right care, it may thrive in a diversified soil types and climatic environments. Cooler temperatures and higher moisture trigger the start of active development from germination of seeds or existing plants, and growth continues until nighttime temperatures reach freezing (Zhang et al., 2007).

Allotetraploid *Trifolium repens* L. is extensively used as forage for livestocks and an important target for marker-assisted breeding. Using SSR markers based on sequences from numerous *Trifolieae* species, containing red clover, *Medicago truncatula*, white clover, and soybean (*Glycine max* L.), a white clover genetic linkage map was produced by Zhang et al., (2007). A cross between 2 highly heterozygous genotypes produced an F1 generation of 179 individuals, which was used for genetic mapping. Using DNA from 2 parents and 14 F1 from mapping population, total 1,571 SSR markers were checked for amplification and polymorphism. The map consists of 415 loci amplified from 343 SSR primer pairs, containing 181 from red clover, 83 from white clover, 77 from *Medicago truncatula*, and 2 from soybean. Red clover, white clover, and alfalfa (*M. sativa* L.) markers were compared to their locations on maps, revealing probable macro-colinearity between the three species of *Trifolieae*. This map may help to connect QTL with SSR markers and speed up the breeding and selection of white clovers using markers.

Using RAPD markers to generate genetic fingerprints, Bortolini et al. (2006) attempted to evaluate the genetic heterogeneity of the USDA core collection of white clover, which consisted of 78 accessions covering 50 countries and 2 well-known cultivars. DNA bulks produced by extracting and combining 20 randomly selected individuals from each accession were used. The findings demonstrated genetic similarities between the accessions, enabling the identification of each accession separately by only 3 primers. The findings also revealed significant genetic variation within white clover core collection, likely as a result of its reproductive strategy and ploidy level.

Trifolium alexandrinum L., often known as Egyptian clover (or Berseem), has been used as a pasture crop widely throughout western Asia and northern Africa. Using AFLP data, origin and the ancestry of Egyptian clover were investigated by Badr et al., (2008). The evidence suggests that *T. alexandrinum* accessions from Syria and Egypt are closely related to *T. apertum*, *T. berytheum*, and *T. salmoneum*. But, geographical distributions and crossability indicate that *T. apertum* is an improbable progenitor. Although despite having a tight relationship with T. berytheum, T. salmoneum seems to be the most likely progenitor for Syrian material of Egyptian clover. Due to its propensity for interbreeding, T. berytheum and T. salmoneum may be regarded as two main species that human domesticated Egyptian clover in Syria by artificial selection.

5. Marker studies at *Vigna* spp.

The cowpea, or *Vigna unguiculata* L., is a fodder crop that can be fed either fresh or as hay. Using 22 RAPD and ISSR markers, the genetic

diversity and connections among eleven cowpea genotypes representing 2 cultivars and 9 elite genotypes were examined by Gajera et al., (2014). Regarding the detection of polymorphism, ISSR markers outperformed RAPD test. However, it was discovered that RAPD outperformed ISSR in terms of resolution power and the average number of polymorphic loci/primer. Additionally, RAPD markers were more dominant than ISSR markers in terms of total genotype-specific marker loci, Shannon's information index, Nei's genetic diversity, average heterozygosity, and total average heterozygosity. The most diversified genotypes can be recognized using the cultivar-specific or genotype-specific unique DNA fingerprint produced by RAPD and ISSR. According to a cluster analysis, the most diverse genotypes were distinguished from cultivars and genotypes that were only moderately diverse.

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CHAPTER 4

ANALYSIS OF POSTGRADUATE THESES ON MEDICINAL LEECHES IN THE FIELD OF SCIENCE IN TÜRKİYE

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INTRODUCTION

The use of leeches for medicinal purposes dates back to ancient times (Elliott and Kutschera, 2011). There is a long history of leech treatment, which has functioned as an important element of folk medicine (Parlakpınar and Polat, 2021). With the US Food and Drug Administration's approval of the use of leeches in plastic and reconstructive surgery in 2004, medicinal leeches have started to take their place in modern medicine (Singh, 2010; Mumcuoğlu, 2014). In addition to the findings of scientific studies on the qualitative and quantitative determination of bioactive substances in the salivary glands of leeches, proving the effectiveness of bioactive substances in treatments with evidence-based clinical applications has played an important function in the replacement of leeches in modern medicine (Singh, 2010; Lemke & Vilcinskas, 2020). In Türkiye, the "Regulation on Traditional and Complementary Medicine Practices (GETAT)", which also covers leech applications, entered into force in 2014 (Biçer & Yalçın Balçık, 2019). The intensive use of leeches in modern medicine as well as traditional medicine increases the demand for leeches day by day, which is reflected in the prices significantly (Ceylan et al., 2019). The sales price of leeches, which was 0.75 Turkish Liras/piece (0.30 USD$/piece) on average in 2014 when the GETAT regulation came into force, increased approximately 5 times to 40 Turkish Liras/piece (1.50 USD$/piece) as of 2023-September.

Leeches are used not only in medicine but also in veterinary medicine for the treatment of some diseases and in cosmetics for the production of creams (Nikonov et al., 1999; Sobczak and Kantyka,

2014). In addition to the medical field, to meet the increasing demand for leeches due to the need arising in the field of veterinary medicine and cosmetics, both the hunting pressure on leech stocks in nature and the deterioration of the shallow sensitive habitats where they live due to global climate change and human interventions and the danger of drying out have been included in the category of endangered species (Utevsky et al., 2010; Sağlam, 2018). As a result of this situation, international trade of medicinal leeches is carried out with certain quotas allocated to countries under the "Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)" (Sağlam, 2011; Ceylan and Çetinkaya, 2017).

According to the records of the General Directorate of Fisheries and Aquaculture of the Ministry of Agriculture and Forestry of Türkiye, there are 11 licensed medicinal leech farms in Türkiye with a project capacity of 7,792 kg/year (6,592,000 pieces/year) (General Directorate of Fisheries and Aquaculture, 2023). Unfortunately, records of the actual production volume are not available. Although our country is a leader in the international trade of leeches collected from nature, it is not at the desired level in aquaculture (Sağlam, 2011). According to current CITES data, in 2021, a total of 3,825,735 leeches were subject to international trade, of which 3,302,435 leeches were cultivated and 523,300 leeches were collected from nature. While Türkiye's share in the trade of leeches collected from nature was 85.4% with 447,000 leeches, this share was only 0.7% with 23,300 leeches from aquaculture (CITES, 2023).

This chapter aims to analyze postgraduate theses in Türkiye produced on medicinal leeches, which have both economic and

ecological importance, in the field of Science and to provide a basis for future theses.

MATERIAL AND METHODS

Theses produced in the Science group in Türkiye were accessed from the Thesis Centre of the Council of Higher Education of Türkiye. Using the Detailed Search option, the keywords medicinal leech, leech, *Hirudo*, *Hirudo verbana*, *Hirudo sulukii*, *Hirudo medicinalis*, and Hirudinea were entered in the Title and Abstract sections. Theses were examined in terms of university, institute, main science department, thesis type, year, author, the undergraduate program graduated by the authors, supervisor, subject, scope, page, language, and keyword. The concrete outputs obtained are summarized by giving the imprint of the theses. In accordance with professional ethics, only theses in the Science group were taken into consideration, theses grouped as Medicine and Social Sciences were not included in the scope of this study.

Graphics were prepared in the GraphPad Prism 8 program and the word cloud was generated from https://www.jasondavies.com/wordcloud/ by using the keywords specified in the theses.

RESULTS

Universities

A total of 11 universities published theses in this field. Ege University and Fırat University published the greatest number of theses in this field with 3 theses each. Kahramanmaraş Sütçü Imam University and Sinop University produced 2 theses, while the other universities

produced one thesis each. The distribution of theses produced in the field of Science based on universities is given in Figure 1.

Institutes

Theses in this field were produced under two different institutes. While 16 theses were produced in the Graduate School of Natural and Applied Sciences, one thesis was produced in the Graduate School of Postgraduate Education.

Main Science Departments

Theses in this field were conducted under 8 different Departments of Main Science. The Department of Biology produced the greatest number of theses in this field with 5 theses. The departments of "Aquaculture and Diseases" and Fisheries produced 3 theses, the Aquaculture Department produced 2 theses and the others produced one thesis each. The distribution of theses based on the main science department is given in Figure 2.

Thesis type

Thirteen theses were written for a master's degree and four for a Ph.D. degree.

Years

The first thesis in this field was published in 2006 and the first Ph.D. thesis was written in 2016. The increase in the number of theses in recent years draws attention. More than half of the theses were written after 2020. The year in which the most theses were written was 2020

with 4 theses. The cumulative total of theses produced in this field according to years is shown in Figure 3.

Authors

Except for two of the theses published in this field, they were written by different authors. One author is studying in the field of Hirudiculture (medicinal leech breeding) who has produced both a master's and doctoral thesis. When the undergraduate graduation of the authors is examined, it is seen that the graduates of Biology (n: 7) and Fisheries Engineering (n: 6) are the undergraduate programs of the authors who produced the most theses in this field (Figure 4).

Thesis Supervisors

Theses produced in this field were supervised by 14 academicians. Considering their academic titles at the time of publication of the thesis, it is seen that the supervisors mostly have the academic title of Professor. With 10 theses, the title of Professor was followed by Associate Professor with 6 theses, Assistant Professor with 2 theses, and Aquaculture Engineer (Ph.D.) with one thesis. Only two of the theses had a second thesis supervisor. The distribution of thesis supervisors according to their academic titles is given in Figure 5.

In terms of their current academic title, Prof. Dr. Naim SAĞLAM (n: 3), Prof. Dr. Özlem ÇAKICI (n: 2), Assoc. Prof. Dr. Seval DERNEKBAŞI (n: 2) and Assoc. Prof. Dr. Mustafa CEYLAN (n: 2) were the academician who advised the theses produced in this field in the greatest number. The academicians who supervised the theses are given in Figure 6.

Thesis writing languages

Only one of the theses was written in English and the others were written in Turkish.

Number of pages

Theses were written on average 75 ± 52 (min: 17, max: 231) pages. When analyzed according to the thesis type, it is seen that Ph.D. theses are 138 ± 74 (min: 62, max: 231) pages and master's theses are 56 ± 19 (min: 17, max: 89) pages.

Subject and scope

Theses produced in this field included in Aquatic products, Biochemistry, Biology, Chemistry, Fisheries, Polymer science and technology, and Zoology. The subject of Aquatic products was the most classified subject with 10 theses. The distribution of theses according to the subjects is given in Figure 7.

When their scopes are analyzed, it is seen that the theses in this field are mostly within the scope of Hirudiculture (n: 5) and Systematics/Fauna (n: 4). The distribution of theses according to their scope is given in Figure 8.

Keywords

In the writing of the theses, 72 different words were used. *Hirudo verbana* (n: 9), medicinal leech (n: 8), reproduction (n: 4), Hirudinea (n: 3), and *Hirudo medicinalis* (n: 3) were the most preferred keywords. The keywords used in the theses are given in Figure 9.
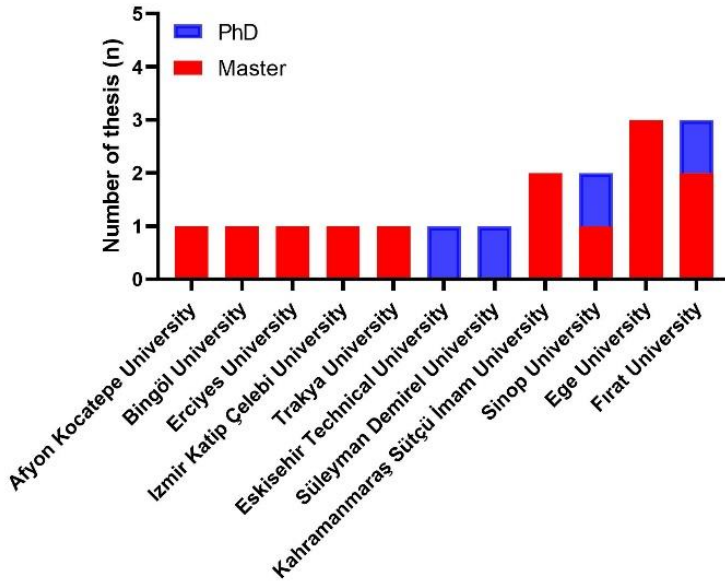
Figure 1. Theses by universities.
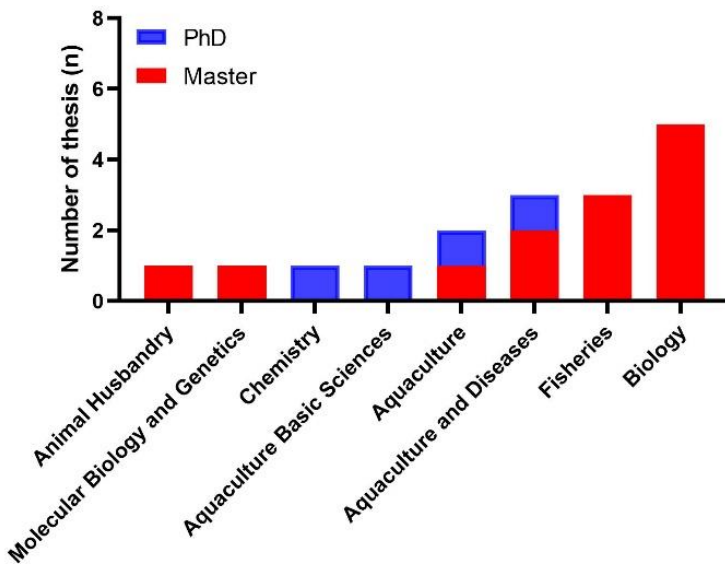
Figure 2. Theses by main science departments.
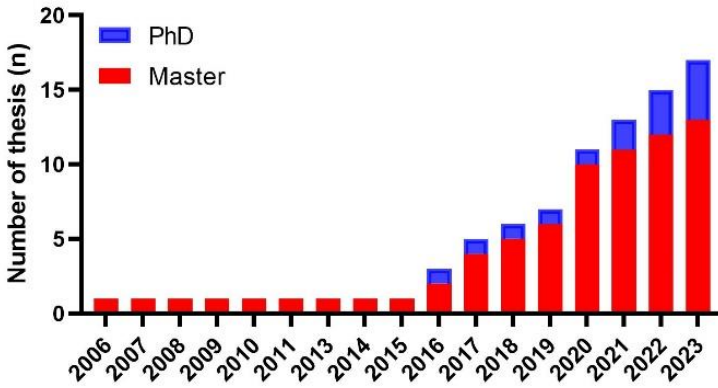
Figure 3. Theses by years.
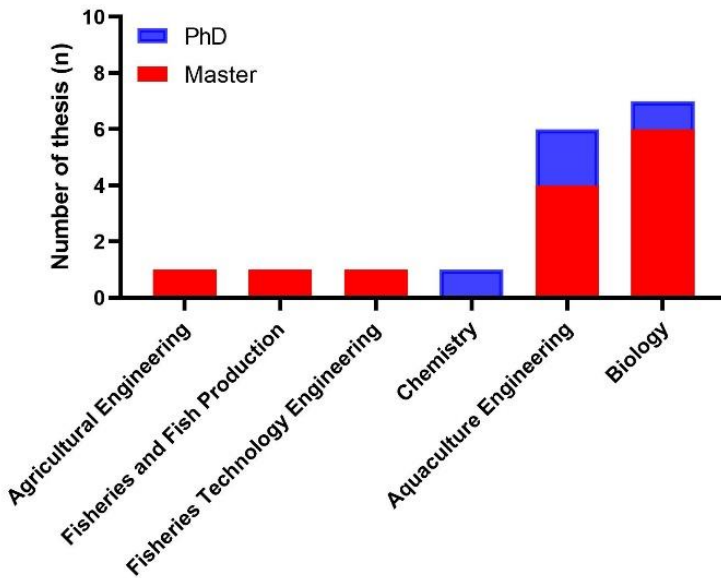
Figure 4. Theses according to the undergraduate programs graduated by the authors.
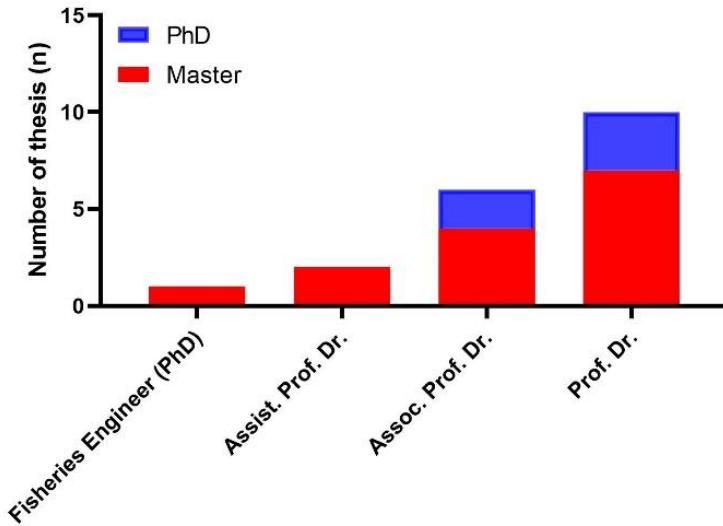
Figure 5. Theses according to the academic titles of supervisors.
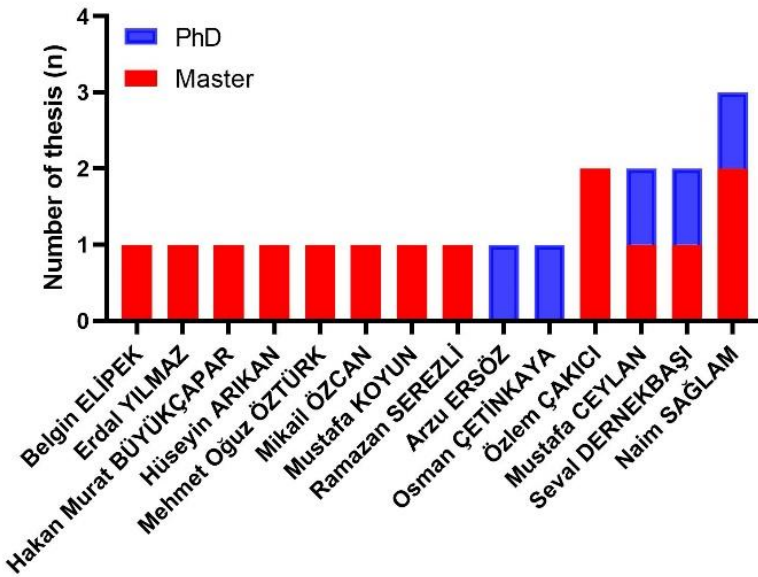
Figure 6. Theses according to the supervisors.
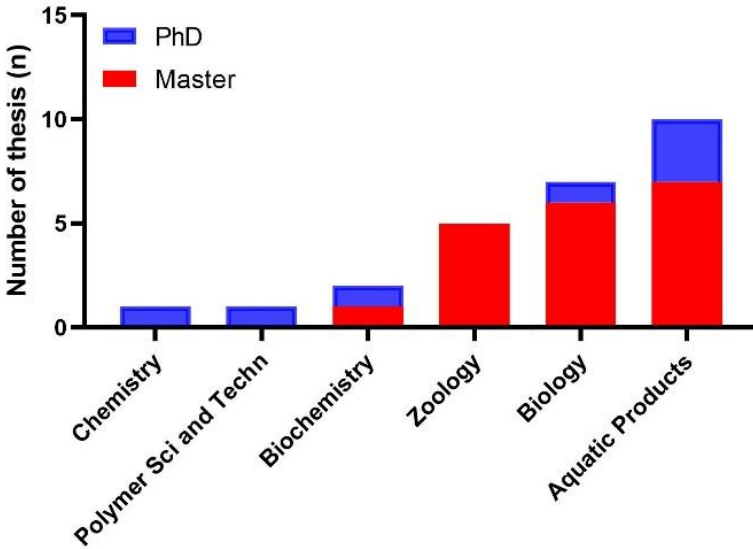
Figure 7. Theses according to the subjects.
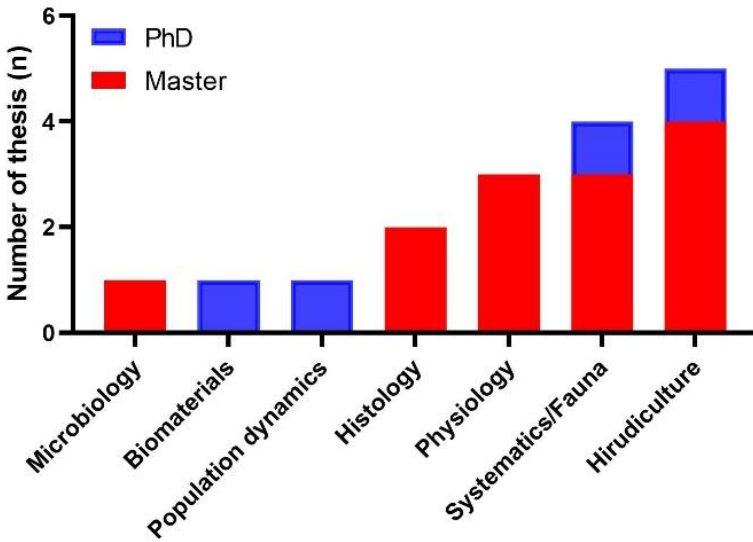
Figure 8. Theses according to the scopes.

Figure 9. Keywords used in theses. The font size of the words reflects the frequency level.

Details and outstanding outputs of theses

Details of the theses and outstanding outputs in chronological order are listed below.

1. Thesis

Title: The investigation of hyaluronidase activity in muscle of medicine leech (*Hirudo medicinalis*)

Author: Ayşegül ŞAHİN

Supervisor: Assoc. Prof. Dr. Naim SAĞLAM

University: Fırat University

Year: 2006

Thesis type: Master

Outputs: This study aimed to investigate the amount of hyaluronidase in the tissue of the medicinal leech *H. medicinalis* species by spectrophotometric method and its changes according to months. Hyaluronidase content in the muscle tissue of leeches was highest in

March and lowest in September. It was found that the distribution of Hyaluronidase content according to months was statistically significant.

2. Thesis

Title: Determination of ecology, population size and catch efficiency of medicinal leech (*Hirudo verbana* Carena, 1820) populations in wetlands around Lake Eğirdir

Author: Mustafa CEYLAN

Supervisor: Prof. Dr. Osman ÇETİNKAYA

University: Süleyman Demirel University

Year: 2016

Thesis type: Ph.D.

Outputs: This study aimed to determine the ecology, population size, and collection efficiency of the populations of the medicinal leech *H. verbana* living in the wetlands around Lake Eğirdir. It was determined that medicinal leeches are one of the important components of the wetlands around Lake Eğirdir and have a commercial value with a stock size of 593 kg. Clustered in 4 different age groups, leech populations were found to breed in July-September. The average annual catch yield of leeches was 53 ± 71 leeches/hour/collector, and the greatest number of leeches was caught in June.

3. Thesis

Title: Investigation of the immune system cells of *Hirudo medicinalis* Linnaeus, 1758 (Hirudinidae, Clitellata) with light and fluorescence microscopy

Author: Irmak ATALAYIN

Supervisor: Prof. Dr. Hüseyin ARIKAN

University: Ege University

Year: 2016

Thesis type: Master

Outputs: This study was carried out to determine the immune system cells of *H. medicinalis* by both light and fluorescence microscopy. Four different haemocyte types (hyaline hemocytes, granular hemocytes, and eleocytes), which are thought to originate from prohaemocytes, were identified in medicinal leeches. These hemocytes play important roles in antibacterial and antiparasitic defense mechanisms, phagocytic activity, melanization/capsule formation, clot formation, and wound healing.

4. Thesis

Title: The effects of various environments on the number of cocoons and juveniles in controlled medicinal leech breeding

Author: Bahadır UĞURAL

Supervisor: Assoc. Prof. Dr. Ramazan SEREZLİ

University: İzmir Kâtip Çelebi University

Year: 2017

Thesis type: Master

Outputs: This study aimed to investigate the effects of different media on the number of cocoons and offspring of medicinal leeches and to compare the reproduction efficiency of new media (water retention materials and cropped sponges) as an alternative to peat for hygienic medicinal leech breeding. Peat was determined more efficient than other media in both summer and winter trials.

5. Thesis

Title: A research on the culture of medicinal leech (*Hirudo verbana* Carena, 1820) in aquarium conditions

Author: Emin KARATAŞ

Supervisor: Assist. Prof. Dr. Seval DERNEKBAŞI

University: Sinop University

Year: 2017

Thesis type: Master

Outputs: In this study, the effects of cocopeat and floating material (styrofoam) on the reproductive efficiency of medicinal leeches were investigated. In the study in which 58 cocoons were obtained in a cocopeat platform, it was determined that no cocoons were left in the styrofoam and the broodstock leech mortality rate was more than doubled in the styrofoam platform.

6. Thesis

Title: Bacterial flora of the medical leech (*Hirudo verbana*, Carena, 1820) present in Karagöl (Kahramanmaraş)

Author: Gürkan TEMİZ

Supervisor: Assist. Prof. Dr. Mikail ÖZCAN

University: Kahramanmaraş Sütçü İmam University

Year: 2018

Thesis type: Master

Outputs: This study aimed to investigate the bacterial flora of medicinal leeches. In the samples taken from blood, skin, stomach, intestine, and mouth parts, *Aeromonas hydrophila* DNA group 2, *Pseudomonas alcaligenes*, *Aeromonas veronii/sobria* DNA group 8, *Aeromonas*

hydrophila DNA group 1, *Aeromonas ichthiosmia*, *Aeromonas sobria* DNA group 7, *Providencia alcalifaciens*, *Listeria seeligeri*, *Chryseobacterium scophthalmum*, *Enterobacter nimipressuralis*, *Flavobacterium resinovorum* and *Aeromonas veronii* DNA group 10 bacterial species were detected. This information will help to take necessary precautions for the safe application of medicinal leech treatment.

7. Thesis

Title: Determination of growth and reproduction properties of the medicinal leech (*Hirudo verbana* Carena, 1820) feeding by cattle and chicken bloods

Author: Murat MANAV

Supervisors: Prof. Dr. Hakan Murat BÜYÜKÇAPAR, Dr. Mustafa CEYLAN

University: Kahramanmaraş Sütçü İmam University

Year: 2019

Thesis type: Master

Outputs: This study aimed to determine the effects of two different blood types, mammalian (bovine blood) and poultry (chicken blood), on the growth and reproductive performance of medicinal leeches. It was determined that blood type had no significant effect on the growth performance and survival rates of leeches; however, in the process of gravidity formation and reproductive performance of gravid leeches, the chicken group performed approximately 2.5 times more than the bovine group. It was stated that the high reproductive performance of the leeches in the chicken group may be because chicken blood contains 50% more

blood glucose, which is an important energy source, compared to bovine blood.

8. Thesis

Title: Investigation of epidermis histologic structure of two leeches species *Hirudo sulukii* and *Hirudo verbana* (Hirudinida: Hirudinidae)

Author: Müge Uncu GÜL

Supervisor: Assoc. Prof. Dr. Özlem ÇAKICI

University: Ege University

Year: 2020

Thesis type: Master

Outputs: This study aimed to investigate the epidermis histology and secretory cell types in the body wall of *H. verbana* and *H. sulukii*. It was determined that the epidermis of both species consisted of simple single-layer cylindrical epithelium and secretory cells. The areas of epithelial and glandular cells were generally larger in *H. sulukii*, suggesting that *H. sulukii* is a more resistant species than *H. verbana*. The fact that the secretory area of the glandular cells is generally larger in *H. sulukii* indicates that the mucus content components are at different densities between the two species.

9. Thesis

Title: Investigation of Hirudinea (Annelida) fauna of inland waters in Edirne province

Author: Damla KAÇMAZ

Supervisor: Prof. Dr. Belgin ELİPEK

University: Trakya University

Year: 2020

Thesis type: Master

Outputs: The study was carried out to investigate the leech fauna in 254 localities in Edirne province. A total of 6 species (*Haemopis sanguisuga*, *H. verbana*, *Limnatis nilotica*, *Erpobdella octoculata*, *Erpobdella* sp., and *Placobdella costata*) belonging to 4 families (Haemopidae, Hirudinidae, Erpobdellidae and Glossiphoniidae) were identified.

10. Thesis

Title: Determination of the Hirudinea (Annelida) fauna of some water areas in Bingöl

Author: Tuba ELALTUNKARA

Supervisor: Prof. Dr. Mustafa KOYUN

University: Bingöl University

Year: 2020

Thesis type: Master

Outputs: The study was carried out to investigate the leech fauna of some wetlands in Bingöl. In the study, 8 different species including *H. verbana*, *Glossiphonia complanata*, *Theromyzon tessulatum*, *Placopdella ornata*, *Placopdella costata*, *Erpobdella octoculata*, *Erpobdella testacea* and *Piscicola geometra* were identified.

11. Thesis

Title: The investigation of the production of medicinal leech *Hirudo verbana* fed with bovine and chicken blood under laboratory conditions

Author: Boyukkhanım KARIMOVA

Supervisor: Prof. Dr. Naim SAĞLAM

University: Fırat University

Year: 2020

Thesis type: Master

Outputs: This study aimed to investigate the effects of feeding bovine and chicken blood by sieve and sausage intestine methods on the reproductive performance of medicinal leeches. Although more cocoons were obtained from leeches fed with bovine blood and intestine method, it was determined that the cocoons of leeches receiving chicken blood by sieve method were larger.

12. Thesis

Title: Investigation of the uses of bionanoconjugates for artificial blood purposes

Author: Umut ÇELİKOĞLU

Supervisor: Prof. Dr. Arzu ERSÖZ

University: Eskişehir Technical University

Year: 2021

Thesis type: Ph.D.

Outputs: In this thesis, the use of bionanoconjugates synthesized for artificial blood as food by the medicinal leech *H. verbana* and their effect on haemoglobin species (oxyHb, metHb, deoxyHb) were investigated in detail.

13. Thesis

Title: Serological and histological investigations on medicinal leech species *Hirudo verbana* and *Hirudo sulukii* (Hirudinida: Hirudinidae)

Author: Zalihe KÜRTÜRAL

Supervisor: Assoc. Prof. Dr. Özlem ÇAKICI

University: Ege University

Year: 2021

Thesis type: Master

Outputs: This study aimed to make amino acid, total protein, and total carbohydrate analyses in the mucus of *H. verbana* and *H. sulukii*, to determine the types of hemocytes and the fractions of hemolymph proteins, and to examine the histological structures of the anterior and posterior suction cups as contracted and relaxed. Analyses of the mucus and hemolymph of *H. verbana* and *H. sulukii* revealed some differences between the two species. As a result of the analyses performed in the mucus of leeches belonging to *H. verbana* and *H. sulukii* species, tryptophan was not detected in *H. verbana*, while arginine was not detected in *H. sulukii*. Tryptophan was detected in *H. sulukii* even at a low level, but there was no statistically significant difference between the two leech species. Four types of haemocyte cells (prohaemocyte, plasmatocyte, granulocyte, and eleocyte) were detected in both species and the cell areas of haemocyte types did not differ between the two species.

14. Thesis

Title: Morphological and molecular identification of leech species of Lankaran-Astara (Azerbaijan) region

Author: Shabnam FARZALI

Supervisor: Prof. Dr. Naim SAĞLAM

University: Fırat University

Year: 2022

Thesis type: Ph.D.

Outputs: This study was carried out to determine the morphological and molecular characteristics of Hirudinea fauna living in Lankaran-Astara region of Azerbaijan Republic. As a result of the study, 6 leech species belonging to 3 families Erpobdellidae (*Dina lineata*, *Trocheta* n.sp.), Glossiphoniidae (*Helobdella stagnalis*, *Hemiclepsis marginata*, *Placobdella costata*) and Hirudinidae (*H. orientalis*) were identified.

15. Thesis

Title: Investigation of saliva antimicrobial properties in medical leeches (*Hirudo verbana*) exposed to two different heavy metals (copper and zinc)

Author: Ebubekir ATICI

Supervisor: Prof. Dr. Erdal YILMAZ

University: Erciyes University

Year: 2022

Thesis type: Master

Outputs: The study was carried out to determine the effects of different concentrations of zinc and copper metals on the antimicrobial properties of the saliva of medicinal leeches. It was found that the amount of heavy metals stored in the tissues of medicinal leeches increased when they were exposed to zinc and copper metals. It was also found that malondialdehyde values in leech saliva increased in different concentrations and durations of zinc and copper exposure. From the data obtained, it was revealed that *H. verbana* is a useful biomonitor organism for zinc and copper heavy metals.

16. Thesis

Title: Investigation of the effects of feeding with different levels of D-glucose monohydrate added bovine blood in various growth and reproduction performances of medicinal leeches (*Hirudo verbana*, Carena 1820)

Author: Emin KARATAŞ

Supervisors: Assoc. Prof. Dr. Seval DERNEKBAŞI, Assoc. Prof. Dr. Mustafa CEYLAN

University: Sinop University

Year: 2023

Thesis type: Ph.D.

Outputs: This study aimed to compare the growth, reproduction, performance, and survival rates of medicinal leech *H. verbana* individuals fed bovine blood with different glucose levels. The greatest growth performance was found in the 2500 mg/dL group with a specific growth rate of 2.34, while the greatest survival rate and the greatest gravidity rate were found in the 750 mg/dL group. Although the greatest growth performance was found in the 2500 mg/dL group, when the survival and gravidity rates were taken into consideration, it was revealed that blood should be used with 750 mg/dL glucose level in medicinal leech breeding. Approximately 2.80 g/L D-glucose monohydrate was recommended to be added to bovine blood to obtain 750 mg/dL glucose level.

17. Thesis

Title: Molecular description of medicinal leech (*Hirudo verbana* Carena, 1820) based on DNA sequences

Author: Al-Hussein Hameed Hussein OKBI

Supervisors: Prof. Dr. Mehmet Oğuz ÖZTÜRK

University: Afyon Kocatepe University

Year: 2023

Thesis type: Master

Outputs: This study aimed to describe the anatomical and morphological characteristics of *H. verbana* specimens sampled from Karamık Lake and the sequence data of 18S rRNA and mitochondrial cytochrome C oxidase subunit I loci (mt COI). The mt COI sequences of *H. verbana* obtained during the study showed complete agreement with each other (OM721927-30 and OM033510) and with the mtCOI locus sequences of *H. verbana* isolates in the NCBI database (AY763151, AY763150, JN083801, KT692947, EF446694, KU216244). Thus, the molecular taxonomic position of the specimens belonging to the species *H. verbana* from Karamık Lake, whose anatomical and morphological characteristics were described, was confirmed by mtCOI sequence data.

DISCUSSION and CONCLUSION

Medicinal leeches have an important place in history and the first records date back to Ancient Egypt and the beginning of civilization (Whitaker et al., 2004; Elliott & Kutschera, 2011). For this reason, it is noteworthy that the first thesis on medicinal leeches in the group of Science in our Türkiye was produced in 2006 and there are currently few. The most important basis for the increase in the number of theses on

medicinal leeches in recent years has been the entry into force of the Regulation on Traditional and Complementary Medicine Practices in 2004, which authorizes the use of 15 different traditional treatment methods including medicinal leech treatment only by physicians in the treatment of certain diseases in hospitals and clinics. The pioneering efforts of Prof. Dr. Naim Sağlam in scientific studies on medicinal leeches in Türkiye, which started before the regulation, are admirable. When we look at the fields of expertise of the academicians who advised the theses produced in this field, it is seen that only two of the 14 academicians (Prof. Dr. Naim Sağlam and Assoc. Prof. Dr. Mustafa Ceylan) have medicinal leech-centred studies, while the others combine their different fields of expertise with the subject of medicinal leech from time to time. For this reason, increasing the specializations in the field of medicinal leeches in the academy with a multidisciplinary approach will bring about a qualitative and quantitative increase in the works in the field of medicinal leeches.

It is noteworthy that there is only one author (Dr. Emin KARATAŞ) who studied medicinal leeches in both master's and doctoral education. The study of complementary subjects not at the master's level, but at the undergraduate level starting from the graduation homework will make important contributions to the meaning, integrity, quality, and sectoral outputs of the theses produced as well as the accumulation of experience. In this respect, it is important to direct students to this field at the undergraduate level. In addition to graduation assignments, directing students to the research ecosystem within the scope of 2209-A - University Students Research Projects Support Program supported by

the Scientific and Technical Research Council of Türkiye (TÜBİTAK) will make effective contributions to the creation of scientific curiosity in students.

The authors who graduated from the programs of Biology and Fisheries Engineering come to the forefront of the theses produced. It is thought that the fact that the course contents in the relevant programs are directly or indirectly related to medicinal leeches and that the lecturers in the undergraduate program are close to the subject are effective in this. Especially considering the recent need for leech enzymes, it is important to focus on multidisciplinary studies, especially chemistry.

The words in the title are frequently used in the Keywords section. As a reflection of this situation, it is noteworthy that the words "medicinal leech" and "*Hirudo verbana*", which are frequently used in the titles, are repeated in the keywords section. However, the fact that keywords are different from the words in the title or the use of variants of the words in the title contributes significantly to the prominence of search engines in the results list (Kate et al., 2017; Bahadoran et al., 2020).

When the subject and scope of the theses are analyzed and the problems and needs in the field of medicinal leeches are taken into consideration, it is suggested to produce postgraduate theses on the following subjects.

- Theses on the biology and physiology of medicinal leeches are primarily needed. It will not be possible to obtain effective results in this field without obtaining basic information.

- Molecular-based studies on leech taxonomy will reveal new species existing in Türkiye as well as verification of existing ones.

- Determining the size and structure of medicinal leech populations will pave the way for the establishment of sustainable conservation policies.

- Theses on the marketing and international trade of medicinal leeches will contribute to the strengthening of state policies in this field, the prevention of illegal leech trade (Bio-smuggling), the protection of the generation of medicinal leeches, the continuation of the leech economy at the legal level and the correct guidance of the economic volume.

- Theses on the improvement of medicinal leech breeding techniques will contribute significantly to the solution of production problems currently experienced in the sector.

- Theses on the improvement and dissemination of methods for obtaining high-value-added products such as bioactive substances obtained from medicinal leeches will contribute to the increase of our country's foreign exchange income by preventing leeches from functioning only as raw materials.

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CHAPTER 5

EFFECTS OF THE COVID-19 PANDEMIC ON BARLEY AGRICULTURE AND TRADE IN THE WORLD AND TURKIYE

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1. INRODUCTION

Barley ranks fourth among the most produced plants in the World after wheat, corn and rice (Just and Echaust, 2022). Although it is mainly used in animal nutrition, barley is also used in the nutrition of millions of people in the World (Tricase et. al., 2018). Barley is one of the plants that spreads to the widest areas in the agricultural sector (Cao and Cheng, 2021; Hung, 2021). At the same time, barley trade is one of the products that experiences intense ups and downs (Chevallier and Ielpo, 2013; Śmiech et al., 2020). This is due to the fact that barley is preferred because it is resistant to harsh climate (Aktas, 2017) and soil (Akdeniz and Koc, 2022; Tabur and Demir, 2008) conditions, as well as being rich in essential nutrients (Koten et al., 2013).

Epidemics have caused serious losses in the World when they were effective. Asian, Hong Kong and Spanish flu, HIV and COVID-19 pandemics have been quite effective in the last century. Spanish Flu caused 50 million deaths between 1918-1919 (Johnson and Mueller, 2002), Asian Flu 1.1 million deaths between 1957-1958 (CDC, 2020a), Hong Kong Flu 1 million deaths between 1968-1970 (CDC, 2020b), HIV 32 million deaths between 1981 -2018 (WHO, 2020) and the Covid-19 pandemic 6.4 million deaths between 2019-2022.

The Covid-19 pandemic negatively affected economic growth figures and caused the growth rate to decrease from 2.9% to 1.5% (OECD, 2020). The negative effects on the World economy, it has been observed that it was at its most effective level after World War II (ILO, 2020). Quarantine, social distance etc. even though some measures such as these partially prevented the spread of the virus, they caused the

production of goods and services to stop and supply chains to break all over the World (Bandyopadhyay, 2020; Del Rio-Chanona et al., 2020).

A significant part of the World's population earns their living from agriculture (Zavatta et al., 2014). With the impact of the measures implemented, the Covid-19 pandemic has caused primarily a decrease in agricultural production, disruptions in the transportation of products, thus creating uncertainties and crises regarding access to food and pricing (Siche, 2020). Like all agricultural activities such as plant and animal production and fishing, grain agriculture has been negatively affected by the pandemic (Marlow, 2020).

The effects of the Covid-19 virus have decreased as important measures have been taken to combat the pandemic Worldwide and vaccinations have reached high levels that will create social immunity. In this study, the effects of Covid-19 on barley agriculture Worldwide, in leading countries and in Türkiye were examined as the 2018-2019 pre-pandemic period, the 2019-2021 pandemic period and the 2022 post-pandemic period.

2. BARLEY AGRICULTURE AND TRADE DURING THE COVID-19 PANDEMIC
2.1. Barley Beginning Stocks Before, During and After the Pandemic

Barley beginning stocks in the World, in the ten leading countries and in Türkiye in the pre-Covid-19 Pandemic (2018-2019), pandemic period (2019-2021) and post-pandemic periods are shown in Table 1.

Beginning stocks in the 2018-2019 season before the pandemic were 21.46 million tons (mt) of barley in the World, 16.99 mt in the top ten countries and 0.72 mt in Türkiye. In this period, the ratio of the top ten leading countries to the World's starting stocks is 0.79. In other words, the top ten countries constitute 79% of the World's starting stocks. In the same period, Türkiye has only 0.33% of the starting stock compared to the World. In the 2018-2019 pre-pandemic period, the European Union (EU: 5.59 mt) constituted approximately 25% of the World's initial stocks. This is followed by the United States of America (USA). Türkiye ranks tenth.

At the beginning of the pandemic process (2019-2020), barley beginning stocks were 20.19 mt in the World, 15.57 mt in the top ten leading countries and 0.46 mt in Türkiye (Table 1). In this period, the ratio of the initial stocks of the top ten leading countries to the World's total stocks is 0.77. In other words, the top ten countries in terms of barley beginning stocks have 77% of the World's total beginning stocks. Türkiye, on the other hand, had only 0.23% of the World's beginning stocks in this period. EU, which ranks first in this respect, has a beginning stock of 5.45 mt of barley. This amount corresponds to more than 25% of the total beginning stocks in the World during the period. Australia follows this, and USA, which ranked second in the previous period, fell to third place in this period. Türkiye, on the other hand, ranks 12th with its initial barley stock of around 0.46 mt in this first period of the pandemic.

In the 2020-2021 period, when the effects of the pandemic are felt more intensely, the World's beginning stocks are around 21.8 mt in total,

the leading ten countries are around 16.1 mt in total, and Türkiye is around 0.77 mt. The beginning stocks of the ten leading countries correspond to 74% of the World. Türkiye's beginning stocks in this period correspond to 0.35% of the World's. In this period, EU leads the ranking with 5.21 mt, followed by Australia with 2.7 mt. It is noteworthy that Australia's beginning stocks increased significantly by around 0.8 mt compared to the previous period. In this period, when Saudia Arabia fell one place, the initial stock amount decreased by around 0.3 mt. Türkiye, on the other hand, rose to the 8th place by recording an increase of around 0.3 mt in initial stocks (Table 1).

In the 2021-2022 period, when the effects of the pandemic diminish, initial stocks are at 20.3 mt in the World, 14.87 mt in the leading ten countries and 0.52 mt in Türkiye. The ten leading countries held 73.2% of the World's beginning stocks during this period. Türkiye appears to have 0.26% of the World's beginning stocks. In this period, the country with the highest initial stocks of 5 mt is EU, as in previous periods, followed by Australia (1.97 mt). It is interesting that China, which had not been among the top ten in previous periods, ranked 4th with 1.37 m in this period. Türkiye fell to 14th place in this period, the most backward position in all periods. However, it is noteworthy that although it ranked 12th in the 2019-2020 period, its beginning stock was lower than in the 2021-2022 season.

When the 2018-2022 periods are examined in terms of beginning stocks, it is seen that there is no serious change in the World, the leading countries generally remain unchanged, and Türkiye follows a fluctuating course but does not affect the general situation much.

Table 1. Barley beginning stocks of the World, Top 10 countries and Türkiye between 2018-2022 (USDA, 2022a).

2018-2019		2019-2020		2020-2021		2021-2022	
Country	Quantity (1000 MT*)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)
1.European Union	5592	1.European Union	5457	1.European Union	5207	1.European Union	5002
2.United States	2057	2.Australia	1908	2.Australia	2711	2.Australia	1969
3.Saudi Arabia	1841	3.United States	1884	3.United States	1747	3.United States	1555
4.Australia	1776	4.Saudi Arabia	1327	4.United Kingdom	1357	4.China	1374
5.Canada	1244	5.United Kingdom	1091	5.Saudi Arabia	1016	5.United Kingdom	1058
6.Ukraine	1149	6.Ukraine	1008	6.Canada	957	6.Saudi Arabia	1002
7.United Kingdom	1076	7.Canada	863	7.Ukraine	953	7.Iran	782
8.Iran	782	8.Algeria	797	8.Türkiye	766	8.Russia	757
9.Russia	750	9.Russia	640	9.Russia	743	9.Canada	711
10.Türkiye	724	10.Argentina	598	10.Algeria	653	10.Ukraine	661
		12.Türkiye	465			14.Türkiye	522
World Total	21460		20193		21802		20310
Türkiye/World	0.033		0.023		0.035		0.026
Top 10 Countries	16991		15573		16110		14871
Top10/World	0.79		0.77		0.74		0.73

*Metric Ton (~1000 kg), 1000 MT: 1 milyon kg

2.2. Changing in Barley Harvested Areas

Barley harvested areas in the World, in the ten leading countries and in Türkiye before the Covid-19 Pandemic (2018-2019), during the

pandemic period (2019-2021) and after the pandemic are shown in Table 2.

Harvested areas in the 2018-2019 season before the pandemic were 48.8 million ha in the World, 38.76 million ha in the top ten countries and 3.6 million ha in Türkiye. In this period, the ratio of the top ten leading countries to the World's harvested area is 0.79. In other words, the top ten countries constitute 79% of the World's harvested areas. In the same period, Türkiye has only 0.73% of the harvest area compared to the World. In the 2018-2019 pre-pandemic period, the EU constituted approximately 23% of the World's harvested area with 11.18 million ha. This is followed by Russia (7.78 million ha). Türkiye ranks 4th.

At the beginning of the pandemic process (2019-2020), harvested areas were 52.6 million ha in the World, 41.29 million ha in the top ten countries and 3.8 million ha in Türkiye. In this period, the ratio of the top ten leading countries to the World's harvested area is 0.78. In other words, the top ten countries constitute 78% of the World's harvested areas. In the same period, Türkiye has only 0.72% of the harvest area compared to the World. During this period, the EU constitutes approximately 21% of the World's harvested area with 11.16 million ha. This is followed by Russia (8.4 million ha). Türkiye is again ranked 4th.

In the 2020-2021 period, when the effects of the pandemic are felt more intensely, 51.37 million ha in the World, 40.09 million ha in the top ten countries and 3.8 million ha in Türkiye. In this period, the ratio of the top ten leading countries to the World's harvested area is 0.78. In other words, the top ten countries constitute 78% of the World's

harvested areas. In the same period, Türkiye has only 0.74% of the harvest area compared to the World. During this period, the EU constitutes approximately 21.5% of the World's harvested area with 11.04 million hectares. This is followed by Russia (8.16 million ha). Türkiye ranks 4th.

In the 2021-2022 period, when the effects of the pandemic diminish, harvested areas are 48.59 million hectares in the World, 38.45 million hectares in the ten leading countries and 3.7 million hectares in Türkiye. The ten leading countries accounted for 79.1% of the World's harvested area during this period. Türkiye appears to have 0.76% of the World's harvested areas. In this period, the country with the highest harvested area with 10.34 million ha is EU, as in previous periods, followed by Russia (7.69 million ha). During this period, the EU's harvest area constitutes approximately 21.3% of the World's harvest area.

When the 2018-2022 periods are examined in terms of harvested areas, it is seen that there is no serious change in the World, the leading countries generally remain unchanged, and Türkiye's table remains close to the same level throughout the periods.

Table 2. Areas harvesed of the World, Top 10 Countries and Türkiye between 2018-2022 (USDA, 2022a).

2018-2019		2019-2020		2020-2021		2021-2022	
Country	Quantity (1000 HA)	Country	Quantity (1000 HA)	Country	Quantity (1000 HA)	Country	Quantity (1000 HA)
1.European Union	11178	1.European Union	11161	1.European Union	11043	1.European Union	10344
2.Russia	7784	2.Russia	8403	2.Russia	8160	2.Russia	7688
3.Australia	4437	3.Australia	5041	3.Australia	4400	3.Australia	4350
4.Türkiye	3600	4.Türkiye	3800	4.Türkiye	3800	4.Türkiye	3700
5.Ukraine	2569	5.Kazakhstan	2977	5.Canada	2809	5.Canada	3002
6.Kazakhstan	2516	6.Ukraine	2782	6.Kazakhstan	2729	6.Ukraine	2680
7.Canada	2395	7.Canada	2728	7.Ukraine	2584	7.Kazakhstan	2157
8.Morocco	1599	8.Iran	1700	8.Iran	1600	8.Iran	1700
9.Iran	1550	9..Syria	1500	9.Syria	1500	9.Morocco	1490
10.United Kingdom	1138	10.Iraq	1200	10.Morocco	1467	10.Argentina	1340
World Total	48858		52614		51374		48589
Türkiye/World	0.074		0.072		0.074		0.076
Top 10 Countries	38766		41292		40092		38451
Top10/World	0.79		0.78		0.78		0.79

2.3. Changing in Barley Production

Barley production in the World, in the ten leading countries and in Türkiye before the Covid-19 Pandemic (2018-2019), during the pandemic period (2019-2021) and in the post-pandemic periods is shown in Table 3.

Production in the 2018-2019 season before the pandemic was 139.62 mt in the World, 109.47 mt in the top ten countries and 7 mt in Türkiye. In this period, the ratio of the top ten leading countries to World

production is 0.78. In other words, the top ten countries have a production share of 78.4% of the World. In the same period, Türkiye has only 0.5% of the World's production. In the 2018-2019 pre-pandemic period, the EU accounted for approximately 35.4% of the World's production with 49.47 mt. This is followed by Russia (16.73 mt). Türkiye ranks 6th.

At the beginning of the pandemic process (2019-2020), barley production was 158,376 mt in the World, 124.41 mt in the first ten countries and 7.9 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley production is 0.78. In other words, the top ten countries account for 78.5% of the World's production share. In the same period, Türkiye has only 0.49% of the harvested area compared to the World. In this period, EU constitutes approximately 34.8% of the World's barley production with 55.18 mt. This is followed by Russia (19.94 mt). Türkiye ranks 7th.

In the 2020-2021 period, when the effects of the pandemic were felt more intensely, barley production was 159.41 mt in the World, 126.27 mt in the top ten countries and 8.1 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley production is 0.79. In other words, the top ten countries have a production share of 79.2% of the World. In the same period, Türkiye has only a 0.51% production share compared to the World. In this period, EU accounts for approximately 34.1% of the World's production with 54.32 mt. This is followed by Russia (20.63 mt). Türkiye ranks 7th.

In the 2021-2022 period, when the effects of the pandemic diminish, barley production is 154.08 mt in the World, 117.79 mt in the

leading ten countries and 4.5 mt in Türkiye. The ten leading countries accounted for 81.2% of the World barley production in this period. Türkiye seems to have 0.3% of the World barley production. In this period, the country with the highest barley production is EU, with 51.97 mt, as in previous periods, followed by Russia (17.5 mt). In this period, EU production constitutes approximately 35.8% of the World barley production.

When the periods of 2018-2022 are examined in terms of barley production, it is seen that barley production in the World during the pandemic periods (2019-2021) increased by around 20 mt compared to the previous period, and the production of the leading countries increased by 14-15 mt in these periods, and Türkiye's table is also similar to this. It is seen that there was a similar increase over the periods and then the production decreased to half the level. It is understood that the increase in barley production during pandemic periods is largely due to the increase in the production of the top ten countries.

Table 3. Barley production of the World, Top 10 countries and Türkiye between 2018-2022 (USDA, 2022a).

2018-2019		2019-2020		2020-2021		2021-2022	
Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)
1.European Union	49470	1.European Union	55180	1.European Union	54324	1.European Union	51972
2.Russia	16737	2.Russia	19939	2.Russia	20629	2.Russia	17505
3.Australia	8819	3.Canada	10383	3.Australia	13100	3.Australia	13700
4.Canada	8380	4.Australia	10127	4.Canada	10741	4.Ukraine	9923
5.Ukraine	7604	5.Ukraine	9528	5.United Kingdom	8117	5.United Kingdom	6961
7.United Kingdom	6510	6.United Kingdom	8048	7.Ukraine	7947	6.Canada	6948
8.Argentina	4635	8.Kazakhstan	3830	8.Argentina	4035	7.Argentina	5300
9.Kazakhstan	3971	9.United States	3756	9.United States	3719	9.Morocco	2780
10.United States	3343	10.Argentina	3615	10.Kazakhstan	3659	10.Iran	2700
6.Türkiye	7000	7.Türkiye	7900	6.Türkiye	8100	8.Türkiye	4500
World Total	139623		158376		159408		145079
Türkiye/World	0.05		0.05		0.05		0.03
Top 10 Countries	109469		124406		126271		117789
Top10/World	0.78		0.79		0.79		0.81

2.4. Changing in Barley Exports

Barley export information in the World, in the ten leading countries and in Türkiye before the Covid-19 Pandemic (2018-2019), during the pandemic period (2019-2021) and in the post-pandemic periods is shown in Table 4.

Exports in the 2018-2019 season before the pandemic were 25.59 mt in the World, 25.33 mt in the top ten countries and 0.15 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley exports is 0.99. In other words, the top ten countries have an export share of 98.9% of the World. In the same period, Türkiye has an export rate of only 0.06% compared to the World. In the 2018-2019 pre-pandemic period, EU constituted approximately 19.14% of World exports with 4.90 mt. This is followed by Russia (4.67 mt). Türkiye ranks 9th.

At the beginning of the pandemic process (2019-2020), barley exports were 29 mt in the World, 28.73 mt in the top ten countries and 0.002 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley exports is 0.99. In other words, the top ten countries account for 99% of the World's export share. During this period, EU constituted approximately 26.78% of the World's barley exports with 7.77 mt. This is followed by Ukraine (4.98 mt). On the other hand, it is stated that Russia and Ukraine had 32% of the World barley exports between 2016 and 2021 (Just and Echaust, 2022). Türkiye ranks 12th.

In the 2020-2021 period, when the effects of the pandemic were felt more intensely, barley export amounts were 36.27 mt in the World, 35.44 mt in the top ten countries and 0.06 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley exports is 0.98. In other words, the top ten countries have a 97.73% export share of the World. In the same period, Türkiye has an export share of only 0.02% compared to the World. Australia ranked first in this period, accounting

for approximately 23% of the World's total exports with 8.34 mt. This is followed by EU (7.40 mt). Türkiye ranks 15th.

In the 2021-2022 period, when the effects of the pandemic diminish, barley exports are 33.17 mt in the World, 32.62 mt in the leading ten countries and 0.17 mt in Türkiye. The ten leading countries accounted for 98.35% of World barley exports in this period. Türkiye seems to have 0.05% of World barley exports. In this period, the country with the highest barley exports was Australia, with 9 mt, as in the previous period. This is followed by EU (7.2 mt). During this period, Australia accounted for approximately 27.13% of World barley exports.

When the 2018-2022 periods are examined in terms of barley exports, it is seen that the World's total barley exports, which were 25.6 mt in the 2018-2019 period before the pandemic, increased by 4.5 mt in the first period of the pandemic and 10.6 mt in the second period. The nearly two-fold increase in exports to Australia and the EU has a great impact on this increase. Although it decreased to 33.2 mt in the post-pandemic period (2020-2021), this figure is 7.6 mt more than the pre-pandemic period. The decrease in the increase in barley exports experienced during the pandemic period in the following period is largely due to the decreases in barley exports of Russia (Anonymous, 2022), Canada, the United Kingdom and Kazakhstan. Although Türkiye's barley exports decreased during the pandemic period, it increased significantly in the post-pandemic period.

Table 4. Exports of barley of the World, Top 10 countries and Türkiye between 2018-2022 (USDA, 2022b).

2018-2019		2019-2020		2020-2021		2021-2022	
Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)
1.European Union	4898	1.European Union	7767	1.Australia	8342	1.Australia	9000
2.Russia	4661	2.Ukraine	4984	2.European Union	7399	2.European Union	7200
3Australia	3687	3.Russia	4470	3.Russia	6259	3.Ukraine	5800
4.Ukraine	3561	4.Australia	3324	4.Ukraine	4187	4.Argentina	3600
5.Argentina	3237	5.Argentina	2421	5.Canada	3534	5.Russia	3500
6.Canada	2296	6.Canada	2244	6.Argentina	2336	6.Canada	1800
7.Kazakhstan	1820	7.United Kingdom	1876	7.United Kingdom	1303	7.United Kingdom	800
8.United Kingdom	916	8.Kazakhstan	1366	8.Kazakhstan	1085	8.Kazakhstan	500
9.Türkiye	148	9.Syria	150	9.Iraq	700	9.Uruguay	250
10.United States	107	10.United States	125	10.United States	300	10.Türkiye	173
		17.Türkiye	2	15.Türkiye	60		
World Total	25590		29004		36270		33171
Türkiye/World	0.006		0		0.002		0.005
Top 10 Countries	25331		28727		35445		32623
Top10/World	0.99		0.99		0.97		0.98

2.5. Changing in Barley Imports

Barley import information in the World, in the ten leading countries and in Türkiye before the Covid-19 Pandemic (2018-2019), during the pandemic period (2019-2021) and in the post-pandemic periods is shown in Table 5.

Before the pandemic, imports in the 2018-2019 season were 25.27 mt in the World, 20.39 mt in the top ten countries and 0.38 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley

imports is 0.81. In other words, the top ten countries have an import share of 80.67% of the World. In the same period, Türkiye has an import rate of only 0.01% compared to the World. In the 2018-2019 pre-pandemic period, Saudi Arabia accounted for approximately 22.6% of World imports with 5.7 mt. This is followed by China (5.18 mt). Türkiye ranks 13th.

At the beginning of the pandemic process (2019-2020), barley imports were 28 mt in the World, 22.32 mt in the top ten countries and 1 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley imports is 0.79. In other words, the top ten countries account for 79% of the World's import share. In this period, Saudi Arabia increased its imports to 7.3 mt, with an increase of 1.6 mt compared to the previous period, and constituted approximately 26% of the World's barley imports. This is again followed by China (5.97 mt). Türkiye ranks 7th.

In the 2020-2021 period, when the effects of the pandemic were felt more intensely, barley import amounts were 36.97 mt in the World, 29.67 mt in the top ten countries and 1.63 mt in Türkiye. In this period, the ratio of the top ten leading countries to World barley imports is 0.80. In other words, the top ten countries have an 80.24% import share of the World. In the same period, Türkiye has an export share of only 0.44% compared to the World. China ranked first in this period, accounting for approximately 32.6% of the World's total imports with 12.04 mt. This is followed by Saudi Arabia (6.1 mt). Türkiye ranks 4th.

In the 2021-2022 period, when the effects of the pandemic diminish, barley imports are 29.94 mt in the World, 22.55 mt in the

leading ten countries and 2.1 mt in Türkiye. The ten leading countries account for 75.33% of World barley imports in this period. Türkiye appears to have 0.7% of World barley imports. In this period, the country with the highest barley imports was China, as in the previous period, with 8.5 mt. This is followed by Saudi Arabia (4.5 mt). During this period, China constituted approximately 28.4% of World barley imports.

Table 5. Imports of barley of the World, Top 10 countries and Türkiye between 2018-2022 (USDA, 2022b).

2018-2019		2019-2020		2020-2021		2021-2022	
Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)	Country	Quantity (1000 MT)
1.Saudi Arabia	5700	1.Saudi Arabia	7300	1.China	12049	1.China	8500
2.China	5181	2.China	5969	2.Saudi Arabia	6100	2.Saudi Arabia	4,00
3.Iran	3200	3.Iran	2300	3.Iran	3700	3.Iran	2200
4.European Union	1762	4.Japan	1253	4.Türkiye	1634	4.Türkiye	2100
5.Japan	1158	5.European Union	1089	5.European Union	1150	5.Japan	1200
6.Jordan	928	6.Morocco	1073	6.Japan	1132	6.European Union	900
7.Libya	888	7.Türkiye	1007	7.Thailand	1043	7.Tunisia	800
8.Brazil	608	8.Libya	891	8.Libya	1039	8.Jordan	800
9.Tunisia	487	9.Tunisia	751	9.Tunisia	1038	9.Morocco	800
10.United Arab Emirates	476	10.Thailand	685	10.Algeria	780	10.Libya	750
13.Türkiye	375						
World Total	25274		28030		36968		29936
Türkiye/World	0.001		0.036		0.044		0.07
Top 10 Countries	20388		22318		29665		22550
Top10/World	0.80		079		0.80		0.75

When the 2018-2022 periods are examined in terms of barley imports, it is seen that the World's total barley imports, which were 25.27

mt in the 2018-2019 period before the pandemic, increased by 2.7 mt in the first period of the pandemic and 11.7 mt in the second period. Especially the increases in imports of China and Saudi Arabia have a great impact on this increase. Although it decreased to 29.94 mt in the post-pandemic period (2020-2021), this figure is 4.5 mt more than the pre-pandemic period. The decrease in the increase in barley imports during the pandemic period in the following period is largely due to the decreases in the imports of China, Saudi Arabia and Iran (Fgure 1). Türkiye's barley imports reached 6 times with gradual increases during and after the pandemic period.
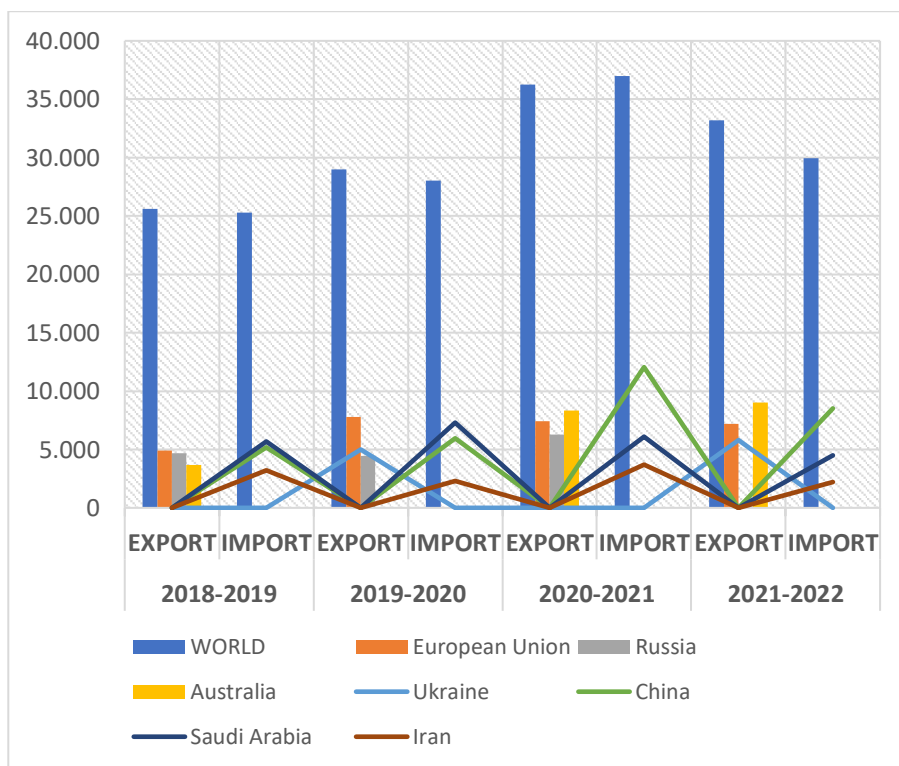

Figure 1. Comparison chart of import and export situations of the World and leading countries between 2018-2022.

CONCLUSION

When the effects of the Covid-19 pandemic process on barley agriculture, import and export of the World, leading countries and Türkiye are examined;

It has been observed that barley starting stocks did not undergo a serious change in the period before, during and after the pandemic, and the ups and downs in Türkiye were not at a level that would change the general course. Similarly, it was observed that the harvest areas did not change much during this period. It has been observed that barley production in the World increased by around 20 mt due to serious increases in 10 leading countries during the pandemic period, and although a similar increase was recorded in Türkiye, production decreased in the post-pandemic period.

Barley exports increased significantly during the pandemic period and first increased to 29 mt and subsequently to around 36.3 mt, but decreased by 3.1 mt in the post-pandemic period. This decrease was largely due to the decrease in exports of Russia, the United Kingdom and Kazakhstan. Türkiye's barley exports decreased during the pandemic period. Similarly, barley imports increased by 2.7 mt and then by 11.7 mt during the pandemic period. These increases were due to the increases in the imports of China and Saudi Arabia. As a matter of fact, after the pandemic, World barley imports decreased by around 7 mt due to the decrease in imports of China, Saudi Arabia and China. Türkiye's barley imports gradually increased during and after the pandemic period, reaching a level of 6 times.

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CHAPTER 6

IMPORTANCE AND USES OF COMPOST MADE FROM WASTE IN SUSTAINABLE AGRICULTURE IN OUR COUNTRY

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INTRODUCTION

Türkiye possesses significant agricultural potential due to its ecological structure. This potential allows for promising prospects in the agricultural sector. Türkiye holds a prominent position among the world's leading countries in various production sectors. To ensure sustainable production in Türkiye, a country known for its diverse range of cultivated crops, safeguarding the soil and implementing appropriate cultural practices are crucial. Preserving the soil's physical and chemical composition is also of utmost importance. However, continuous sowing and planting without incorporating organic matter into the soil, coupled with the unregulated use of chemical fertilizers, contribute to a decline in soil fertility (Çelik et al., 2020) and the chemical fertilisers used cause pollution of soils and groundwater. Although the intensive use of chemical fertilisers in crop production provides temporary increases in yields and high incomes, it causes pollution and barrenness of agricultural land, depriving it of long-term sustainable cultivation. It also poses serious risks to plant, human and environmental health. For this reason, sustainable agricultural practices that use organic waste and improve soil quality are a step ahead (Bellitürk and Goldmann Benardete, 2020; Çelik et al., 2020).

Unconscious agriculture for many years has led to loss of organic matter in soils. In order to maintain soil fertility and sustainability, it is necessary to add sufficient organic matter to the soil in agricultural areas. It has been observed that the organic matter content in agricultural areas in Türkiye has fallen below 2% and is gradually decreasing and the

physical, chemical and biological properties of soils to deteriorate (Demirtaş, 2004).

Recently, the use of organic wastes in agricultural production has become widespread, both for the recycling of organic wastes and for the prevention of environmental pollution. Plant-based wastes are used in pure form or in certain mixtures as growing media or directly applied to the soil as a source of organic matter and plant nutrients (Özenç, 2004; Benito, 2005, 2006). Mixing organic or vegetable wastes directly or especially after composting, increases the content of plant nutrients, especially organic matter and nitrogen, in the soil, provides an increase in yield and quality, improves soil structure, air-water balance and microbiological activities (Eriksen, 2005; Randhawa et al., 2005; Alagöz, 2006; Candemir and Gülser, 2007; Chaturvedi et al., 2008; Okur et al., 2008; Yılmaz and Alagöz, 2010).

Figure 1. Agricultural areas

The fresh fruit and vegetable chain system in Türkiye consists of five main stages: harvesting, preservation, transport, marketing and consumption. In this process, due to faulty practices, losses varying between 25-40% depending on the product occur or turn into waste. Approximately 9-10 million tonnes of fresh fruit and vegetable waste are generated annually due to the problems experienced in the fresh fruit and vegetable chain. According to the data of the Food and Agriculture Organization of the United Nations (FAO), approximately 1.3 billion tonnes of the 4.5 billion tonnes of food produced in the world is wasted as waste and loss (Anonymous, 2017). It is not preferred to add organic wastes, herbal wastes and fruit and vegetable processing industry wastes directly to the growing environment. These wastes must undergo certain processes in order to be used in agriculture. Thus, organic wastes turned into compost are successfully used in different areas of crop production.

Compost is a variety of biochemically decomposable organic materials stabilised by microorganisms. Composting is the biochemical decomposition of organic materials by microorganisms under appropriate temperature, humidity and oxygen conditions. Compost improves the physical, chemical and biological structure of the soil and provides significant increases in yield and quality. In addition to its advantages as an organic material, the fact that it is cheap and sustainable and not dependent on foreign sources increases the importance of compost. Today, composting has become a solid waste management procedure rather than just a stable organic material. Composting is increasingly attracting attention as an efficient and cost-effective process with minimal environmental risks. Composting has become one of the

most popular ways to replace sending biodegradable waste to landfills (Pankhurst et al., 2011). It is an accepted practice everywhere and one of the most effective means of recycling organic waste for agricultural use (Raj and Antil, 2011). This valuable process of waste removal completes the natural cycle and provides economic benefits, while at the same time stabilising and reducing organic waste and returning valuable nutrients to the soil (Rose, 1992: Arslan, 2011).

The integration of recycled organic and municipal solid waste compost into modern agricultural systems is recognised as a clean and sustainable technology with high positive impacts on the environment, plant productivity and food safety (Neugart et al., 2018; Vinci et al., 2018; Chaudhary et al., 2020). These organic materials are reservoirs of essential plant nutrients and biomolecules such as macro- and micronutrients, amino acids, carbohydrates, lipids and minerals that are essential for plant metabolism. Depending on the quality of the material and the organic matter content, organic matter has a positive effect on the physical, chemical and biological structure of the soil, increasing hydraulic conductivity, infiltration rate, porosity and aggregate stability in the soil whıle reducing bulk density (Flaig et al., 1977; Albiach et al., 2000; Ünsal and Ok, 2001; Logsdon et al., 2017).

The introduction of new and environmentally friendly techniques such as sustainable agriculture, organic agriculture and good agricultural practicses in the world, especially in recent years, has made the use of compost even more important. Compost can be added to the soil as organic matter in agricultural production and can also be used in different ways. The materials used in the production of compost, which is obtained

by decomposing various organic wastes, include green wastes, wood wastes, sewage sludge, barnyard manures and other animal wastes as well as solid fruit wastes in the form of pulp from vegetable and fruit processing factories. For this purpose, there are also enterprises in the world that use different fruit and vegetable wastes as compost (Sayara et al., 2020).

1.1.1. Compost use in Türkiye

The increasing population density in Türkiye is leading to a continuous increase in food consumption and production. The process from production to consumption generates a large amount of organic waste. In Türkiye, tomato paste, yeast, fruit juice, olive oil and other vegetable oil producing factories as well as walnut, hazelnut, almond, grape, sugar beet and other similar food producing factories produce a large amount of organic waste suitable for composting. In particular, waste from the sugar, tomato paste and fruit juice industries is used as animal feed, and waste from shelled products such as walnuts, almonds and hazelnuts is used as fuel. In addition, domestic waste, municipal waste and herbal waste released in the process from production to marketing are major concern. Decomposing these wastes and turning them into compost not only eliminates environmental pollution and economic burden but also reduces the need for synthetic fertilisers, which we depend on from foreign sources.

The "Waste Management Regulation" of 02.04.2015 and the "Compost Communiqué" of 05.03.2015 entered into force. In order to reduce the amount of waste, prevent waste, recycle waste and use

resources in a beneficial way, the "Zero Waste" project has been launched in Türkiye and composting is being popularised with more modern techniques. According to TurkStat reports, 78.3 million tonnes of 127.4 million tonnes of waste were disposed of and 49.1 million tonnes were recycled in Türkiye in 2020, and the total amount of waste processed increased by 22% compared to 2018. It was reported that there were 9 composting facilities in Türkiye in 2020 and 127 thousand tonnes of waste were processed in these facilities (TÜİK, 2020). These data published by TÜİK show that we are still far behind in terms of the importance and production of compost. It is thought-provoking that out of millions of tonnes of organic waste, only 127.000 tonnes are used as compost, while we continue to depend on foreign sources for synthetic fertilisers and the cost of synthetic fertilisers is very high. Much more serious studies and measures are needed in this regard.

Figure 2. Composts obtained from organic wastes

Regarding organic matter, it has been found that about 75.6% of the soils in our country are deficient in organic matter and the organic matter content is low (< 2%) (Pilanalı, 2001). Therefore, organic matter content is the most important factor in terms of the productivity and efficiency of soils in our country. The most widely used material as a source of organic matter in Türkiye is livestock manure (Bayındır et al., 2004). Problems such as not being able to find the required amount of farmyard manure in the required period or not reaching sufficient maturity are encountered. In this case, alternative approaches or materials are needed. This situation shows the importance of using waste materials as a source of organic matter in agricultural areas (Özgüven et al., 1996). Studies have shown that many vegetable wastes can be used as sources of organic matter in Türkiye (Kaçar et al., 1996; Sönmez et al., 2002; Kütük and Çaycı, 2005).

1.1.2. Use of Compost in Agriculture

Stability and maturity are two important characteristics required for compost to be used for agricultural purposes. Mature compost increases soil organic matter content more than fresh or immature compost due to its high stable carbon (C) content (Bouajila and Sanaa, 2011). Otherwise, insufficient decomposition of organic matter leads to accumulation of pollutants in the soil and toxic effects on plants. In this respect, the quality, maturity and stability of the compost should be well adjusted to avoid such side effects. Although various tests are used to assess the stability and maturity of compost, one of the most practical methods is the germination test (Flavel et al., 2005; Guo et al., 2012; Liu et al., 2018).

Today, as the demand for agricultural products increases day by day, intensive agricultural practicses negatively affect soil fertility and health and cause various environmental problems. The production of compost from organic waste and its incorporation into the soil meets the need for soil organic matter, provide waste management and are considered a reliable sustainable approach.

Figure 3. Recycling of organic waste

The effect of compost use on soil depends on the materials from which the compost is made and the amount applied. However, the expected effect of compost may not be seen in a short time due to the slow release of nutrients into the soil (Bouajila and Sanaa, 2011; Duong et al., 2012). The application of compost to normal or saline soils, especially in arid and semi-arid conditions, is very important to increase the amount of soil organic matter and soil mineralisation. Soil organic matter is essential for maintaining soil fertility and reducing nutrient loss. In addition, organic matter improves soil water-holding capacity, soil

fertility, biological activity, soil aeration and soil structure. Mature compost produced from organic waste contains good levels of nitrogen. Therefore, adding compost to the soil enriches the soil with nutrients such as nitrogen, carbon, sulphur and phosphorus (Bouajila and Sanaa, 2011).

Low levels of organic matter in the soil decrease the efficiency of fertilisers applied to the soil and cause a decrease in the yield and quality of the product. The use of organic and chemical fertilisers together provides a balance between plant nutrient elements due to the increase in soil fertility and the fact that they contain more or less of all nutrient elements. It is especially desired that the amount of organic matter in the soils where vegetable cultivation will be carried out be high. If successful cultivation is desired, the amount of organic matter in the soil should be increased. Therefore, organic wastes should be applied to the soil homogeneously and in a matured state (Kaçar and Katkat, 2007). In addition, the addition of organic wastes to the cultivation environment increases the structure, air-water balance and microbial activity of the environment (Eriksen, 2005; Randhawa et al., 2005; Chaturvedi et al., 2008).

1.1.3. Use of Compost as Soil Conditioner and Fertiliser

Organic wastes such as food wastes, aquatic wastes, biowastes and plant-based wastes are important resources as they can be used as soil conditioners and fertilisers. Humus, which is abundant in wastes, can be very valuable for sustainable soil fertility (Raj and Antil, 2011). Compost from crop waste not only contributes as a source of organic matter and soil conditioner in agricultural lands (Raj and Antil, 2011), but also

provides the transformation and recovery of organic matter, N, P and other nutrients (Jedidi, 1998; Achiba et al., 2010). Plant-based wastes and food wastes are a source of high quality compost as they contain abundant carbohydrates, proteins, lipids and organic acids as well as nutrients such as nitrogen, phosphorus and potassium (Ma and Liu, 2019). It is also stated that composts obtained from biowastes can be used as organic fertiliser when their nutrient content is high as well as soil structure regulators (Mieldazys et al., 2019).

Compost used for agricultural purposes is reported to accelerate soil microbial activity, increase nutrient uptake, reduce plant pathogens and increase water retention, soil porosity and cation exchange capacity (Jedidi, 1998; Çelik et al., 2004; Hargreaves et al., 2008). The nutrient content of compost varies depending on its source. It is generally recommended to use composts with low nutrient content as soil improvers, while composts to be used as organic or organic fertilisers should have a high nutrient content. It has been noted that food waste compost has a higher nutrient content than livestock manure, making it more beneficial to use as a fertiliser, while livestock manure is more suitable as a soil conditioner (Kelley et al., 2020).

1.1.4. Use of Compost in Soilless Agriculture

Although various materials are used as growing media in soilless cultivation around the world, and especially in Europe, sphagnum peat accounts for 80% of the growing media used (Bohlin, 2002; Schmilewski, 2009; IPS, 2007; EU Commission). Peat-based media are generally characterised by high water holding capacity, good aeration, good drainage, high cation exchange capacity and therefore have the

effect of increasing nutrient uptake by plant roots throughout the vegetation period (Rainbow and Wilson, 1998; Barrett et al., 2016). Although there is a wide variety of materials used in soilless agriculture, only 0.1-1.0% of these media in Europe do not contain peat. Therefore, approximately 99% of the substrates used in soilless agriculture consist of or contain peat (Schmilewski, 2009). In Europe, the European Commission Decision 92/43/EEC of 21 May 1992 on the protection of the natural environment and wild fauna and flora prohibits the opening and licensing of new peat operations. In addition, the fact that peat soils are a potential source of atmospheric carbon emissions, and thus one of the factors causing climate change, is one of the reasons that make it difficult to use peat soils as a source of peat (Mäkiranta et al., 2010). Although peat ecosystems make up a third of all soils in the world, their regeneration is very slow (Schaller and Kantelhardt, 2009).

Sustainable development is defined by the United Nations as meeting the needs of the present without compromising the ability of future generations to meet their own needs. This definition was later broadened to recognise that sustainability requires the reconciliation of environmental, social and economic needs. Environmental concerns about plant growing media (particularly peat-based media) have necessitated effective action, with plans to reduce the use of peat-based media in agriculture by up to 90 per cent.

Figure 4. Hydroponics cultivation

The increasing price of good quality Sphagnum peat and environmental awareness have led the hydroponics sector to look for more sustainable media. In Europe, the substitution of alternative media for peat in soilless agriculture is progressing very slowly. When the properties of the new materials to be used are not similar to peat, the transition to an alternative material is often very complex and expensive. This is because peat-producing countries in Europe have built their industrial infrastructure based on peat, such as the processing, crushing and packaging of peat soils (Barrett et al., 2016). In this case, peat importing countries are more advantageous.

Waste from coconut processing plants and coconut fibre are also prominent as growing media. However, there are reasons that make it difficult to use coconut fibre a growing medium, although not as difficult as the difficulties mentioned for peat beds. Many countries engaged in soilless cultivation are dependent on coconut fibre. This poses many

challenges. Furthermore, the proximity of coconut plantations to the sea increases the sodium and potassium content to very high levels, which requires industrial washing of the product before marketing (Poulter, 2014). This is necessitated by the large transport distances from countries such as the Philippines, Indonesia, India and Sri Lanka (Barrett et al., 2016) to Europe, which significantly increase costs and make environmental sustainability difficult.

Different materials are being tried instead of peat and coconut fibre. In many European countries and the USA, pine bark is used as an alternative to peat (Lu et al., 2006; Bilderback et al., 2013). However, pine bark is a by-product of the wood industry. Since it cannot be produced as a growing substrate like coir by-products, its physical and chemical properties vary significantly depending on its origin and production method (Barrett et al., 2016). Furthermore, its extremely high drainage and low water-holding capacity (Burés, 1997) limit its use in soilless agriculture. Similarly, wood sawdust does not provide the desired productivity due to its low water holding capacity and high compaction tendency (Domeño et al., 2010). Many organic media have been tried in soilless agriculture for this purpose, but they could not be substituted for peat or coconut fibre due to similar negative properties. The search for many years and the studies carried out to date show that compost is an important alternative in this field (Landis and Morgan, 2009). The use of compost as a growing medium in soilless agriculture has grown steadily over the last 40-50 years (Poole, 1970; Sanderson, 1980; Raviv, 2013).

1.1.5. Effect of Compost Applications on Soilborne Pathogens

One of the important benefits of compost in agriculture is the suppression of soil-borne diseases and pests. In crop production, many cultivated plants are adversely affected by soil-borne pathogens and diseases. Controlling soil-borne diseases and pests is difficult, expensive and causes environmental pollution. Adding compost to the soil can suppress soil-borne diseases and pests. The suppressive effect of compost against phytopathogenic microorganisms is due to the microbial activity in compost (Borrero et al., 2004; Moral et al., 2009).

Compost can increase the amount of substances toxic to fungi, such as organic acids, in the soil while providing nutrients to plants. More importantly, it increases the population of beneficial microorganisms that can suppress soil pathogens through antagonism, antibiosis and competition (Hoitink and Boehm, 1999; De Clercq et al., 2004). Compost and compost extracts obtained from different materials are also effective in suppressing some soil pathogens and pathogens that are problematic in the above-ground parts of plants (Craft and Nelson, 1996; Diab et al., 2003; Borrero et al., 2004; Scheuerell et al., 2005; Termorshuizen et al., 2006; Bonanomi et al., 2007; Malandraki et al., 2008; Papasotiriou et al., 2013). Organic fertilisers reduce the incidence and severity of many soil-borne diseases in some economically important species and the mechanism of this effect has been reported by different researchers (Aryantha et al., 2000; Nelson et al., 2002; Shaikh and Ghaffar, 2004; Jenana et al., 2009; Saadi et al., 2010; Pane et al., 2011; Ahmed 2017).

Figure 5. Plant damage by soilborne pathogens

Marull et al (1997), who investigated the effect of olive pomace, poultry manure and municipal compost wastes on the suppression of root-knot nematodes in tomato and pepper and on plant growth in soil contaminated with nematodes, found that nematode growth in pepper roots was significantly reduced in organic matter treated soils compared to the control. Subsequent studies have also shown that the use of compost suppresses soilborne pathogens such as Rhizoctonia solani, Fusarium oxysporum and Pythium spp. (Van Assche and Uyttebroeck, 1981; Kuter et al., 1988; Gorodeck and Hadar, 1990).

Ntougias et al. (2008), who investigated the effect of olive oil waste compost, grape pomace compost and mushroom compost on soil-borne diseases in tomato by mixing them separately with peat in a 1:3 ratio, reported that the addition of compost to the medium played a suppressive role on Phytophthora nicotianae, Fusarium oxysporium and Septoria lycopersici and that this effect differed according to the compost material.

In addition to suppressing soil-borne diseases, compost also contributes to the elimination of soil pollution caused by pollutants such as pesticides, petroleum waste and chlorinated compounds. It also has a preventive effect on heavy metal pollution such as Cd and Ni in the soil. Compost provides immobilisation of heavy metals in the soil (Arnesen et al., 1998; Angelova and Singh, 2013).

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CHAPTER 7

A REVIEW OF CULTURAL AND MOLECULAR DETECTION OF
Listeria monocytogenes IN FOOD

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INTRODUCTION

Listeria species (spp.) are short Gram-positive, facultative anaerobic microorganisms and can grow at a temperature between 0°C and 45°C, and at 37°C optimum heat (Allerberger, 2003). *Listeria* spp. can grow in poor water conditions and high salt conditions (NaCl) up to 20% and at a pH range between 4.6 and 9.5 (Buchanan et al., 1989). The genus *Listeria* was believed to include only eight species and two subspecies. However, currently, out of 17 identified Listeria species, two of them, *Listeria monocytogenes* a serious pathogen for humans and animals, whereas *Listeria ivanovii* causes listeriosis in ruminants (Gouin et al.,1994; Orsi et al., 2011) and rarely is related to human infections. However, there are few reports on listeriosis cases caused by *L. seeligeri* ([Acute Purulent Listeria Seelingeri Meningitis in an Immunocompetent Adult], 1986). Nevertheless, it was shown that some strains of *L. innocua* can lead to severe infections in human Caco-2 cells (Johnson et al., 2004). The capability to survive and to multiply under extreme environmental conditions makes *L. monocytogenes* a crucial food-related pathogen giving rise to sporadic infections or large-scale outbreaks worldwide. Human listeriosis cases due to *L. monocytogenes* can be divided into two groups: severe or mild invasive form and noninvasive form (Buchanan et al., 2017) and infections in these two forms mainly rely on the virulence factors of contaminated strain, patient's age, the immune system of the patient, and the taken dose (Poimenidou et al., 2018; Pouillot et al., 2015).

1. *Listeria monocytogenes* IN FOOD AND ENVIRONMENTS

L. monocytogenes is a ubiquitous bacteria and is found in water, soil, and fee, and it can survive in the environment, i.e., sewage, sewage sludge, and river water for up to 8 weeks (Watkins & Sleath, 1981). In addition, *L.monocytogenes* can be carried by animals for instance, goats, cattle, or sheep leading to contaminating the environment (Dhama et al., 2015). Nevertheless, *L. monocytogenes* is mostly prevalent in food manufacturing facilities. Because of poor sanitation conditions and inadequate control of types of equipment or people, the bacteria can survive for a long time (Buchanan et al., 2017; Ferreira et al., 2014). Strains of *L. monocytogenes* are persistent in wide environmental conditions, such as low oxygen, low humidity, high salt, and acidity in food. For this reason, the persistence of bacteria in food manufacturing facilities can result in prolonged contamination in food production (Ferreira et al., 2014; Hoelzer et al., 2012; Malley et al., 2015). In addition, inadequate hygiene conditions or ineffective sanitizers in food plants or the ability of biofilm formation by *L. monocytogenes* may lead to the permanence of this organism in food operation environments (Lee et al., 2019).

2. *Listeria monocytogenes* CULTURAL DETECTION METHODS

Listeria monocytogenes is a crucial pathogen leading to a foodborne infection known as listeriosis by contaminated food with this pathogen. The fact that this pathogen survives in extreme conditions becomes a potential risk for food production environments. Various enrichment and plating methods are utilized to identify and isolate

L.monocytogenes from several food samples. Various selective enrichment media, for instance, Fraser broth, Buffered Listeria Enrichment Broth Supplement (BLEB), and UVM Listeria Selective Enrichment Broth are proposed by Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM), US Food Safety and Inspection Service (USDA-FSIS), and International Organization for Standardization (ISO). Concerning reference methods like ISO 11290, FDA-BAM, and USDA-FSIS method, these methods can identify one *Listeria* organism per 25 g of sample. In these procedures, food samples are subject to the enrichment step ensuring the bacteria reach a certain number before plating the culture on the media (*ISO 11290-2:2017*, n.d. and USDA-FSIS 2019). Antimicrobial agents are added to the medium to suppress competitive microflora. One of the most commonly used agents is acriflavine preventing Gram-positive bacteria growth except *L. monocytogenes* by inhibiting RNA synthesis. In addition, nalidixic acid prevents the synthesis of DNA and suppresses Gram-positive bacteria growth, and cycloheximide is applied to inhibit the process of protein synthesis by blocking translation elongation by binding to 60S ribosomal unit. Thus, it hinders the growth of molds and yeasts (Beumer & Curtis, 2003; Jantzen et al., 2006; Schneider-Poetsch et al., 2010). In addition, *L. monocytogenes* is capable of hydrolyzing esculin. All *Listeria* spp. perform esculin hydrolysis and in the presence of ferric ammonium citrate, esculin generates a black residue which facilitates the identification of *Listeria* species (Fraser & Sperber, 1988). Thus, esculin is an important agent added to Listeria enrichment media.

PALCAM (polymyxin acriflavin lithium chloride ceftazidime aesculin mannitol) Listeria Selective Agar and Oxford Listeria Selective Agar (Law et al., 2015) are both recommended by FDA-BAM and ISO. The distinctiveness of PALCAM agar is associated with the fermentation of mannitol and the hydrolysis of esculin (Van Netten et al., 1989). Because of the ability of *Listeria* spp. to hydrolyze esculin, gray-green color colonies with a dark halo in black appear in the medium. Nevertheless, *Staphylococcus* spp. or *Enterococcus* spp. can grow on PALCAM agar, but their colonies are able to easily differentiate from *Listeria* spp. due to the fermentation of mannitol. These contaminants ferment mannitol and produce a gray or red-to-yellow color with a yellow halo or brown-green halo because of the production of acids. (Osek et al., 2022 and Van Netten et al., 1989).

Similar to PALCAM agar, several selective agents exist in Oxford agar such as acriflavine, cycloheximide, lithium chloride, cefotetan, and fosfomycin. They can impede the undesired microflora growth. The distinction of *Listeria* strains on Oxford agar depends on the hydrolysis of esculin similarly on PALCAM agar. After 24h of incubation, *L. monocytogenes* appeared as green colonies surrounding a black halo, whereas *Listeria* spp. colonies are black with a black halo (Curtis et al., 1989 and Magalhães et al., 2014). Whereas after 48h of the incubation time, colonies of *L. monocytogenes* become black in color with black zones and other *Listeria* spp. colonies do not show any color change. Additionally, there are various chromogenic media capable of identifying *L. monocytogenes* strain providing quicker characterization.

One of the most known media, Agar Listeria concerning Ottaviani and Agosti (ALOA) is sensitive, time and cost-saving in *L. monocytogenes* detection. (Vlaemynck et al., 2000). This medium includes X-glucoside, 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside, a chromogenic substrate used to detect β-glucosidase enzyme activity. This enzyme is capable of hydrolyzing β-glucosidic linkages in oligosaccharides and disaccharides to generate glucose (Ottaviani, 1997). All *Listeria* spp. exhibit β-D-glucosidase activity appearing as blue-green colonies on the ALOA medium. *L. monocytogenes* differs from other *Listeria* spp. because of the ability to secrete phosphatidylinositol-specific phospholipase C (PI-PLC) encoded by the *plcA* gene. This ubiquitous protein in *L. monocytogenes* and some *L. ivanovii* strains generates green-blue colonies surrounded by an opaque halo (Magalhães et al., 2014; Ottaviani, 1997; Quereda et al., 2018; Schwan et al., 1994).

BBL CHROMagar Listeria produced by Becton Dickson Diagnostics is a selectively enhanced ALOA type medium to isolate and identify *L. monocytogenes*. *L.ivanovii* and *L. monocytogenes* species produce blue colonies with a white halo and other *Listeria* spp. species produce blue colonies but without halo (Hegde et al., 2007). Several classes of ALOA medium are commercially available including Biosynth Chromogenic Medium (BCM) Plating Medium (Biosynth), Compass and Confirm L. Mono Agar (Biokar Diagnostics), Brilliance Listeria Agar (Oxoid) and chromID Lmono (BioMérieux) (Osek et al., 2022; Ottaviani, 1997).

Another chromogenic medium is Rapid' L.mono agar (Bio-Rad). The principle of this medium depends on the detection of phospholipase C activity of *L. monocytogenes* with the different substrates (X-IP) and the inability of metabolizing xylose (Beumer et al., 2003 and Zunabovic-Pichler et al., 2011). *L.ivanovii* can metabolize xylose producing blue colonies with a yellow halo. On the other hand, *L. monocytogenes* appears as blue colonies without a yellow halo because they cannot metabolize xylose. Colonies formed by other *Listeria* spp. are white with or without a yellow halo (Jantzen et al., 2006; Magalhães et al., 2014; Osek et al., 2022). Therefore, to distinguish and isolate *L. monocytogenes* from other *Listeria* species, it is important to utilize both selective and chromogenic media in food samples.

3. ALTERNATIVE DETECTION METHODS

3.1. Immunoassays

Various kinds of immunoassays are preferred because of their easy accessibility and availability as commercial kits approved by FDA. The principle of the immunoassays to detect *L. monocytogenes* is based on the reaction between antibodies and *L. monocytogenes* target-specific proteins such as flagella, listeriolysin O, phosphatidylcholine - specific phospholipase C and p60 protein (encoded by the *iap* invasion-associated protein) (Jantzen et al., 2006; Pilgrim et al., 2003). The most known enzyme-linked immunosorbent assays (ELISA) for *L. monocytogenes* identification are TRANSIA™ PLATE *L. monocytogenes* (BioControl Systems) and VIDAS® LMO2 (Bio-Mérieux) and both methods are validated by AOAC International in terms of ISO protocol.

3.2. Spectrometry Methods

The VITEK 2 Compact® is a fast and robust microorganism identification system. The system utilizes barcode cards allowing kinetic analysis by reading each biochemical test every 15 min and combines fluorimeter and photometer readings to yield fluorescence, turbidity, and colorimetric signals. (*AOAC Approves bioMérieux VITEK® 2 Identification Cards for Biological Threat Organisms, E. Coli O157, Listeria and Staph*, n.d.). For the detection of *Listeria* spp., the GP card, utilized for Gram-positive bacteria applies 43 biochemical tests for instance carbon source utilization, resistance, and enzymatic activity. Even though The VITEK® system is an acceptable and precise automated method, it failed to identify certain *L. monocytogenes* species as *L. innocua* and showed difficulty in identifying atypical strains of the *Listeria* genus (De Lappe et al., 2014). Recently, the usage of MALDI-TOF/MS (Matrix-Assisted Laser Desorption Ionization technology – Time of Flight/Mass Spectrometry) based on the molecular mass profiling of bacteria protein biomarkers has been gaining interest in *L. monocytogenes* identification and differentiation. In the recent study, MALDI-TOF MS correctly identified *L. monocytogenes, L. welshimeri, L. fleischmannii, L. ivanovii, L. innocua, L. seeligeri, L. grayi,* and *L. weihenstephanensis* and these results are validated by whole-genome sequence analyses (Thouvenot et al., 2018). In addition, Reis et al. (2022) have demonstrated that MALDI-TOF/MS identified accurately 73% of *L. monocytogenes*, but the VITEK system exhibited correctly 16% of L. *monocytogenes* emphasizing low sensitivity and false-negative results for VITEK system. However, MALDI-TOF/MS exhibited more

sensitivity at the species level and demonstrated some repeatability issues.

3.3. Molecular Detection Methods

L. monocytogenes identification in food samples by molecular methods maintains precise and reliable results in comparison to the conventional methods but requires specialized instruments and highly trained personnel. Various molecular methods was established for *L. monocytogenes* identification such as PCR, mPCR (multiplex polymerase chain reaction), qPCR (real-time/quantitative PCR), NASBA (nucleic acid sequence-based amplification), LAMP (loop-mediated isothermal amplification), DNA microarray and NGS (next generation sequencing).

3.3.1. Conventional Polymerase Chain Reaction

The polymerase chain reaction (PCR) method is broadly utilized to detect *L. monocytogenes* because it is simple and maintains quick results. The most commonly used target genes of *L. monocytogenes* in PCR protocol are *plcA, plcB, inlA, inlB, iap, hly,* 16S, and 23S rRNA genes. On the other hand, it cannot differentiate viable from dead cells and is unable to detect an accurate number of *L. monocytogenes* giving an elevated number of false-positive data (Chen et al., 2017; Jadhav et al., 2012).

3.3.2. Multiplex Polymerase Chain Reaction

A high proportion of human listeria cases is related to *L. monocytogenes* serotypes 1/2a and 4b infection. The multiplex PCR

method is a useful tool to detect serotype- and virulence-specific *L. monocytogenes* strains which allows identifying of several *Listeria* isolates from the same sample tube. Bubert et al. (1999) developed a multiplex PCR assay that enables the identification of several *Listeria* strains such as *L. monocytogenes, L. innocua, L. grayi, L. ivanovii, L. seeligeri*, and *L. welshimeri,* respectively. In several studies, the detection of *L. monocytogenes* serotype 1/2a or 4b and the lineage-specific identification of *L.monocytogenes* isolates is achieved (Borucki et al., 2004 and Call et al., 2003).

3.3.3. Real-time Polymerase Chain Reaction (Real-time PCR, qPCR)

The real-time PCR method provides high sensitivity and accuracy and unlike the conventional PCR, amplicons (amplified DNA product) are produced in each qPCR cycle with a fluorescent dye for instance SYBR Green, TaqMan, or FRET hybridization probes. The increased fluorescent signal generated is in proportion to the amount of amplicon, which in turn is proportional to the target DNA concentration of bacteria without any post-PCR procedures. However, the main obstacle of this assay is the fluorescent probe binds to all double-stranded DNA such as primers and PCR products, and hinders the accurate identification of the target sequence. Using a real-time PCR method, it is possible to detect *L.monocytogenes* in several kinds of foods. The most common target genes to differentiate *L.monocytogenes* from other *Listeria ssp.* are *actA* gene expressing virulence factor ActA which provides intracellular motility and cell-to-cell spread of the bacteria (Darji et al., 1998; Pistor

et al., 1994; Travier et al., 2013) and *hlyA* gene essential for listeriolysin O production (Hage et al., 2014; Liming et al., 2004).

3.3.4. LAMP- Loop-Mediated Isothermal Amplification, NASBA- Nucleic Acid Sequence-Based Amplification, DNA Microarrays, And NGS- Next Generation Sequencing Technology

There are various LAMP methods developed recently for instance reverse-transcription LAMP, multiplex LAMP, in situ LAMP, and real-time LAMP. LAMP assay can provide higher specificity and sensitivity than conventional PCR methods by targeting the *hlyA* gene. According to Tang et al. (2011) the LAMP assay was studied with the traditional PCR method to identify *L.monocytogenes* strain indicating that the LAMP assay provides more specific and sensitive quantification than the traditional PCR method. In another study, a real-time quantitative LAMP assay has been utilized to identify *L.monocytogenes* in various food samples. The study showed that the LAMP assay exhibited a greater sensitivity than the PCR assay (Shan et al., 2012). In addition, a double LAMP assay targeting the *hlyA* and *iap* genes was quite successful to detect *L. monocytogenes* (Wu et al., 2014).

NASBA maintains selective amplification of mRNA transcript and was implemented successfully to quantify viral RNA molecules avoiding DNA-free RNA. Several studies showed that NASBA is highly selective and consistently identifies viable *L.monocytogenes* cells independently of the presence of DNA in several food samples (Leone et al., 1998; Nadal et al., 2007).

DNA microarray technology enables a determination of a complete genomic map of any microorganism by using the randomly fragmented genomic DNA. DNA microarray studies demonstrated that *L.monocytogenes* can be distinguished simultaneously by targeting virulence factor genes for instance *iap, hly, plcA, plcB, clpE*, and *inlB*. This potential robust genotyping method can be applied not only for *L. monocytogenes* but also for most foodborne pathogens including *Salmonella Typhimurium* and *E. Coli O157:H7* (Bang et al., 2013; Suo et al., 2010).

Next-generation sequencing (NGS) technology allows rapid and simultaneous identification of pathogens and applies to bacteria, viruses, parasites, fungi, animal, and human vectors. This technology revealed the cause of several listeriosis outbreaks by detecting serotype and strain-specific characteristics of *L. monocytogenes*. Molecular detection methods are powerful tools providing high sensitivity and specificity. However, the main drawbacks of these methods are requiring highly trained personnel and relatively high cost, especially for NGS technology.

4.CONCLUSION

Classical culture-based methods, simple PCR, and multiplex PCR are substantially used for routine testing and screening of *L.monocytogenes* in food samples. However, they do not help characterize and type bacterial strains. qPCR, DNA microarray, and especially whole-genome sequence-based techniques such as NGS are

useful for subtyping microorganisms and maintaining a deeper gene analysis and epidemiology of the microbiome.

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CHAPTER 8

ECONOMICALLY AND CULTURALLY IMPORTANT USAGE POTENTIAL OF CHICORY (*Cichorium intybus* **L.**)

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INTRODUCTION

Cichorium intybus L., commonly known as cichory in English and white chicory in Turkish, is an important medicinal plant. It belongs to the Asteraceae family. The most used parts of this plant are its aerial parts, flowers, seeds and roots. All parts of this plant are rich in essential oil, fatty acids, unsaturated sterols, alkaloids, triterpenes, vitamins, flavonoids, tannins, coumarins, saponins, terpenoids, cardiac glycosides, sesquiterpenes, lactones, anthocyanins and phenols. With its rich content, chicory (*Cichorium intybus* L.) is a very valuable healing plant with various pharmacological activities such as antimicrobial, anthelmintic, antimalarial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, analgesic, antioxidant, anti-tumor and anti-allergic. The leaves of the plant can be used as a vegetable, the roots as a coffee ingredient, inulin as a prebiotic fiber, and sometimes as animal feed. (Munoz, 2004). Blue chicory, which was introduced to the world as a forage plant in the 70s and whose varieties were developed, is a plant that attracts attention with its natural spread and density on earth. Blue flowers bloom between April and September. Chicory, which is naturally found in cultivated areas, meadows, rocky slopes and roadsides, can produce large amounts of high-quality forage under suitable conditions. It is quite tolerant to drought. The performance of chicory in animal nutrition is similar to legume plants. All parts of the plant are a very rich source of energy. Chicory seeds, aerial parts and roots contain significant amounts of mineral substances (Fe, Cu, Zn and Mn). Again, different parts of chicory contain varying amounts of ascorbic acid, starch, tannins, reducing and non-reducing sugars. Also

industrial chicory, it is economical because it is used as an inulin source in many agricultural regions around the World (Wang and Cui 2011). Fresh chicory leaves have high it has a special economical importance as a growth factor in probiotic bacteria with its inulin content (Abbas et al. 2015). In recent years, the increase in interest in the use of natural dyes in textile coloring has increased the interest and need for dye plants. Chicory is also a plant rich in natural dyes. Its leaves and roots are used as natural dye. Chicory, which has many different areas of use, reduces nitrate leakage with its deep-rooted roots, thus preventing the formation of acidification and salinity.

1. ORIGIN AND GEOGRAPHICAL DISTRIBUTION

It is a word of ancient Greek origin and was used to describe edible endives. Derived from Greek and Latin, chicory takes its name from the words "cichorium" meaning "field" and "intybus" meaning "cutting" leaves and hollow stems. Pliny records that the plant was called "cichorium" in Egypt. It is stated that it was a widely consumed vegetable in Ancient Egypt. However, this plant is probably *C. endivia*, which is closely related to *C. intybus*. The plant, thought to originate from Egypt and Indonesia, has been cultivated in Europe since the 16th century. Chicory is native to Western Asia, North Africa and Europe, but geographically it is widely distributed in Africa, Asia, Europe, Australia, North America and South America. (Nieddu et al., 1999). The wild chicory plant, which is distributed along roadsides and waste areas, was brought to North America by European colonists. Also common in China and Australia (Street et al., 2013).

There are 10 species worldwide and 5 species (5 taxa) in our country. In our country's geography, *Cichorium endivia* L. (bostan hindibası*), Cichorium glandulosum* Boiss. & A.Huet (akkanak), *Cichorium pumilum* Jacq. (dünek), *Cichorium spinosum* L. çukur otu) *Cichorium intybus* L. hindiba) species are distributed and their synonymous are given in Table 1.

Table 1. *Cichorium* species and their synonyms distributed in Türkiye
(Bizimbitkilerhttps://bizimbitkiler.org.tr/list.html)

Synonym name	Valid name
Cichorium byzantinum Clem.	*Cichorium intybus*
Cichorium intybus var. *eglandulosa* Freyn & Sint.	*Cichorium intybus*
Cichorium glabratum C.Presl	*Cichorium intybus*
Cichorium intybus var. *glabrum* (C.Presl) Gren. & Godr.	*Cichorium intybus*
Cichorium perenne Stokes	*Cichorium intybus*
Cichorium rigidum Salisb.	*Cichorium intybus*
Cichorium divaricatum Schousb.	*Cichorium pumilum*
Cichorium endivia subsp. *divaricatum* (Schousb.) P.D.Sell	*Cichorium pumilum*
Cichorium intybus subsp. *pumilum* (Jacq.) Ball	*Cichorium pumilum*
Cichorium endivia subsp. *sativum* Cout.	*Cichorium endivia*

Although *C. intybus* is distributed in all regions of our country, other species are not common. *C. endivia* is produced as a salad plant, especially in the Marmara region (Figure 1).
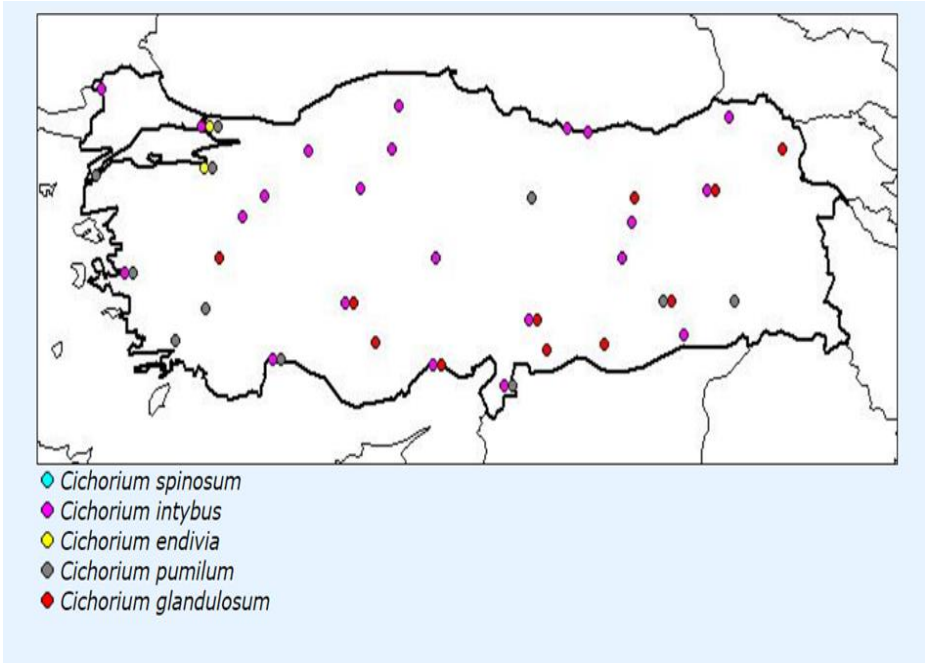
Figure 1. Cichorium species distributed in the geography of our country (Turkish Plants Data Service)

2. MORPHOLOGY

Cichorium intybus L., known as chicory, is an upright, very woody and perennial plant. The plant is 1-1.5 m tall, has a fleshy taproot, is approximately 75 cm long and has broad basal leaves. The leaves have a stalked-lanceolate structure and are unlobed. Its flowers are generally purple-blue, but rarely pink or white. Flowers are hereditary. (Figure 2). Chicory is a plant that is extremely resistant to high temperatures and drought and can remain green for a long time due to its rosette growth habit and deep and wide root system (Kiers et al., 1999). It is also resistant to cold and can survive at -40 degrees.

Figure 2. General appearance of chicory (*C. intybus*) (original photo)

3. CHICORY AS A MEDICINAL PLANT

In the past, Chicory was grown as a medicinal herb by the ancient Egyptians. In the 1970s, it was discovered that the root of *C. intybus* contained up to 40% inulin. It is estimated that approximately 36.000 plant species contain inulin, and most of the inulin is commercially obtained from the roots of the chicory plant. *C. intybus* has been grown as an industrial source of inulin from past to present. Inulins are a group of natural polysaccharides produced by many types of plants, belonging to a class of dietary fibers known as fructans. Inulin is used by some

plants to store energy and is typically found in their roots or rhizomes. It has a negligible effect on blood sugar and is therefore said to be suitable for diabetics. The use of white chicory for therapeutic purposes began with human history. *C. intybus* has traditionally been used to treat fever, diarrhea, jaundice and gall stones. *C. intybus* has various pharmacological activities such as antimicrobial, anthelmintic, antimalarial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, analgesic, antioxidant, anti-tumor, anti-allergic. (Street et al., 2013; Al-Snafi, 2016). Despite its long tradition of use, the herb is not described in the European Pharmacopoeia or any official pharmacopoeia of a European Union Member State (Al-Snafi, 2016). In folk medicine in our country, the roots are boiled for cancer and kidney stones, the leaves are used as an ointment for wounds, the ash obtained by burning the roots in the oven is mixed with butter for wound healing and as an ointment for the treatment of skin disorders, and the aboveground parts are used as tea for hemorrhoids, liver diseases and urinary tract infections. is used   (Street et al., 2013; Sezik et al., 1991).

4. CHICORY IN ANIMAL NUTRITION

Chicory (*Cichorium intybus* L.) is a perennial plant used as animal feed in many parts of the world. Forage chicory produces large amounts of high-quality forage under favorable conditions in the warm season. Animal performance on chicory is similar to that on legumes and is superior to grass-based pastures.

Figure 3. Animals grazing on the roadside (original photo)

Providing quality feed at the end of the summer season, chicory has a high digestibility rate, non-structural carbohydrate content and is rich in minerals. It prevents swelling and parasite formation in animals (Athanasiadou et al., 2007).

Chicory, a common plant of natural pastures, has been included in breeding studies and varieties have been developed due to its high forage potential. The first variety, "Grasslands Puna", was registered in New Zealand. Later, Puna II and Choice varieties were developed in 2002.

Forage chicory varieties: Puna (Grasslands Puna), Forage Feast, Choice, Oasis, Puna II, Grouse,'Six Point. Others varieties known include; Chico, Ceres Grouse, Good Hunt, El Nino and Lacerta. As an alternative forage plant, its use has increased in recent years, especially in grazing.

Şekil 4. Chicory/clover pasture (httpswwwagricomconz/management-advice/pasture-management/chicory/chicory-grazing-dairy-cows)

Chicory is a forage plant that is rich in minerals, has high feed quality, is delicious, but has low fiber content (ADF/NDF). It can also be used by growing it together with other forage plants to increase fiber concentration. In arid conditions, it produces grass with high nutritional value in rotationally grazed pastures. Its production is good in the summer period and the hay yield is around 800-1300 kg/da. In these areas, it creates successful mixtures with *T. repens, T. pratense, L. corniculatus, L. perenne, D. glomerata, S. halepense* (Figure 4).

The dry matter ratio is 25-30% and silage can be made successfully. In some countries, its roots are soaked and used to feed horses. Additionally, grazing chicory may reduce some internal parasites in animals and therefore has the potential to reduce the use of anthelmintics. (Li & Kemp, 2005).

5. CHICORY IN HUMAN NUTRITION

White chicory plant is very rich in nutrients. It contains a number of important compounds such as carbohydrates, phenolic compounds, flavonoids, fat and amino acids, lactones, vitamins, minerals, anthocyanins, inulin, alkaloids, coumarins, sesquiterpenes, unsaturated sterols, chlorophyll pigments, saponins, cinnamic and quinic acid derivatives. Therefore, it is used in many different areas (Molan et al., 2003; Muthusamy et al., 2008). Endive has high potassium, fiber and water content. Its leaves and roots are consumed raw or cooked. It is generally consumed as a fresh salad with a sauce consisting of olive oil and lemon juice. Edible flowers; It is part of many regional cuisines, including Asian, European and Middle Eastern cuisines. In some regions of our country, blue flowers are collected to make jam and syrup. When young roots are roasted, a slightly bitter caramel taste is obtained. It is used as a coffee substitute.

6. AS A NATURAL DYE CHICORY

Dyeing is the art of imparting color to textiles in the form of loose fibre, yarn, fabric or clothing by treating them with a dye. Archaeologists have found evidence of textile dyeing with natural dyes dating back to the Neolithic period. Plant-based dyes such as woad, indigo, and madder were important elements of the economies of Asia and Europe until the discovery of man-made synthetic dyes in the mid-19th century. Although synthetic dyes rapidly replaced natural dyes for the large-scale commercial textile production enabled by the industrial revolution, natural dyes continued to be used by traditional cultures around the

world. In recent years, interest in the use of natural dyes in textile coloring has been increasing. Natural dyes are very compatible with the environment because they are easily degraded. Chicory (*Cichorium intybus* L.) is a plant rich in natural dyes. In some parts of Europe the leaves are used to a limited extent in the production of blue dye. The flowers and leaves of the chicory plant, which has a long history of use, can be used to obtain blue tones from light sky blue to dark dark blue. The flowers and leaves of the plant are collected and boiled in water for a few minutes, then the dye is filtered and used to color fabric, thread or other materials. Natural dyes, which are old and environmentally friendly, also have problems such as the complexity of the dyeing process, limited color tones and inadequate color fastness.

Figure 5. Chicories in the garden and on the roadside (original photo)

However, these problems can be eliminated by using new natural dyes, different mordants and other parameters effective in dyeing. Different colors can be obtained with different mordant or different plant parts. The roots may yield light brown dye. Fresh roots are chopped into small pieces and boiled in water for at least an hour. After straining, alum is added as a mordant, or iron can be added to obtain a darker brown shade.

7. AS ORNAMENTAL PLANT CHICORY

This durable plant, which can grow up to 1.5 meters tall, prefers rich but well-drained soil, preferably in an open and sunny location. Its seeds, which are sown indoors in the spring and then planted later, start to grow with any support from April to the end of September and even the first weeks of October. With its bright, vibrantly colored flowers, it can be used as an ornamental plant in rock gardens, coastal areas, roadsides and parks in landscaping works. It is also an environmentally friendly plant that provides nectar and pollen to bees and other insects.

8. SOIL PROTECTION AND CHICORY

Chicory, a deep-rooted perennial plant, can reduce nitrate leaching and deep drainage, thus reducing the acidification rate of soil and the occurrence of dryland salinity. Chicory, which is an alternative plant that can be used in the evaluation of marginal areas and idle agricultural areas in Türkiye, is a plant with high economic importance and potential, especially in terms of inulin.

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CHAPTER 9

THE IMPORTANCE OF ANNUAL FORAGE LEGUMES: A REVIEW

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Introduction

Sustainability of agricultural production and ensuring healthy food production are the priority problems in the world. Legumes, with their ability to fix atmospheric N_2 through a symbiotic relationship with soil bacteria (*Rhizobium* spp.), have an important role in agriculture by reducing the need for fertilizer N while maintaining high yields of high-quality crop. Legumes have been used in crop rotations for centuries to reduce soil erosion and improve organic matter and physical properties in soil (Woll, 1915; Semple, 1928).

In addition, annual legumes in a mixture with cereals can play an important role in integrated weed management through plant competition (Schoofs and Entz, 2000). However, the system of incorporating legumes into crop rotations has reduced since the development of chemical fertilizers and herbicides (Doran and Smith, 1991). The rising cost of fossil fuels needed to produce chemical fertilizers and pesticides, and awareness of the harmful impact of soil erosion and environmental pollution have highlighted a renewed interest in the utilization of annual legumes in agriculture systems around the world.

This led researchers to re-examine alternative production systems. Because of the great number of species available, the variation in climate, and agricultural practices, research on annual legumes around the world. There is a requirement for exploring the integration of legumes into farming systems. This review synthesizes research results on the agronomic potential of annual legumes with a particular focus on their use for livestock grazing and animal feeding. For utilization, annual

legumes can be divided into two groups based on the size of seeds (small or medium-sized seeds: *Medicago*, *Trifolium, Melilotus*, and *Lens*, and relatively large seeds: *Lupinus, Pisum, Glycine, Phaseolus*, and *Lathyrus*).

Annual Legumes as Forage in Türkiye

Although farming has an important place in Türkiye's economy, the use of livestock products, which are indispensable components of adequate and balanced nutrition, is quite low. Although Türkiye has a good potential livestock population, the productivity is quite low. This situation is caused by genetic factors and our underfeeding. The main reason for this is that feed stuff, which constitutes 60-75% of production inputs, cannot be obtained cheaply and easily. There is a deficit of concentrate and roughage in Türkiye. The quality forage needs of the livestock are met by forage plants grown in agricultural fields and pasture areas.

Pastures constitute Türkeye's most important natural resources and cover approximately 25% of its surface area. Yield potential has decreased significantly as a result of excessive and untimely grazing that has been going on for years. This situation affects pastures negatively leading to water and wind erosion occurring in these areas negatively affecting our soil and water resources thereby a reduction in livestock production.

Türkiye's agriculture system, the cultivation area of forage crops (except silage corn) has been stagnant in recent years, therefore the amount of forage production is limited. When considering climate, soil,

and plant characteristics, forage production can be increased by rehabilitating existing pastures. When natural pastures cannot meet the needs for forage production, or when their structures are damaged, it is vital to establish sown pastures in order to reduce the pressure on these areas and provide roughage.

There are lots of annual legumes in the flora of Türkiye. Medics are some of the annual legumes that contribute to the productivity of the pasture in a certain period. (*Medicago h*ispida Gaertn., *Medicago arabica* L., *Medicago orbicularus* L., *Medicago rigidula* L., *Medicago lupulina* L.). In addition, annual clovers are another annual legume crop that increases the productivity and quality of pastures. (*Trifolium subterraneum* L., *Trifolium meneghinianum* Clem., *Trifoium alexandrinum* L., *Trifolium resupinatum* L., *Trifolium campestre* L. and *Trifolium michelianum* Savi.). Many species of annual legumes, whose origins are reported to be in the Mediterranean floristic region, are found in almost all regions in Türkiye. 23 of 30 Medicago species and 65 of 94 Trifolium species are naturally grown in Türkiye (Davis, 1970).

In a study conducted in a plowed pasture under Samsun ecological conditions, after a few years, the rate of legumes was determined to be 28.43%, and within this ratio, gelemen clover 4.48%, alexandria clover 4.02%, anatolian clover 3.24%, subterranean clover 3.71%, bur clover 2.15%, T. dubium 1.57% and spotted medick was 0.77% (Mut and Ayan, 2009).

The Importance of Annual legumes and Some Properties

In addition to their high adaptability to different soil and climate conditions, they grow early and quickly in the spring, thanks to their tolerance to drought and their ability to escape from drought. Therefore, they extend the grazing period. Their growth form is prostrate or semi-prostrate and they could produce high amounts of seeds in a short time during and post grazing period. They contribute to the sustainability of pastures and animal production with their features such as high nutritional and palatability (Moot, et al, 2003; Striker and Colmer, 2016; Gultekin, et al, 2021). Annual legumes are used especially in lamb finishing pastures due to their rapid growth characteristics in early spring. It has been reported that annual legumes such as subterranean clover are more productive than white clover, especially in dry conditions, thanks to their drought escape mechanisms (Chapman et al., 1989). These crops can survive even in regions where rainfall is 200 - 300 mm. Annual legumes that germinate in autumn under suitable climatic conditions can continue to grow throughout the winter and provide a good feed source for livestock.

Grazing Potential of Annual Legumes

The quality of forage in pastures is largely assessed by the botanical composition (Samuel and Hart, 1998). The grazing behavior of animals, feed consumption, digestibility of feed, and conversion into animal products vary depending on the quality of the feed (Van Soest, 1994; Pavlu et al., 2006). A study conducted by Fraser et al. (2004) in Canada-Alberta reported that the crude protein ratio during the flowering period varied between 15-17% in medicks and 14-16% in annual clover.

Aydın et al., 2010 reported that in Burr medick (*M. hispida* L.), the crude protein ratio is 14.61-26.41%, ADF is 22.20-36.82%, NDF is 33.18-50.60%; In Spotted medick (*M. arabica* L.), crude protein ratio is 15.47-29.00%, ADF is 22.60-32.93%, NDF is 30.54-46.39%, in black medick (*M. lupulina* L.) is 17.50-25.29%, the ADF is 27.06-40.35%, and the NDF is 38.26-55.34%. It has been reported that annual legumes, which are bred and cultivated, are widely used in sown pasture mixtures in countries such as Australia, New Zealand, Canada, and the USA (McCartney and Fraser, 2010; Ateş et al., 2013; Gültekin et al., 2021) and numerous studies on it continue intensively. A study was conducted that 9-12 tons/ha of dry matter can be produced annually from sown pastures established with different pasture plants under Konya conditions. It has been reported that the daily live weight gain of lambs fed on these sown pastures varies between 65 and 285 g/head on average, depending on factors such as seasonal conditions, sheep genotype, and the mixture of plants that make up the pasture (Ates et al., 2013). A study conducted in America states that the sustainability of perennial legumes in pastures is less permanent than annual legumes (Alison and Hoveland, 1989).

Conclusion and Recommendation

When natural pastures cannot meet the needs for forage production, or when their structures are damaged, it is vital to establish sown pastures to reduce the pressure on these areas and provide roughage. Annual legumes extend the grazing period in pastures due to their high adaptability to different soil and climate conditions, their tolerance to drought and their ability to escape from drought, and their

early and rapid growth characteristics in spring. Their permanence in pastures is good thanks to prostrate or semi-prostrate growth, their ability to produce high amounts of seeds in a short time during and post the grazing period, and their hard-seeded feature. Features such as their high nutritional value and high digestibility have a positive impact on animal performance. Annual legumes have a very high potential for use in inter-seeding, especially in natural pastures.

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CHAPTER 10

THE IMPORTANCE OF GRASSLANDS AS CARBON SINKS

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Introduction

The rapid rise in atmospheric carbon (CO_2) concentrations that continued throughout the industrial period is due to anthropogenic (human activities). Today, the most important sectors that cause greenhouse gas emissions are energy, transportation, industry and agriculture. Grasslands, which are vitally important for livestock farming practices that lead in the production of greenhouse gas emissions from the agricultural sector, are natural assets and rural-ecological commons that are directly affected by climate change (Kalonya, 2022). Grasslands have planetary importance in terms of their biodiversity, high carbon absorption and erosion prevention capacity. In addition, they are important terrestrial carbon sinks, thanks to their plant diversity and soil structure, as noted by the Intergovernmental Panels on Climate Change (IPCC). It is known that carbon sinks in the ocean and on land slow the increase of CO_2 in the atmosphere (IPCC, 2019).

The concept of "sink", first defined in the UN Framework Convention on Climate Change, is defined as any process, activity or mechanism that removes a greenhouse gas, an aerosol or a precursor of a greenhouse gas from the atmosphere (UNFCCC, 1992). Kyoto Protocol Land Use, Land Use Change and Forestry Guide (LULUCF) divides sink areas into six groups; It is categorized as (1) forest areas, (2) meadow and pasture areas, (3) agricultural areas, (4) wetlands, (5) residential areas and (6) other areas (IPCC, 2003).

Numerous ecosystem services in grasslands are supported by the movement of carbon (C) among plants, microorganisms, soils, and the atmosphere, which serves as the principal medium for ecosystem energy

flow and biogeochemical cycle (Chapin et al., 2002). Thus, a key component of grassland conservation and sustainable use is understanding the mechanisms that regulate C fluxes and pools in grassland (Liu et al., 2022).

Although the C pool in grasslands is less than that in forests, it nevertheless contributes roughly 25–34% of the total terrestrial C supply in the world (Bai and Cotrufo, 2022; IPCC, 2007). The difference between C uptake, primarily by photosynthesis, and losses, primarily by respiration, leaching, and erosion, determines the C balance of grasslands, just like it does in forests. However, due to their lower density and shorter stature of plants compared to forests, grasslands can be subject to stronger winds, more intense sun radiation, and larger temperature swings. Therefore, the regulation of the grassland C balance is more heavily influenced by abiotic processes (Austin, 2011; Grünzweig et al., 2022).

It is estimated that grasslands retain at least 10% of the global carbon stock in soil (Anderson, 1991; Eswaran et al., 1993). It is known that grasslands are important carbon sinks with a level of 200-300 billion tons (Pg) of CO_2 (Batjes and Sombroek, 1997). Even small changes in grassland carbon absorption inputs can cause greater and longer-lasting carbon sequestration in pastures (Scurlock and Hall, 1998).

Interactions between climate change and rangeland biogeochemistry have received less attention in the literature compared to forest areas. Alteration effects such as temperature, water and nutrients have recently been relatively well understood, but it has only been possible to assess the consequences of interactions between long-term

CO_2 fertilization and global carbon cycles (Hall and Scurlock, 1991). Rangelands are often seen as static areas. However, the loss of carbon absorption of apparently infertile and/or semi-deserted pastures can be compensated by transforming marginal forest areas into humid/semi-humid pastures, which are potential carbon sinks. For this reason, different land use decisions need to be made according to different meadow-pasture characteristics (Schimel et al., 1990; Thornley et al., 1991).

Organic Carbon Cycling in Grasslands

The C balance of grasslands is significantly influenced by the nature, frequency, and degree of disturbance. A significant portion of the primary production in a cutting regime is exported from the plot as hay or silage, although some of these C exports may be offset by organic C imports through the application of farm manure and slurry (Soussana et al., 2010).

Domestic herbivores consume up to 60% of the above-ground dry-matter output when grazing is intense (Lemaire and Chapman, 1996). Under heavy grazing, however, this proportion may be considerably smaller. The majority of the C that is consumed is digested and is therefore breathed out quickly after ingestion. The non-digestible C (25–40% of intake depending on how easily the grazed herbage can be digested) is excreted back into the pasture, primarily as feces. Agricultural techniques include regular grazing and the use of forage cultivars that are highly digestible tend to maximize the herbage digestibility in the majority of productive husbandry systems. As a result, in these systems, the grazing pressure—defined as the average number

of livestock units per unit area—variates with the yearly stocking rate and is the main variable that alters the C flux returned to the soil through excreta (Soussana et al., 2004). The following are some of the secondary effects of grazing on a pasture's carbon cycle: (i) the contribution of excretal returns, which, at a moderate rate of grazing intensity, could favor nutrient cycling and increase primary production, especially in nutrient-poor grasslands (De Mazancourt et al., 1998); and (ii) the contribution of defoliation intensity and frequency, as well as animal treading, which both reduce leaf area and subsequently atmospheric CO_2 capture.

Processes Controlling Soil Organic Carbon Accumulation

Grassland ecosystems primarily accumulate carbon underground. Grassland soils are typically rich in SOC, in part due to active rhizodeposition (Jones and Donnelly, 2004) and in part due to the activity of earthworms, which encourage macro-aggregate formation and the emergence of micro-aggregates that stabilize SOC for long periods of time (Six et al., 2002; Bossuyt et al., 2005). C storage is preferred by rhizodeposites (Balesdent and Balabane, 1996) because it can be physically stabilised in the soil matrix. The transformation of root litter affects both the quality of root litter and the activity of rhizospheres, which can affect the C cycle of grassland ecosystems (Personeni and Loiseau, 2004 and 2005).

According to Jones and Donnelly (2004), the majority of the organic C found in soils (humic substances) is created when plant litter is converted into more durable organic compounds, which results in below-ground C having slower turnover rates than above-ground C.

According to Klump et al. (2007), intensive management reduces the mean residence time of carbon in coarse soil organic matter fractions (above 0.2 mm), which have a quick turnover in soils.

Role of Management for Carbon Sequestration in Grasslands

Although C sequestration or loss may also occur in deeper soil layers, the majority of research primarily focus on the topsoil (e.g., 0 to 30 cm), according to Fontaine et al. (2007). It is frequently believed that management effects are greatest at the surface and diminish when a profile is descended deeper (Ogle et al., 2004). For 20-year time spans, some of the potential soil C sequestration opportunities for temperate grasslands in France have been computed and compared. These calculations yield yearly C storage rates between 20 and 50 g C/m^2 per year. On the other hand, the intensification of nutrient-poor grasslands grown on organic soils may result in significant losses of carbon, and the transformation of permanent grasslands into leys with a medium life span also encourages the release of soil carbon (Soussana et al., 2004).

According to studies conducted in Belgium (Lettens et al., 2005; Goidts and van Wesemael, 2007, respectively), grasslands either sequester carbon in soils at rates of 22 or 44 g C/m2 per year or lose it at 90 g C/m2 per year on podzolic, clayey, and loam soils (Lettens et al., 2005). Because a little change in bulk density can have a significant impact on the stock of SOC, these investigations used pedo-transfer functions to estimate soil bulk density, which increases the degree of uncertainty (Smith et al., 2007).

In a study by Follet and Schuman (2005), in which the carbon sequestration capabilities of pasture areas grazed in 19 different regions were determined, it was concluded that approximately 200 megatons of soil organic carbon are retained annually in 3.5 billion hectares of permanent pasture. Piao et al. (2009) calculated that C stocks in Chinese rangelands increased by 117 and 101 g C/m^2, respectively, vegetation and soil over the previous 20 years.

In a study investigating the European C balance, Jansens et al. (2003) concluded that pastures are a rather uncertain component of the C balance compared to forests and croplands. They estimated that pastures sequestered 66–90 g C/m^2 net C per year in Europe. However, this result was determined using a simple model based on yield and land use data (Vleeshouwers and Verhagen, 2002).

In grassland ecosystems, approximately 60% of net primary productivity is allocated belowground. This is due to the chemical composition of belowground carbon inputs, such as aliphatic compounds and root exudates, as well as their presence in the soil (Fig. 1). It is worth noting that root carbon inputs have a SOC stabilization efficiency that is five times greater than aboveground carbon inputs on average. Therefore, it is recommended to prioritize the incorporation of belowground carbon inputs into soil organic carbon (Jackson et al., 2017).
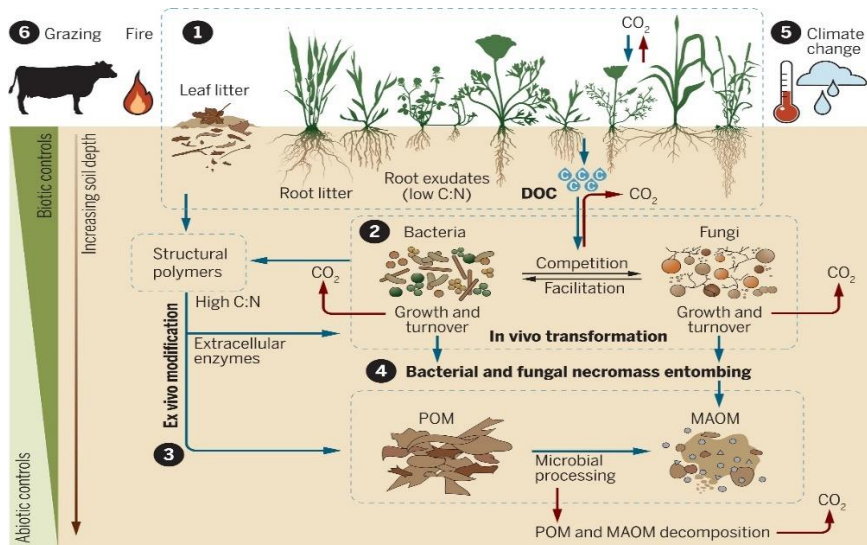
Fig. 1. Conceptual framework for key factors and mechanisms controlling SOC sequestration in grassland ecosystems (Bai and Cotrufo, 2022).

Conclusion

In order to reduce greenhouse gas emissions, it is necessary to focus on reducing emissions caused by livestock farming and land use changes (Niles et al., 2002; Ringius, 2002; Scharlemann et al., 2014). For this reason, it is important that plants and trees store atmospheric CO_2 in the soil and within themselves by photosynthesis, and to increase natural sink areas such as forests and pastures (Pan et al., 2011; McDermot and Elavarthi, 2014).

According to many studies, great progress has been made in the strategies developed to determine the capacity of pastures to retain and preserve carbon in the soil, with the aim of reducing the impact of climate change (Bai and Cotrufo, 2022). The importance of pastures as a carbon sink is not only in reducing the effects of climate change, but also in increasing land productivity and improving livelihoods. In addition,

increasing the effectiveness of rangelands as carbon sinks is a cost-effective process to be used to reduce the impact of climate change.

Above-ground vegetation and organic carbon protect the soil from erosion and increase the water retention and nutrient capacity of the soil. The most desired way to regain C, which has been released from the soil into the atmosphere through various means (fossil fuels and degradation), is to reconnect it to the organic structure by plants through photosynthesis. Preventing and restoring this rapid increase in CO_2 concentration in the atmosphere requires regional-scale global land use planning rather than local-scale planning (Jacobs and Graham, 2000).

The role of pastures in carbon sequestration depends on how effectively pastures can be protected and how well degraded pastures can be repaired. For this reason, more research needs to be done to increase the role of rangelands as carbon sinks in reducing the effects of climate change.
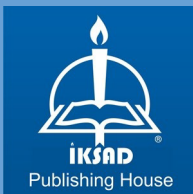
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