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PREFACE

More than other fields, agricultural science deals with complex issues such how the environment is impacted by an increase in the demand for high-quality agricultural goods. New and sustainable approaches to research data management (RDM) are required to meet present and upcoming difficulties and promote integrated and comprehensive research approaches in agricultural sciences. A key element in their development is the participation of scientific users. Since the very beginning of the human species, agriculture has played a central role in human life, and the need for agricultural knowledge is almost certainly just as old. Clay tablets with agricultural knowledge have been found during excavations in the city of Babylon. Modern research data management and imaginative combining of varied relevant data can, among other things, assist to conduct complicated relationships that call for well-connected scientific sub-disciplines that extend beyond agricultural research and cannot be addressed by isolated study. The complexity of research environments is rising in the twenty-first century, and data production is rising along with it. Agricultural engineers need to be knowledgeable about the methods for preventing data loss as well as gathering and managing huge, complicated data sets.

Assist. Prof. Dr. Zeynep DUMANOĞLU

CHAPTER 1

SOME NANOTECHNOLOGICAL RESEARCH TOPICS IN CROP PRODUCTION

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1. INTRODUCTION

With the increasing population, access to safe and sustainable food is getting harder day by day. The problems experienced in agricultural production, which emerged as a reflection of global climate change, directly affect human and animal nutrition negatively. Lots of research is being done to prevent this situation and to prevent the negative effect (Dumanoğlu, 2022).

In sustainable agricultural production, technological developments are used in reducing the amount of pesticides and fertilizers used, and in monitoring the products that are produced with a healthy, high quality and standard production.

In recent years, technological developments such as smart biosensors, controlled release systems, and nano-structured catalysts have been used by many sectors. In the agricultural field; Studies are carried out on issues such as combating diseases and pests, increasing the development and germination capabilities of seeds, and evaluating renewable energy sources (Gogos et al., 2012; Erol Demirbilek, 2015; Uslan, 2023).

In the modern sense, biotechnology includes many approaches that allow understanding and development of the genetics of organisms involved in the agricultural production process (Demirel, 2020). In terms of plant production, abiotic and biotic stress factors, diseases and pests, utilizing plant food materials (James, 2002; Khan & Khan, 2010; Kole, 2011; Coghlan, 2003; Cordell et al., 2009); in animal production;

Issues such as increase in yield and quality by meeting the needs of animals and animal health come to the fore (Fu et al., 2005; Tanaka et al., 2005).

2. NANOTECHNOLOGY

The word "nano", which means "dwarf" in Greek, is mostly used to indicate material dimensions. Metal, ceramic, organic molecular structures, polymeric or composite materials with dimensions of 1-100 nm (nanometers) are expressed as nanomaterials (Özer, 2008) (Figure 1). Materials of these sizes can exhibit properties such as optics, physical strength, chemical reactivity, electrical conductivity, and magnetism. Nanotechnological developments that emerged with the cooperation of many disciplines such as physics, chemistry, engineering, medicine, biology, agriculture and material sciences are actively used (Joseph & Morrison, 2006; Erol Demirbilek, 2015).

Figure 1. Core elements of nanomaterials (https://chemicalwatch.com/)

2.1. Nanotechnology in Agriculture

New methods are applied to ensure that healthy, high quality and standard products are delivered to the consumer by preventing the negative situations (climatic and environmental factors) that arise in sustainable agricultural production as much as possible. It focuses on green production practices such as avoiding fossil fuels, preventing greenhouse gas emissions, and reducing the amount of carbon footprint, especially with the greater use of renewable energy sources. However, cleaning the land and water resources that are polluted or contaminated with diseases and pests under current conditions, and reopening them to the use of producers; Nanotechnology is used in many areas such as cleaning wastewater and including it in the production chain.

Today, nanotechnology in agricultural understanding is carried out to solve problems such as plant breeding, development of plant and animal production factors, inputs such as drugs and fertilizers, early detection of diseases and pests, processing and storage of the product obtained after harvest with yield, and combating soil and water pollution (Boz et al, 2017; Nazir et al, 2020; Jiang et al., 2021; Tüylek, 2021; Uslan, 2023).

Nanotechnology in Crop Production

Plant production from seeds is a method that requires care. Each seed has its own vital characteristics. It is possible to obtain products of the targeted quality from the seed whose needs are fully met.

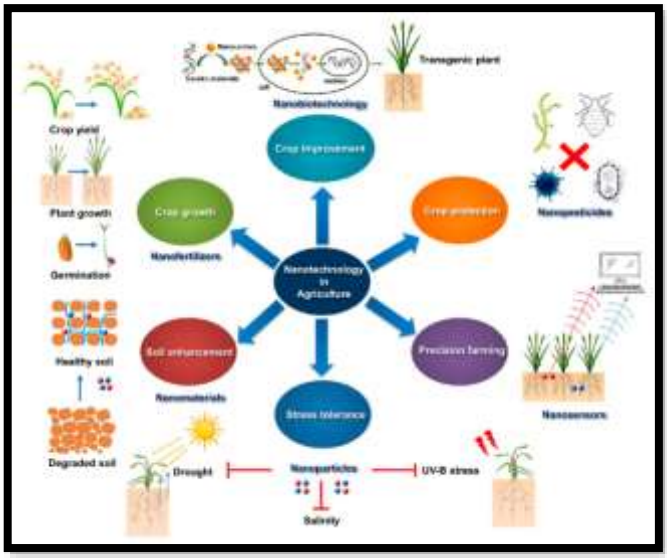

Figure 2. Nanotechnology in agriculture

Some seeds have the ability to germinate easily, while others may take a long time. When biotic and abiotic environmental factors affect negatively, the desired efficiency cannot be achieved. For this reason, seeds are covered with nanomaterials that can withstand predicted/unforeseen environmental factors and increase the germination ability of seeds (Uslan, 2023) (Figure 2).

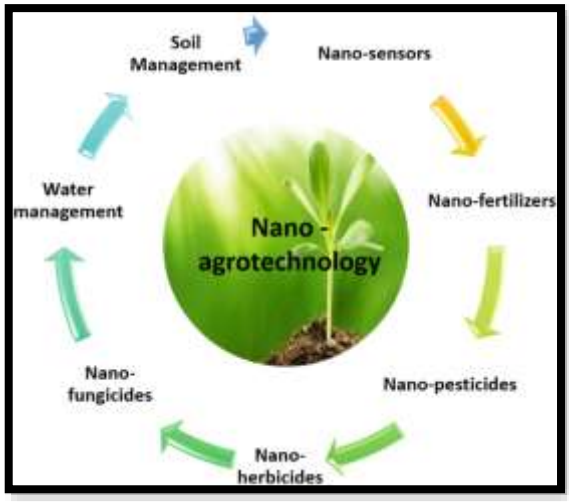

Figure 3. Nanotechnology for using agriculture (Nazir et al, 2020)

After the seeds germinate, the plant needs to reach the plant nutrients it needs for its development. However, the use of much more fertilizer than the rates recommended by agricultural engineers causes both soil and water pollution. In addition, this excessive use does more harm than good to the plant. For this reason, nano fertilizers, which can be easily used and controlled, have been used in recent years (Butt & Naseer, 2020). In addition, researches are carried out on the development of nano-sized materials that can carry important chemicals that can be used in biotechnical control such as the development of pesticides, different pheromones and inhibitors (Figure 3) (Younas et al., 2020).

In addition to production, storage and preservation is another topic that is emphasized in nanotechnological research. During this process, methods such as coating the seed surfaces with nanomaterials containing elements such as silver (Ag), gold (Au), zinc (Zn),

manganese (Mn) and titanium (Ti) are being researched to protect seeds from diseases and pests (Ansari et al., 2020).

In addition to pesticides and fertilizers used in an uncontrolled way in agriculture, nanomaterials are also used in determining the form and rates of pollution of soil and water resources that are polluted industrially, removing pollutants from the environment or reducing them to a minimum level (Scott ve Chen, 2002; Uslan, 2023). In addition, nanomaterials are also used in the removal of deterioration in the soil structure (Shang et al., 2019).

3. CONCLUSION

As a result, the need for food is increasing day by day with the increase in population. However, due to the uncontrolled use of existing resources, environmental pollution, and the increase in unavoidable negative situations such as global warming, transportation to the targeted level and quality food becomes difficult. In order to prevent this, first of all, sustainable measures should be taken and new and modern production methods that emerged with the developing technology should be applied. In recent years, while nanotechnological products have been used more frequently in our daily lives, it is foreseen that these technological developments will be used more and more in agriculture.

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CHAPTER 2

GENETICS AND BREEDING OF HİGH AND LOW TEMPERATURE TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)

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1. Introduction

All stages of the sunflower crops growth are impacted by high temperatures, which ultimately reduces yields. Sunflower crop is most sensitive to heat from early flowering to grain filling. Sunflower can be planted as early as possible in the spring to avoid crop stress at its most vulnerable time during the summer's extreme temperatures. However, low temperatures during the seedling stage of the sunflower induce damage that affects crop growth. Crop improvement for high and low temperature tolerance is a key instrument to combat these stress factors.

Because sunflower (*Helianthus annuus* L.) is a rustic crop, it can grow in a variety of soil types and climates in the temperate zone (Forleo et al., 2018). The primary environmental influences on grain weight and composition in sunflower have been shown to be the temperature and solar radiation during grain filling (Angeloni et al., 2021). Sunflower can survive an early-autumn frost, which often destroys soybeans and maize (Khan et al., 2013). The sunflower development cycle can be divided into the subperiods: sowing to emergence (SEM), emergence to visible miniature floral head (EM-R1), visible miniature floral head to beginning of anthesis (R1-R5.1), beginning to end of anthesis (R5.1-R6), and end of anthesis to physiological maturity (R6-R9). For farmers looking to plan their agricultural practises, the measurement of the thermal time necessary to reach each of these developmental stages is a vital parameter that may be obtained through mathematical models (Maldaner et al., 2018). All stages of the sunflower crops growth are impacted by high temperatures, which ultimately reduces yields. Since

the seed filling stage is extremely sensitive, high temperatures mostly shorten the length of reproductive growth and leaf expansion (Uma & Bharani, 2018).

2. Heat stress

Garca-López et al. (2014) found that sunflower is only marginally resilient to heat stress. It is most sensitive to heat starting from early flowering to grain filling. The specific leaf mass, leaf surface, and soluble protein content all show signs of reduced growth when sunflower is grown at high temperatures (De la Haba et al., 2014). Extreme weather conditions that happen in the estival afternoons at the most crucial periods of sunflower development are occurring more frequently as the climate across the world changes. Sunflower plants may frequently experience short-term temperature and light stress as a result of such circumstances, which is reflected in changes in the ChlF parameters. Sunflower leaf stress has been linked to the synergistic effects of high temperatures and excessive light (Markulj Kulundi et al., 2022). Anthesis is the sensitive stage affected by heat stress because, in sunflowers, pollen and ovule sterility may result when heat stress occurs during the anthesis stage. The threshold temperature varies depending on the genotype and stage of growth. According to reports, sunflowers may experience severe issues with their development rate and embryo survival at temperatures exceeding 27 °C (Soltani & Amirbakhtiar, 2023).

3. Breeding sunflower for heat stress tolerance

For a sustained production under high temperatures, the development of a heat-resistant sunflower breeding population or hybrid is essential. The selection criteria play a major role when breeding for heat resistance. A desirable screening criterion should be rapid enough, highly heritable, and allow the discrimination of resistant and susceptible ecotypes before reproductive maturity so that undesirable plants can be eliminated. In contrast, destructive selection criteria are not applicable for a segregating population. Traits including leaf and head orientation at the time of anthesis are proposed for use in practical sunflower heat-resistant breeding on the basis of an in-depth assessment of the data and experimentation. These traits could be utilized as markers linked with heat avoidance. The traits are simply inherited, non-destructive, non-laborious, and show no impact of the environment and are independent of genotype × environment (Kalyar et al., 2014).

The level of pollen viability reflects sporo-gametophytic heat stress tolerance. Therefore, it is possible to distinguish between resistant and susceptible genotypes by using pollen fertility index under heat stress. To increase pollen viability under heat stress, information about the genetics of pollen viability is required. General combining analysis showed that gametophytic type of heat resistance was important in the inheritance of pollen viability (Razaq et al., 2017).

Diaheliotropism (following the sun) and paraheliotropism (avoiding the sun) are two types of heliotropic movements. Depending on the particular genotypes and environment (high leaf temperature, plant soil

and water potential, photosynthetic photon flux density), a species may exhibit both types of motions. In response to the surrounding environment, the pulvinal tissues around the stem of the sunflower cause heliotropic motions (Kalyar et al., 2013). Intercepted light and a shift in the petiole and lamina angle have connections in sunflower. According to irradiance, plants change their canopy size, and in hot climates, petioles get stiffer (Hernández, 2010).

De la Haba et al. (2014) observed decreased leaf growth (lower specific leaf mass, reduced leaf area) and an increase in soluble protein content during the leaf life span, compared to control plants (70% vs. 45%, respectively) after subjecting sunflower plants to a day/night regime of 33/19°C for 16–42 days. They proposed that hot temperatures encourage the breakdown of soluble proteins in leaves. Additionally, it lowers the rate of net photosynthetic activity, perhaps through reducing the amount of photosynthetic pigments and stomatal conductance. Higher temperatures at the anthesis stage have been shown to have a negative impact on sunflower production because they reduce pollen production and floret fertility (Debaeke et al., 2017). Similar to this, Astiz and Hernández (2013) demonstrated that even in well-watered environments, temperatures exceeding 26°C were supra-optimal for sunflower pollen development. In a modified version of the CropSyst crop model, Moriondo et al. (2011) studied the impact of excessive temperature during anthesis. Two types of sunflower—one drought-tolerant and the other sensitive—were grown by Killi et al. (2016) for 4 weeks in either moderate (25°C) or high temperatures before the

application of a water deficit. They found that the drought-sensitive variety's net photosynthetic rates and stomatal conductance were more negatively impacted by soil drying after being subjected to high temperature treatment than they were after being acclimated to 25°C. Consequently increased temperature could exacerbate the impact of drought stress in sunflower with some genotypic differences to explore (Debaeke et al., 2017).

A recent area of study in higher plants is protein tyrosine nitration, a post-translational modification (PTM) mediated by reactive nitrogen species (RNS). It has been established in the past that high temperature exposure of sunflower seedlings results in both oxidative and nitrosative stress. The investigation of the nitroproteome under these stressful conditions revealed the production of 13 tyrosine-nitrated proteins, of which carbonic anhydrase was one. High temperature examination of carbonic anhydrase activity revealed that this stress reduced carbonic anhydrase activity by 43% (Chaki et al., 2013).

The genome sequence of *Helianthus annuus* (www. heliagene.org) is available. New breeding technologies are reviewed by Ricroch and Henard-Damave, 2016); in addition to these novel technological resources, large genetic diversity within *H. annuus* and across the *Helianthus* genius remains a very promising and largely untapped reservoir of new alleles to adapt sunflower varieties to our needs (Debaeke et al., 2017). Crop wild relatives (CWR) offer the highest diversity for such development, and crop improvement for heat stress tolerance is a key instrument for combating heat stress (Hernández et

al., 2018). Based on expressed sequence tag (EST) sequences, numerous conserved miRNAs in the *Asteraceae* have been predicted (Monavar Feshani et al., 2012). However, little is known about their biological activity and importance (Giacomelli et al., 2012).

The *Asteraceae* family contains unique genes compared with model plants. The haHB4 gene from sunflowers is an example. According to Arce et al. (2011), the encoded transcription factor is a divergent member of the HD-Zip I family that exhibits unusual domains outside of the conserved HD-Zip. HaHB4 has distinct activities that are not shared by its closest *A. thaliana* homologues as a result of this structural difference (Manavella et al., 2008). According to Rushton et al. (2010), WRKY transcription factors have been linked to reactions to both biotic and abiotic stressors. They are distinguished by the presence of the 60 amino acid conserved region known as the WRKY domain, which is made up of motifs similar to the zinc-finger and the WRKY. In sunflower, at least 97 WRKY genes have been found, but it is unclear what their biological functions are. Moreover, phylogenetic analyses of the WRKY genes revealed the existence of *Asteraceae*-specific clades (Giacomelli et al., 2010).

Like many other crops, sunflower is difficult to manipulate. Therefore, it can be very helpful to employ sunflower genes and heterologous systems to shed light on the methods by which these genes function (Chan, 2009). The steps in the pipeline to characterise transcription factors are as follows: 1) isolation of the cDNA and promoter region of the chosen gene, 2) cloning of the cDNA in a suitable vector to

transform *Arabidopsis* plants (under the control of a constitutive promoter like the 35S of CaMV), and 3) obtaining homozygous transgenic plants, and 4) a detailed analysis of these plants using a series of techniques (phenotypic analysis, expression analysis, microarrays, histochemistry, EMSAs, etc.). Last but not least, the knowledge gained in the heterologous system aids in the investigation of the homologous one through temporary transformation of sunflower leaves (Manavella & Chan, 2009). A group of HD-Zip family transcription factors for sunflowers were characterised by using this technology. Finding transcription factors that can change a defence response can help to develop possible biotechnological tools. In this regard, it seems that sunflower HD-Zip proteins are excellent candidates to give tolerance to a number of stressors (Chan, 2009).

With a variety of selection criteria, sunflower segregating and advanced germplasm has been examined for the introduction of heat tolerance in inbred lines. In order to maintain a medium leaf temperature (Tleaf) when under heat stress, Kalyar et al. (2013a) used segregating plants. The characteristics related to leaf gas exchange were of great importance in plants that maintained medium Tleaf. The trait's high heritability, selection gains, and favourable correlation with reproductive biomass all contributed to its importance. Similar to this, Kalyar et al. (2013b) revealed variances in leaf inclination among the F2 population, and they discovered that an upward leaf inclination helped prevent cell membrane damage and high post-noon temperatures. Differences between pre and post noon leaf temperature

was useful criteria for selection of heat resistant plants (Khan et al., 2017).

Sunflower is characterized by high adaptability to high temperatures. Sunflower increases transpiration when the temperature is high, keeping its leaves relatively cool. When there is enough water available, the transpiration rate gets enhanced, necessitating a strong and deep root system. As a result, one crucial factor in the selection of genotypes for sunflowers that can withstand high temperatures is the choosing of genotypes with a strong and deep root system. Tolerance to vigorous transpiration is another essential factor. Breeders should choose genotypes which able to produce large amounts of pollen and preserve pollen viability under such conditions. In order to ensure pollination and seed production, the pistil and its stigma, or the disc flowers as a whole, must also be tolerant of high temperatures (Kori et al., 2012). The capacity for fast seed (formation) filling rates and quick oil synthesis in response to stress conditions is yet another factor in the selection of genotypes adapted to areas with high temperatures and air and soil dryness. Sunflower breeders must have a thorough understanding of how sunflower organs react to high temperatures in order to identify the appropriate breeding techniques, targets, and selection criteria as well as their breeding materials for selection for heat resistance (Škorić, 2016). It is advisable to combine breeding for high-temperature resistance with selection for drought resistance. In countries where extreme temperatures are common, intensive breeding programmes for sunflower heat resistance should be set up. Many

breeding programmes include selection for heat tolerance together with breeding for higher productivity, resistance to dominant diseases, and drought tolerance (Škorić, 2009).

4. Cold stress

Early sowing would enable avoiding unfavourable autumn weather conditions and extending the growing season, improving high-quality seed yields (Hewezi et al. 2006). However, throughout the juvenile stages of sunflower development, seedlings from earlier sowings are typically exposed to extended low or/and fluctuating/unstable temperatures. As a result, it may significantly hinder the emergence, growth, and establishment of seedlings, as well as cause acute chilling injuries and infectious illnesses that lead to a considerable decrease in yield and poor seed quality in sunflowers (Górnik & Lahuta, 2017).

The base temperature for sunflower growth is about 8°C. As a summer crop, sunflowers should be planted as early as possible in the spring to escape stress at its most vulnerable time during the extreme summer temperatures (Garca-López et al., 2014). However, in order to maximise seed and oil yield, early sowing management practises frequently expose seedlings to freezing temperatures. To enable early, successful seeding, it is critical to improve sunflower cold tolerance during the early plant growth and development stages (Kori, 2016). Low temperatures during the seedling stage of the sunflower induce damages that affect crop growth. Some characteristics, like plant chlorophyll content and dry weight, have been linked to a higher tolerance to cold stress in sunflower crops (Allinne et al., 2009). Hewezi

et al. (2006) showed that low temperatures affect plant morphology by altering physiological processes like protein and sugar biosynthesis. They also noted a decrease in the expression of genes that code for glutamate and the production of sugars (sucrose, fructose, and mannose), which are the substances involved in carbon fixation.

5. Breeding sunflower for cold stress tolerance

According to Helena et al. (2017), there is a risk of cold or freeze stress during the early stages of sunflower development at early seeding. Sunflower cold acclimation alterations were examined by Balbuena et al. (2011) using a label-free comparative proteomic approach. Susceptible and tolerant lines were examined. They claimed that tolerant lines had varied proteome responses to cold acclimation and that cold-responsive proteins are mostly engaged in metabolism, protein synthesis, energy, and defence mechanisms. Tetreault et al. (2016) looked at variations in the perennial sunflower species' ability to adapt to cold temperatures and freezing temperatures in three different natural populations: in Texas, Kansas, and Manitoba. Under both non-cold-acclimated and cold-acclimated experimental conditions, plants from the northern latitude had the highest levels of freezing tolerance. Through the process of cold acclimation, the plants from all populations still have the opportunity to increase their freezing tolerance.

In order to study the variations in gene expression connected to two regimes of long-term low-temperature tolerance in sunflower plants, Hewezi et al. (2006) constructed and used a cDNA microarray with >8000 unigenes spotted onto nylon membrane. Two genotypes were

sowed at a temperature of 15°C, and after reaching the one-pair-leaf stage, they were cultivated at a temperature of 7°C until they reached the two-pair-leaf stage. The transcriptome profiles of seedlings cultivated at low temperatures (15°C and 7°C) were compared to those of seedlings grown at typical temperatures (25°C). Only four genes may be regarded to be differentially expressed in both genotypes when comparing gene expression profiles at 15°C and 7°C, showing that identical genetic programmes support the response of sunflower plants to these temperature regimes. The research also showed that early- and late-flowering genotypes react to low-temperature tolerance similarly, which is supported by the small number of genes that exhibit a significant genotype x treatment interaction effect. The susceptibility of sunflower plants to low-temperature tolerance appears to be caused by the down-regulation and/or non-induction of genes that play a vital role in low-temperature tolerance. The data presented give a preliminary assessment of the transcriptome activity of sunflower, a chilling-sensitive plant at suboptimal temperatures. They may be significant in identifying potential distinctions between chilling-sensitive and chilling-tolerant species.

In order to successfully sow sunflower early, it is critical to strengthen its resilience to cold during the plant's early growth and development, namely at the stages of germination, emergence, and the formation of two to three leaf pairs. In order to permit sunflower cultivation at higher elevations and in colder climates, cold resistance at maturation should also be improved. In the mountains where winters are hard and springs

are chilly, wild *Helianthus* species that grow there naturally should only be searched for sources of cold tolerance (Škorić, 2009).

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CHAPTER 3

BREEDING FOR DROUGHT TOLERANCE IN PEANUTS (*Arachis hypogaea* L.): AN OVERVIEW

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1. Introduction

Peanut is one of the most valuable legumes, grown mainly in arid and semi-arid regions, where its production may be hindered by the lack of water. Therefore, breeding drought tolerant varieties is of great importance for peanut breeding programs around the world. it is essential for peanut breeding programmes to understand the molecular basis of drought response especially during the pod formation stage. Development of peanut genotypes with improved drought resistance and water use efficiency, which are interrelated complex traits, is highly desirable. Molecular researches on drought-tolerance mechanisms in peanut is still in a preliminary stage especially because of its huge allotetraploid genome size.

The tropical legume peanut (*Arachis hypogaea* L.) is cultivated under a variety of environmental conditions, with the majority of its commercial production takes place in the northern hemisphere (Wrigley et al., 2004). Water stress can significantly affect crop productivity and quality in arid and semi-arid regions of the world, where peanut is frequently produced (Bertioli et al., 2016). A drop in peanut quality and yield can result from drought, which can have a major impact on peanut plants during flowering and the quality of peanut kernels during the podding stage (Jiang et al., 2022). Consequently, a major factor limiting peanut development and productivity is drought (Jiang et al., 2022).

According to Dang et al. (2013), the timing, severity, and duration of the drought all have an impact on the yield and quality of peanuts. In general, the crop form of peanut is slightly drought-resistant, but in

some specific periods, water shortage seriously affects its yield. Drought can significantly affect peanut production during the pod formation stage because it can significantly lower the number and fullness of pods (Koolachart et al., 2013). Therefore, in order to improve pod production, it is essential for peanut breeding programmes to understand the molecular basis of drought response during the pod formation stage (Zhao et al., 2021).

2. Breeding for drought tolerance in peanuts

The vulnerability of peanut to drought stress depends on genotypic variability (Dinh et al., 2013). Genotypic variations in several physiological characteristics associated with drought tolerance, including transpiration and photosynthesis rate, have been identified and provide opportunities to breed high-yielding drought-tolerant genotypes (Balota et al., 2012).

Different selection criteria have been utilised for breeding drought tolerant peanuts, which depend on physiological surrogates for 1) water use efficiency such as specific leaf area, SPAD chlorophyll meter reading, and carbon isotope discrimination, 2) photosynthesis traits such as photosynthesis rate, stomatal conductance, relative water content, and canopy temperature, and 3) root-related traits such as root length, root volume, nitrogen fixation, 4) yield-related traits such as pod and seed number and above ground biomass, harvest index (Girdthai et al., 2012, Chen et al., 2013). To assess genetic advancement and help the breeding of traits suited to drought in various conditions, crop simulation models have been widely employed (Boote et al., 2021).

These include aiding in the evaluation of advanced peanut breeding lines in multiple environments, understanding the nature of genotype-environment interactions, identifying and evaluating desirable traits, generating a crop ideotype for a particular environment, and assessing breeding methods for drought tolerance. By assessing whether a certain trait is favourable or unfavourable to crop production under long-term climate patterns, crop modelling can be a useful tool for choosing the best traits (Zhen et al., 2022).

Development of peanut genotypes with improved drought resistance and WUE (water use efficiency) is highly desirable compared with those varieties developed only with drought tolerance, as always there would be a yield advantage in earlier case (Sharma and Lavanya, 2002). The interaction of a wide range of genes related to physiological drought-resistance and yield-associated variables affects WUE and yield, which are interrelated complex traits. As a result, it is challenging and time-consuming to simultaneously select many traits for WUE, drought tolerance, yield, and elimination of unwanted genomic areas that were co-transferred throughout breeding programmes (Banavath et al., 2018). Further, transferring desired traits from wild to cultivated *Arachis* species through classical and molecular breeding has been limited owing to ploidy nature and cross-pollination incompatibility barriers (Janila et al., 2013). Marker assisted selection of useful alleles/QTLs could enhance selection power regardless of breeding constraints (Banavath et al., 2018).

3. Transcriptomic studies in peanut for drought tolerance

Drought stress significantly reduces pod yield and nitrogen fixation, and increases aflatoxin contamination in peanut kernels (Arunyanark et al., 2012; Songsri et al., 2008). Investigating the molecular mechanism of drought stress response and further improving drought tolerance of peanuts would be of great significance to the peanut industry (Dai et al., 2019).

Arachis hypogaea is a recent allopolyploid that diverged from the *A. duranensis* and *A. ipansis* subgenome progenitor diploid species. The A and B subgenomes of *A. hypogaea* exhibit significant colinearity, and the gene order is preserved with the exception of a few structural rearrangements, according to genetic maps. After polyploidization, homeologous genes may have various expression patterns that, taken together, give rise to a new phenotype allopolyploid species in comparison to its diploid parents. These changes can manifest in different ways, either as simple homeolog expression bias of one or another subgenome copy, or as expression or genomic dominance of total expression, and can be tissue and developmentally specific (Chu et al., 2016). *Arachis hypogaea* is an allotetraploid species (2n = 4x = 40, AABB) with a very large and complex genome. Cytologically, it behaves mostly as a diploid, but multivalents can result in skewed genetic ratios and likely account for many of the “off types” observed in farmers’ fields (Stalker et al., 2016). This was explained by Leal-Bertioli et al. (2015) who, using both genetic and gene expression data,

demonstrated that peanut display both disomic and tetrasomic genetics recombination.

The genome sequences of ancestral diploid peanuts with ~1.2 Gb genome A in *A. duranensis* or B in *A. ipaensis*, and three cultivars of *A. hypogaea* with genome AABB of 2.6 Gb are made available now (Zhuang et al., 2019), which facilitate genetic and genome-wide gene analysis at the molecule level (Huang et al., 2020).

Transcriptomic research has been done in the past to learn more about the molecular processes that underlie different theories of peanut biology. For instance, Chen et al. (2013) analysed the transcriptomes of young peanut pods of the Yueyo7 variety in an effort to understand why young pod growth can only begin once they contact the soil (Chen et al., 2013). Additionally, Wu et al. (2013) used the Spanish peanut *A. hypogaea* leaves, stems, and roots to characterise the various stages of peanut growth using transcriptome analysis. To further explore the effects of salt-stress on peanuts, Cui et al. (2018) sequenced the transcriptomes of the salt-stressed LH14 shoot and root tissues. Comparatively, there aren't many transcriptome research that focus on the molecular mechanisms of drought in peanuts. Shen et al. (2015) examined the transcriptomes of drought-stressed leaves from the drought-tolerant cultivar FH1, which indicated transcriptional alterations following seven days of drought treatments. Another study by Brasileiro et al. (2015) examined the transcriptomes of tissues from wild peanuts that had been under stress for eleven days. On the other side, Zhao et al. (2018) especially investigated the transcriptome

responses of J1, the second identified drought-tolerant peanut variety, in root tissues to shorter-drought (two days). When the three drought-transcriptomic investigations mentioned above are taken into account, evidence has shown that drought conditions can cause changes in the differential expression of a number of genes, including those linked to ABA, carbon metabolism, proline, and photosynthesis. Nevertheless, molecular researches on drought-tolerance mechanisms in peanut is still in a preliminary stage especially because of its huge allotetraploid genome size (Jiang et al., 2021).

Production of groundnuts is constrained by a number of biotic (such as foliar and fungal diseases, nematodes, and insect pests) and abiotic factors (such as drought, cold, and salinity). The most cost-effective way to produce sustainable groundnut production is to develop resistant/tolerant cultivars to these stressors. Through the introgression of specific genes discovered in compatible diploid wild species or land races, molecular breeding techniques, such as marker-assisted breeding and genetic transformation, enable to accelerate the breeding programme to generate high yielding cultivars with desirable characteristics. Groundnut landraces are a potential source for sustainable groundnut breeding. Land races of *hypogaea*, *hirsuta*, *fastigiata*, *vulgaris*, *peruviana*, and *aequatoriana* were collected from Peru, Mexico, and Brazil and showed rich genetic diversity with less oil content and the ability to sustain under adverse environmental conditions (Alagirisamy, 2016).

Because of ploidy variations and chromosomal barriers between the species, the use of wild *Arachis* species in improvement programmes has been restricted. To address this, wild species with A and B genomes can be artificially hybridised, and then induced chromosomal duplication can be used to restore fertility and the tetraploid state (Dutra et al., 2018). A variety of tetraploids with beneficial features, including resistance to diseases and insect pests, have been produced by the generation of synthetic lines by fusing the A and B genomes (Mallikarjuna et al., 2011). This has opened up new potential for peanut improvement. Varieties such as 'Tamnut 74', 'Coan', 'NemaTAM', 'Tifguard' and Bailey that have a genetic contribution from wild *Arachis* species, were released for cultivation in the USA. In Brazil, currently several synthetics allotetraploid are available and are being evaluated for drought tolerance (Dutra et al., 2018).

WRKY transcription factors are essential in the regulatory processes that allow plants to adapt to their complicated environment. In their study, (Zhao et al., 2020) was combined the drought-tolerant peanut variety 'L422' transcriptome sequencing data with bioinformatic techniques to do a thorough analysis of the AhWRKY family. The procedure of finding WRKYs involved two methods. The *Arachis hypogaea* cv. Tifrunner genome was retrieved in the first method from the peanut genome database (PeanutBase) (https://www.peanutbase.org). Additionally, a Pfam file (PF03106) including various sequence alignments and an HMM corresponding to the WRKY domain was retrieved from the Pfam database

(http://pfam.xfam.org). Using the downloaded genome's default parameters and a probability value of 0.01 for finding WRKY genes, HMMER 3.0 was employed. For the second method, the WRKY protein sequences for *Arabidopsis* (AtWRKY) were downloaded from the website of the Arabidopsis Information Resource (http://www.arabidopsis.org/), while the WRKY sequences for *A. duranensis* (AdWRKY) and *A. ipaensis* (AiWRKY) were acquired from an earlier research (Song et al., 2016). The results from the first strategy were validated using the WRKYs from the second approach. After then, all validated candidate genes with probable WRKY domain were further validated using PFAM and SMART programs (http://smart.embl-heidelberg.de) to retain the only sequences with WRKY domain. Additionally, the primary structure parameters of the genes (length of sequences, molecular weight, and isoelectric point) were analyzed using the ExPASy website (http://web.expasy.org/protparam/). The prediction of subcellular location of identified WRKY proteins was performed using Plant-mPLoc (Chou & Shen, 2010), ProtComp 9.0 and WoLF PSORT (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi; https://wolfpsort.hgc.jp). During the study of gene expression level of AhWRKY family members in response to drought stress, 73 differentially expressed AhWRKY genes were obtained to have been influenced by drought stress. These results provide fundamental insights for further study of WRKY genes in peanut drought resistance (Zhao et al., 2020).

4. Metabolites

Research on metabolomics, which examines all or a subset of the metabolites present in a sample at a certain time, is progressing in plant systems. More over 100.000 primary and secondary metabolites are thought to constitute up the metabolomes of higher plants as a whole, of which only around 10% have been identified so far (Gundaraniya et al., 2020). As a linking factor between genotypes and phenotypes, the quantitative and qualitative contents of plant metabolomes reflect their reactions to biotic and abiotic stimuli, genome, and physiological condition. It makes a significant contribution to the research of stress biology by recognizing various compounds such as byproducts of stress metabolism, stress signal transduction molecules, and molecules that are part of the plant acclimation process (Lv et al., 2022). The most comprehensive reaction to environmental changes might be thought of as metabolites. According to Gundaraniya et al. (2020), drought stress causes changes to a number of cellular metabolites, including soluble sugars, organic acids, phenolics, amino acids, fatty acids, nucleotides, peptides, cofactors, and secondary metabolites. Numerous of these metabolites play crucial roles in the plant's defence mechanism. Significant categories of plant secondary metabolites, such as polyamines and phenolic compounds (phenolic acids and flavonoids), provide tolerance and are referred to as a new class of biostimulants under environmental stress, particularly drought stress conditions (Patel et al., 2022).

5. Proline

Maintaining osmotic equilibrium to mitigate the loss of turgor pressure is one of the key responses to drought that plants have. Plant cells modify their osmotic potential by accumulating more osmolyte solutes to compensate for reduced water potential. Plants include a variety of important osmolytes, such as proline and quaternary ammonium compounds as glycinebetaine and choline. Proline is a crucial and versatile amino acid that can have a major impact on both biotic and abiotic stress responses in addition to plant developmental processes. Proline metabolism and accumulation are linked to plant defence systems against abiotic stress. Proline metabolism can have a variety of impacts, ranging from ATP production to the synthesis of reactive oxygen species, although proline accumulation typically enhances osmotic stress tolerance (Furlan et al., 2020).

Several enzymes are involved in proline metabolism. Two enzymes work together in the chloroplast and/or cytoplasm to produce proline from glutamate during proline biosynthesis. The catabolism of proline occurs in mitochondria. The main regulators of proline levels are stress-induced increases in proline synthesis from glutamate and reduced proline catabolism. Proline may scavenge ROS, which protects plants from injury in arid and salty environments. Plants that were genetically modified to overexpress proline biosynthesis genes showed improved inflorescence structure, higher yields, and increased plant biomass (Ashraf et al., 2018).

High level of synthesis of proline by plants is a common nonenzymatic antioxidant response to a broad range of abiotic stresses, including heat stress (Iqbal et al., 2019), heavy metals stress (Kaur & Goyal, 2022) and salt stress (Ahmed et al., 2021).

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CHAPTER 4

BIOFORTIFICATION IN CEREALS

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INTRODUCTION

The complete development of plants is accomplished through the absorption of nutrients from the environment, including the atmosphere, water, and soil, followed by the utilisation of these nutrients in the process of photosynthesis to create new compounds. Individuals obtain essential compounds and minerals essential for their bodies by consuming animal and plant products, thereby achieving a nutritional balance and proportion commonly known as the nutrient balance.

The issue of insufficient and imbalanced nutrition is widespread in modern times, especially in countries that are developing or underdeveloped. Two discrete types of hunger are prevalent globally. There are two distinct forms of hunger: hunger that arises due to the scarcity or restricted availability of food and "hidden hunger" that emerges from inadequate micronutrients and vitamins. Currently, there is a widespread recognition that approximately 800 million people worldwide suffer from inadequate energy and protein intake, leading to malnutrition. Moreover, an estimated 2 billion individuals face the challenge of hidden hunger, distinguished by insufficient micronutrients (e.g. iron, boron, zinc, selenium) and vitamins (R. A. Welch et al., 2002).

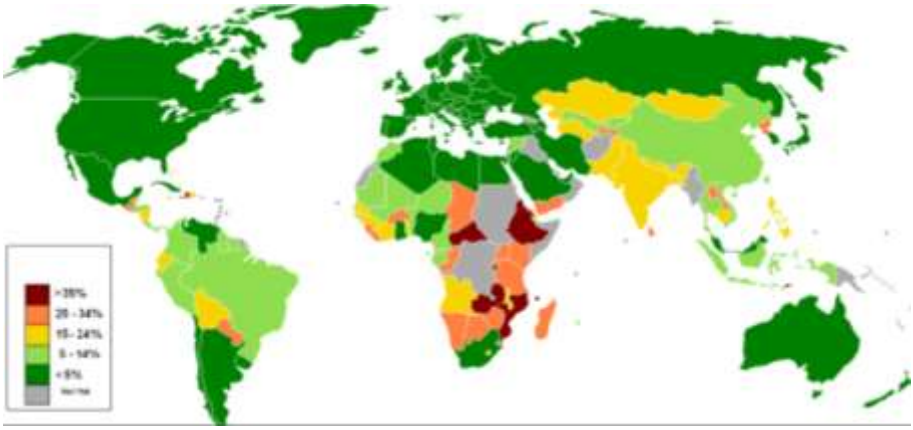

Figure 1. Malnutrition rates by country in the world (%) (FAO, 2016).

Micronutrient deficiency is a significant global health concern affecting more than three billion individuals worldwide, specifically focusing on developing countries (Cakmak et al., 2010; Graham et al., 2001; R. M. Welch & Graham, 2004). Malnutrition is a pervasive global health issue that significantly affects essential developmental outcomes, including cognitive and physical impairment. Research has shown that malnutrition in low-income countries can result in fatalities due to compromised immune systems, infectious diseases, diarrhoea, measles, malaria, and pneumonia.

Figure 2. Distribution of hidden hunger worldwide (HarvestPlus, 2021; WHO, 2009).

Insufficient nutrition can result in long-lasting consequences and could be passed down from generation to generation. Inadequate maternal nutrition during pregnancy increases the risk of maternal mortality and poses a threat to the neonate by increasing the likelihood of low birth weight and mortality. The concerns above persist as a significant matter of public health in countries classified as underdeveloped or developing.

Cereal grains represent an essential proportion of the daily caloric consumption in human diets, comprising more than 50% of the overall necessity. In some geographical areas, such as Southeast Asia and various African countries, this proportion may surpass 70%. However, it is common for these products to lack sufficient amounts of essential minerals and vitamins that are required for proper bodily function.

Currently, modern cultivars exhibit a lower nutritional value with respect to insufficiencies in micronutrients when contrasted with conventional cultivars. Biofortification is regarded as a pragmatic and efficacious approach to furnishing more nutritious commodities for individuals afflicted with malnourishment (Cakmak et al., 2010). In recent years, the development of processed or fortified foods with micronutrients has emerged as a potential solution to mitigate micronutrient deficiencies. However, the implementation of such measures has been accompanied by certain drawbacks, including high costs, limited feasibility in rural areas of developing countries, and challenges in replicating the intervention (Bouis, 2003; Pfeiffer & McClafferty, 2007; Stein et al., 2007). The issue of micronutrient

deficiencies is a global concern, and biofortification has emerged as a promising approach to address this challenge by enhancing the nutritional content of staple food items through biological means. At present, biofortification is the preferred approach in comparison to fortification. The process of biofortification encompasses various strategies, such as the development of novel cereal genotypes that are fortified with micronutrients as well as the augmentation of fertiliser usage that contains micronutrient content (Cakmak, 2008b).

1.BIOFORTIFICATION

The term "biofortification" is derived from the combination of the Greek term "bios," meaning life, and the Latin term "fortificare," which means to enhance or strengthen. The term "biofortification" refers to the genetic or agronomic modification of food items to increase their biologically available micronutrient and vitamin content (Brinch-Pedersen et al., 2007). This process is also referred to as biological strengthening.

According to the definition provided by the World Health Organisation, biofortification refers to the enhancement of the nutritional quality of food crops through various means such as agronomic practices, conventional plant breeding, or modern biotechnology. The objective is to increase the concentration of essential vitamins and minerals, including trace elements, in a food staple to improve human nutrition and promote health benefits while minimising potential health risks. The enhancement of the nutritional value of staple food crops through

biofortification is widely regarded as a sustainable approach for both developing and underdeveloped countries.

The production of nutrient-dense grains is a crucial global concern in the 21st century, particularly with regards to their significance for human dietary requirements. The reason for this is that grains possess an intrinsic deficiency in crucial micronutrients such as iron and zinc.

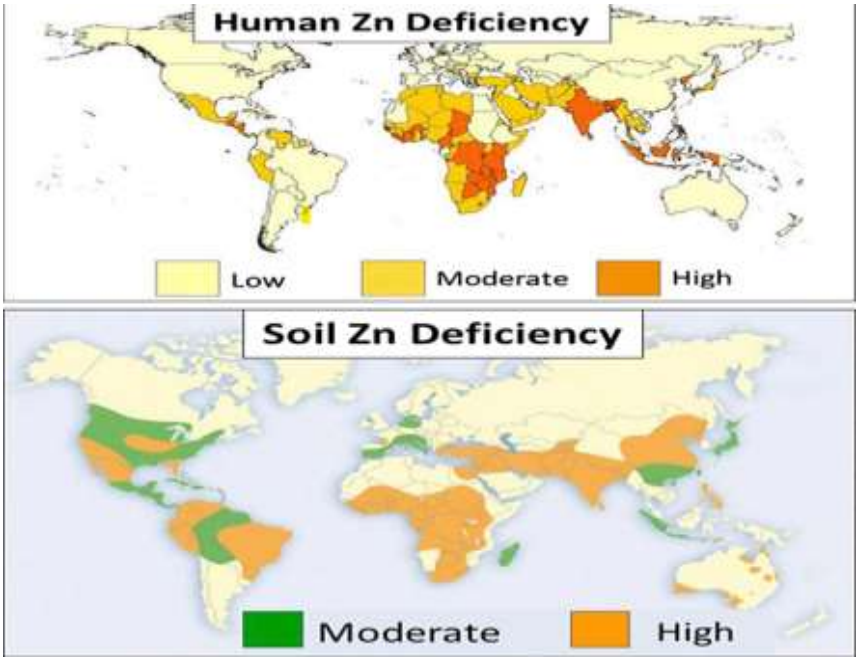

Figure 3. Zn deficiency in agricultural soils and humans = Geographic overlap (Cakmak et al., 2017)

Inadequate zinc intake poses a risk to over 17% of the worldwide populace. Zinc insufficiency is a prominent factor contributing to stunted growth, impacting approximately 22% of the worldwide populace, with a higher prevalence of 33% in Africa and Southeast Asia. According to Wessells & Brown (2012), approximately 30% of

the population in Asia is susceptible to insufficient zinc consumption. As per the World Health Report, zinc deficiency-associated health issues are positioned at the 5th rank among the top 10 health problems in developing countries. Furthermore, Cakmak (2008a) has identified zinc deficiency as the eleventh most prevalent health issue among the top 20. Zinc inadequacy is of particular concern in diets predominantly composed of cereals. According to Black et al. (2008), inadequate zinc intake in the diets of children under 5 can result in mortality rates as high as 4.4% globally. Inadequate zinc levels have been associated with various detrimental outcomes, including impaired fertility, delayed wound healing, cognitive impairment, and other adverse effects. Zinc deficiency poses a risk to preschool-aged children, adolescents, pregnant women, and the elderly. The recommended daily intake of zinc for individuals varies across different life stages. The recommended daily zinc intake for neonates (up to 6 months of age) is 3 mg, whereas this quantity may escalate to 15 mg during adolescence. Furthermore, Mocan (2013) reported that the value above has the potential to increase to 20 mg daily among expectant mothers.

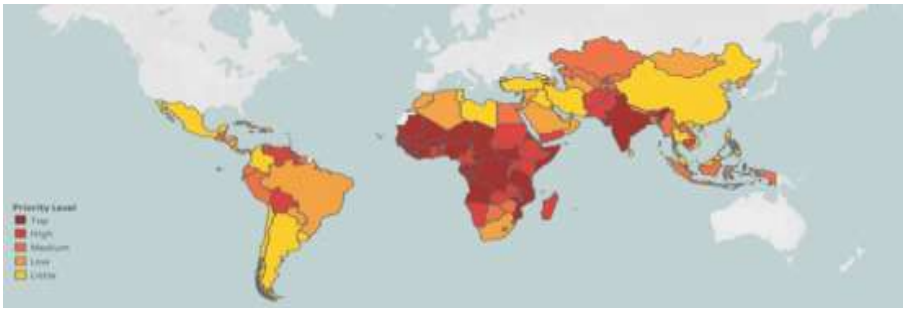

Figure 4. Areas where anaemia due to iron deficiency is prevalent worldwide (HarvestPlus, 2021)

Anaemia significantly impacts women's and children's health worldwide, with a prevalence exceeding 800 million individuals. In the African context, a significant proportion of children under the age of five and women of reproductive age experience limitations in their physical and cognitive capacities, estimated at over 60% and approximately 40%, respectively. Iron deficiency-induced anaemia, which arises from insufficient dietary intake, impacts approximately 32.9% of the global population (FAO & WHO, 2007; Kassebaum et al., 2014).

According to the Lancet series on maternal and child nutrition published in 2015, the insufficiency of vitamin A was responsible for the demise of 157,000 children in 2011. In underdeveloped countries, approximately 7 million expectant mothers suffer from insufficient vitamin A levels, with approximately 6 million experiencing clinical symptoms of night blindness. Most of HarvestPlus's efforts are concentrated in sub-Saharan Africa, where almost 50% of the populace has a vitamin A deficiency.

According to Mayer et al. (2008), biofortified agricultural products can furnish microelements, including iron, zinc, selenium, and vitamin A. Implementing biofortification has been identified as a practical approach to addressing micronutrient deficiencies and enhancing dietary standards (Bouis & Welch, 2010).

2. APPROACHES TO BIOFORTIFICATION

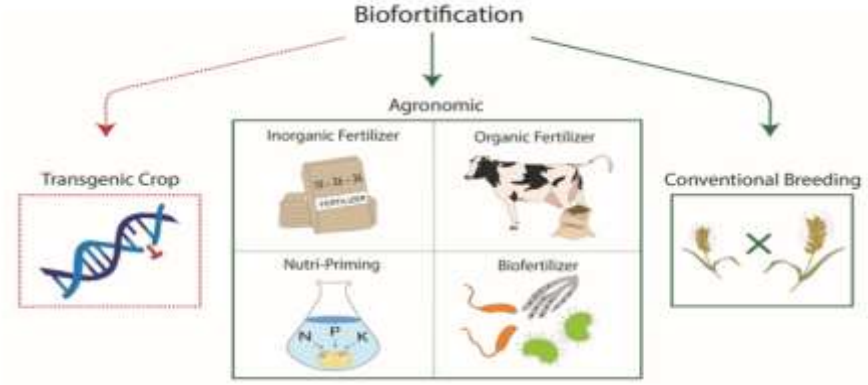

Figure 5. Approaches to Biofortification. Green arrows indicate approaches that are deemed acceptable on a global scale, while the red arrow indicates an approach that is deemed unacceptable (Marques et al., 2021).

2.1. Agronomic Biofortification

Approximately 50% of the global agricultural land is afflicted with soil inadequacies and insufficiencies in various micronutrients. In numerous countries, current fertilization programmes concentrate predominantly on macro-nutrient fertilizers, specifically nitrogen, phosphorus, and potassium. Despite the widespread use of fertilizers, the efficacy of these applications is often limited by various factors, such as the presence of multiple applications and inadequate levels of micronutrients in the soil. Agronomic biofortification is a method that utilizes fertilizers to enhance the nutrient content of crops. This approach involves directly applying fertilizers containing micronutrients to the soil and leaves, either independently or in conjunction with other fertilizers (Manzeke et al., 2014; Voortman & Bindraban, 2015) have suggested that utilizing this approach could

serve as a viable and sustainable means of augmenting crop production and enhancing the nutritional value of crops.

Agronomic biofortification strategies are widely used globally due to their ease of implementation and promptness. The techniques above are characterised as pre-harvest agronomic methodologies that enhance the nutritional value of food (Zou et al., 2012). One limitation of these methodologies is that their execution must occur before harvest to qualify the food as biologically fortified. Upon post-harvest implementation, the food is classified as fortified (Zou et al., 2012). Agronomic biofortification techniques include inorganic fertilisers, organic fertilisers, and biofertilizers applied to the soil or leaves. Applying inorganic fertilisers to either the soil or leaves is a crucial agricultural technique to enhance food crops' natural fortification. Applying these fertilisers has increased the micronutrient levels of diverse crops in varying agro-ecological settings (Garg et al., 2018; Hirschi, 2009; Zou et al., 2012). According to Cakmak et al. (2017), the agronomic approach involves the physical administration of nutrients to crops to enhance their nutritional and health status temporarily. This is followed by the requirement for consumers to improve their nutritional status. Edible plant parts contain varying micronutrients, including iron, zinc, copper, and magnesium, typically assimilated from the soil.

Consequently, enhancing the nutritional quality of the soil has the potential to mitigate insufficiencies and disparities in nutrient intake, commonly referred to as hidden hunger (Cakmak, 2008b). The

methodology above is characterised by its straightforwardness and cost-effectiveness. However, it is imperative to exercise caution when implementing application techniques, selecting nutrient sources, and considering potential ecological ramifications. According to Aciksoz et al. (2011), a positive correlation exists between applying nitrogen and iron to leaves and iron accumulation. According to Yang et al. (2001), the application of zinc to leaves has demonstrated a reduction in the quantity of phytic acid, an antinutrient. Various soil microorganisms, including Azotobacter, Bacillus, Rhizobium, and Pseudomonas, have been identified as potential agents for enhancing nutrient availability in plants (Noori et al., 2014; Ramesh et al., 2014; Smith et al., 2007).

2.2. Conventional Breeding

Genetic biofortification increases crop nutrition by accumulating minerals or chemicals through plant genetic variability. Crossbreeding and gene tracking are used to select adaptable and practical cultivars in various soil and climate conditions. HarvestPlus biofortification has developed current iron and zinc cultivars from wild wheat genetic diversity. Table 1 lists HarvestPlus biofortification successes (White & Broadley, 2009).

Table 1. Biofortified crops developed through the HarvestPlus program

Crops	Nutrient	Country	Year
Wheat	Zinc, Iron	Pakistan, India	2012-2013
Corn	Provitamin A	Zambia	2011-2012
Paddy	Zinc, Iron	India, Bangladesh	2012-2013
Millet	Zinc, Iron	India	2011
Bean	Zinc, Iron	Congo, Rwanda	2010
Kassava	Provitamin A	Congo, Nigeria	2011-2012
Swet Potato	Provitamin A	Uganda, Mozambique	2007

2.3. Genetic Modification Technology

The utilization of transgenic techniques proves advantageous in cases where there exists restricted or negligible genetic diversity among plant species concerning their nutrient composition (Brinch-Pedersen et al., 2007). Hence, the transgenic methodology represents the only viable strategy to augment the levels of a specific nutrient in a crop in instances where it is not inherently available (Pérez-Massot et al., 2013). Utilizing the transgenic approach facilitates the incorporation of genes that augment the levels of micronutrients while concurrently reducing the prevalence of anti-nutritional compounds. Implementing these methods for crop development necessitates a substantial investment of time, resources, and labour. Nevertheless, it represents an economically viable and ecologically sound strategy (Das & Green, 2013).

3. BIOFORTIFICATION IN CEREALS

Grain-producing cereals that pertain to the Poaceae family, previously called Gramineae, are cultivated for their edible seeds. The plant category commonly referred to as "Cereal" encompasses a variety of grains such as wheat, barley, oats, rye, corn, rice, millet, sorghum, teff, and quinoa, among other examples. This particular assemblage of plants holds significant agricultural value in our nation, as it is widely cultivated, produced, and utilized. Its global prominence further underscores its importance. Cereals hold significant strategic value, particularly in their ability to fulfil the nutritional requirements of both

humans and animals and serve as fundamental raw materials for various industries. As a product group, cereals are deemed indispensable.

Cereals are grown on a vast expanse of approximately 721 million hectares, representing 48% of the total agricultural area of 1.5 billion hectares worldwide. This cultivation yields an estimated 3 billion tons of products. Cereals hold a significant strategic value as a crucial and irreplaceable category of products for both human and animal sustenance and industrial purposes. Cereals are categorized into two groups based on their physiological requirements, particularly temperature, namely cool and warm climate cereals. Cereals that thrive in cool climates encompass wheat, barley, oat, and rye, whereas those that flourish in warm climates comprise maize, rice, sorghum, millet, and teff. According to TUİK (2019) and WHO (2019), cool climate cereals constituted 34.9% of the overall cereal cultivation and 32.9% of the total production. In contrast, warm-climate cereals account for 60.5% of the total cereal cultivation and 67.1% of the total production on a global scale.

Table 2. World cereal production quantities (million/tons).

	2010 /11	2011 /12	2012 /13	2013 /14	2014 /15	2015 /16	2016 /17	2017 /18	2018 /19	2019 /20
Wheat	655	701	659	718	732	740	757	763	732	763
Corn	849	907	901	1.032	1.061	1.023	1.132	1.090	1.129	1.119
Barley	124	134	131	145	144	150	148	145	139	156
Oat	20	23	21	24	23	22	24	24	22	23
Rye	12	13	14	17	15	13	13	13	11	13
Paddy	674	700	710	718	720	711	733	739	745	741
Other	5	5	5	4	4	4	4	5	106	102
Total	2.339	2.579	2.539	2.761	2.813	2.769	2.920	2.881	2.884	2.917

Cereal grains represent a significant proportion of the daily caloric intake in human diets in numerous developing countries, with Southeast

Asian and African countries showing an exceptionally high proportion of up to 70%. Nonetheless, it is commonly observed that they do not adequately fulfil the essential requirements of bare minerals and vitamins (Bouis & Welch, 2010).

The literature indicates that while cereal grains are a reliable source of calories, they are deficient in crucial micronutrients such as carotenoids and folates (Bouis & Welch, 2010). Biofortification research is underway to enhance primary cereal crops' nutritional value and promote human health in less developed and developing countries (McGuire, 2015). The current emphasis is on enhancing the levels of micronutrients such as iron and zinc while also striving to augment the concentrations of provitamin A, folate, and carotenoids. The insufficient micronutrients in the cereal grains that are commonly consumed can be attributed to environmental factors. The soil's elevated pH level hinders the plant's ability to absorb micronutrients, diminishes organic matter and moisture, and increases clay and lime composition. According to a study conducted by Eyüpoğlu et al. (1994), an examination of 1511 soil samples obtained from various regions in Turkey revealed that the most prevalent micronutrient deficiency was zinc (Zn) at 49%, followed by iron (Fe) deficiency at 27%. The occurrence of micronutrient deficiencies in plants cultivated in soils that lack micronutrients and in plant-based food items is an inevitable phenomenon.

Table 3. Zinc Concentration Values in Wheat Grains with Zinc Fertilization Application from Leaves in Different Countries (HarvestPlus, 2021).

Country/Location	- Zn mg kg^{-1}	+Zn mg kg^{-1}	Country/Location	- Zn mg kg^{-1}	+Zn mg kg^{-1}
India			Mexico		
Varanasi	29	47	Year 1	21	45
PAU 1	25	81	Year 2	36	60
PAU2	28	77	Turkiye		
PAU 3	26	61	Konya	12	29
PAU4	49	65	Adana	32	57
IARI	33	45	Samsun	23	49
Kazakhstan			Eskişehir	22	43
Location 1	19	54	China		
Location 2	28	73	Location 1	28	54
Pakistan			Location 2	19	26
Location 1	27	48	Australia		
Location 2	28	44	Location 1	18	39
Location 3	30	40	Germany		
Location 4	29	60	Average	20	32
Brezilya			İran		
Average	30	52	Average	17	28

In cases where health issues arise due to insufficient microelements, recommended interventions include administering microelement-containing tablets/medications to individuals or fortifying food during production with microelements. Nevertheless, implementing these solutions can be challenging and expensive, particularly in rural regions. Furthermore, given that they require renewal annually or within a specific timeframe, they do not represent a sustainable solution. Biological enrichment also referred to as biofortification, involves the fertilization of plants or the selection and cultivation of genetically superior varieties to address microelement deficiencies. This approach is favoured over fortification. The approach under

consideration involves the implementation of tactics such as cultivating novel cereal genotypes that are abundant in microelements that may be detrimental to human health and utilising fertilizers that contain microelements to address the issue of microelement insufficiency (Cakmak, 2008a). Various fertilization techniques address microelement deficiencies in soil, plants, and seeds. These techniques include the application of foliar fertilizers, as well as inorganic and organic fertilizers, which have been found to yield more efficient and expeditious outcomes. Furthermore, utilising foliar application techniques serves to mitigate constraints that impede plant roots and soil uptake, such as fixation, pH, salinity, and other related factors.

Nanomaterials refer to materials that are manufactured using various techniques and have dimensions that are less than 100 nanometers. The nanoscale dimensions of nutrients in fertilizers synthesized through nanotechnology have led to the hypothesis that plants can readily take up the root cells and leaf cuticles and subsequently translocate over short and long distances within the plant. However, similar to conventional fertilizers, the cell membrane may impede the ingress of ions in nanotechnology-based fertilizers. Research findings indicate that the diameter of the pores in cell walls ranges from 5-20 nm (Fleischer et al., 1999). Consequently, materials with dimensions smaller than the size above can traverse the plasma membrane easily and access the plant's conducting system.

In recent years, there has been a surge in biofortification research that involves the supplementation of nitrogen to fertilizers. The previous

research indicates that there was a postponement of the plant's harvest period by approximately one week and that the transportation of nitrogen and other essential nutrients from the leaves and stems to the grain persisted for a minimum of one week (Kara & Mujdeci, 2010; Woolfolk et al., 2002; Žemela G.P. & Sklyarn, 1986). Furthermore, scholarly investigations have indicated that the application of nitrogen has a positive effect on the iron concentration of the grain (Cakmak et al., 2010; Kutman et al., 2010; Shi et al., 2010).

3.1. Zinc Enriched Wheat

Research has demonstrated that augmenting wheat with zinc through agronomic and biological means is a financially viable strategy for addressing the worldwide issue of zinc insufficiency. It fulfils half of the daily zinc intake recommendation. A study conducted in India demonstrated that individuals who consumed food items made with wheat enriched with zinc exhibited less severe symptoms of pneumonia and vomiting on the same day. According to HarvestPlus (2021), there are no restrictions on producing zinc-enriched wheat in Bangladesh, India, Nepal, Bolivia, Brazil, and Mexico.

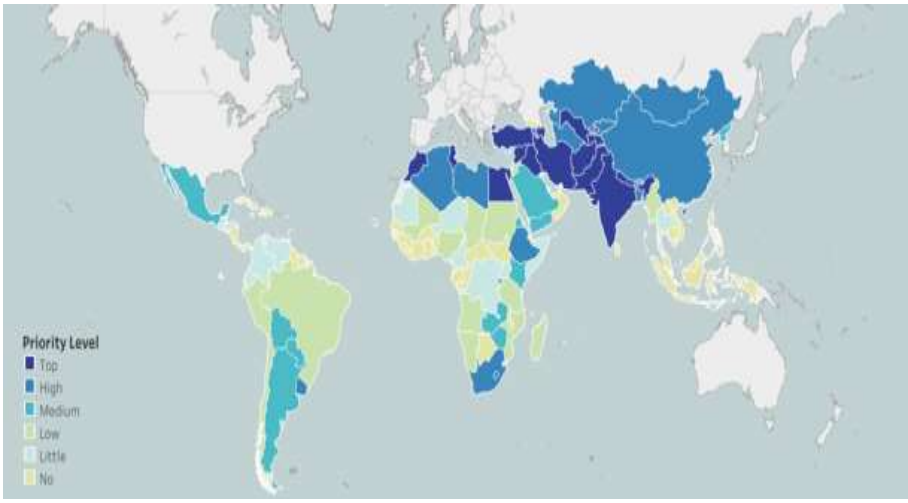

Figure 6. Areas where zinc enriched wheat varieties are used.

3.2. Zinc and Provitamin A-Enriched Rice

Research has demonstrated that the addition of zinc through agronomic and biological means to rice is a viable and economical strategy for addressing the issue of zinc deficiency on a global scale.

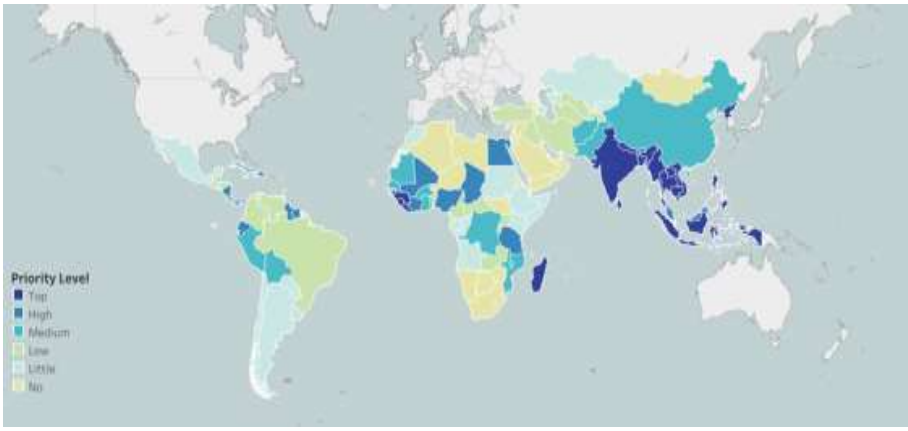

Figure 7. Areas of use of rice varieties enriched with zinc and provitamin A

Rice that is derived from biologically enriched sources containing zinc is associated with a higher bioavailability of zinc. It fulfills 40% of the

daily recommended intake. The cultivation of biologically fortified rice has been authorized in several countries including Bangladesh, India, El Salvador, Indonesia, and Bolivia (HarvestPlus, 2021)

Golden rice is a type of rice that has been genetically modified to contain higher levels of provitamin A, specifically β-carotene, within its genome. The development of β-carotene-enriched golden rice is attributed to the pioneering work of Professor Dr. Ingo Potrykus and Dr. Peter Beyer. The genetic modification of the rice involved the utilization of the crt gene and the lcy gene sourced from the soil bacterium Agrobacterium tumefaciens, which are credited as the foundational elements of this innovation. The acquisition of Golden rice is not achievable through conventional breeding methods. Golden rice can be classified into two distinct types: Golden Rice 1 (GR1) is a type of rice that has been genetically modified to contain 5-7 μg of β-carotene per gram of rice; Golden Rice 2 is a variety of rice that has been genetically modified to contain 31 μg of β-carotene per gram of rice.

3.3. Biofortified Maize Enriched with Zinc and Provitamin A

This food item fulfils 70% of the recommended daily intake of Zinc. The cultivation of Zinc and provitamin A biofortified maize is not subject to any restrictions in Colombia, Guatemala, Honduras, and Nicaragua. The findings of research conducted on children below five years of age in Zambia indicate that consuming maize fortified with Zinc can enhance the daily zinc intake.

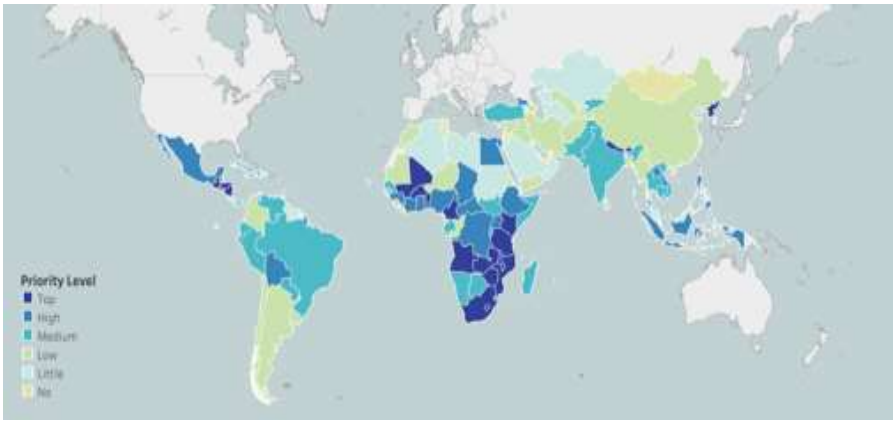

Figure 8. Areas where zinc and provitamin A-enriched maize varieties are used.

The consumption of vitamin A-rich biofortified maize has been found to enhance vitamin A reserves and visual capacity in children. It satisfies half of the recommended daily intake of vitamin A. The production of this item is observed to be unrestricted in Brazil, Cameroon, the Democratic Republic of Congo, Ghana, Mali, Nigeria, Tanzania, Zambia, and Zimbabwe (HarvestPlus, 2021).

3.4. Iron-Biofortified Sorghum

The consumption of iron-biofortified sorghum has been found to mitigate iron deficiency and enhance cognitive function in adolescent children. It satisfies 80% of the recommended daily intake of iron. According to HarvestPlus (2021), the production of this item is unrestricted in Niger and India.

Figure 9. Areas where iron-fortified sorghum varieties are used.

CONCLUSIONS AND RECOMMENDATIONS

Enhancing the nutritional value of food is the goal of biofortification, which also promotes global health and well-being. So far, this field has shown encouraging developments. This complex approach requires the collaboration of many different sectors, with agronomic methodologies playing a crucial role. In contrast to conventional procedures, biofortification is a unique agricultural practice offering a cutting-edge crop fertilisation approach. It is envisaged that the combination of conventional production methods and biofortification will produce a revolutionary agronomic paradigm. Currently, biofortification is being researched, mainly for the agricultural products that are consumed the most. However, it is projected that shortly it will become more productive across a broader range of crops and will be used to address brand-new nutritional concerns. The synergistic fusion of genetic and agronomic techniques is necessary for biofortification to be feasible.

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CHAPTER 5

FORAGE LEGUMES: IMPACTS ON AGRICULTURE AND ENVIRONMENT

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1. INTRODUCTION

We have been faced with a global warming problem that started after the industrial revolution and accelerated in the last century. This is due to the increase in the density of various heat-trapping gases in the atmosphere, primarily carbon dioxide, methane, nitrogen oxide and water vapour. Various industrial activities, motor vehicles, respiration and fermentation, agricultural activities (paddy farming, stubble burning, chemicals, enteric fermentation, agricultural machinery, etc.) accelerate the release of the mentioned gases. As a matter of fact, the International Panel on Climate Change (IPCC), which operates on global warming, reports that the carbon dioxide concentration in the atmosphere increased by 1/3, that is, from 300 ppm to 418 ppm, after the 1950s (Dunn et al., 2022). According to 2021 data in Turkey, it has been determined that there is about 449.72 million tons of CO_2 emissions per year into the atmosphere, the majority of which originates from the energy sector, followed by the industrial sector, emissions from various wastes and agricultural activities (Crippa et al., 2022). The increase in the rate of heat-trapping gases raises the average temperature of the world. Data published by the same institution show that there has been an increase in the average temperature of the world by 10 °C in the last century. It has been determined that the increase in the temperature of the atmosphere causes the rapid melting of the glaciers on the world, especially in the North Pole, and the melting of the glaciers has caused the sea water level to rise by 20 cm in the last century (Anonymous, 2014).

On the other hand, the demand for quality foods, especially animal products, is increasing rapidly around the world due to reasons such as increase in population and urbanization and increase in per capita income (Delgado, 2005). It is expected that the upward trend will continue in the coming years. In order to meet the increasing demand, it is imperative to increase world food production. However, production increases must be environmentally sustainable. In plant and animal production, it is of great importance to include legumes more in the agricultural system and to increase the production of legumes-based quality roughage, due to both increasing production and being environmentally sustainable. In this review, the importance of forage legumes is emphasized in terms of reducing greenhouse gas emissions from the agriculture (mainly livestock) sector and pollution caused by agricultural activities.

2. REDUCING GREENHOUSE GAS EMISSIONS

Agriculture is one of the most important sectors that cause greenhouse gas emissions worldwide. In the agricultural sector, gases such as CO_2, CH_4 and N_2O released into the atmosphere largely originate from the livestock sector. These greenhouse gases are formed because of digestive system fermentation (enteric fermentation), animal feces and urine, respiration and other activities (Hristov et al., 2013). It is estimated that 21-25% of the methane gas released into the atmosphere originating from human activities (anthropogenic) is produced in the digestive system of ruminants (Lascano and Cardenas, 2010). Carbon (C) emissions (C footprint) are explained as CO_2 equivalents and are

estimated as direct and indirect C emissions from products and services (Desjardins et al., 2012). Dairy cows produce 118 kg of methane per year, about twice as much as other cattle. This equates to approximately 2.478 tons of CO_2 (O' Mara, 2004). The ratio of methane gas originating from the livestock sector to other agricultural activities is 38% in Canada (McGinn et al., 2004) and 17.7% in Australia (Eckard and Hegarty, 2004). In general, the effect of methane gas, which is one of the factors that cause global warming, is between 15% and 20% (Gibbs and Hogan, 1990; Zhaoli, 2002). Therefore, the reduction of methane gas emissions from the livestock sector will have an important contribution to reducing global warming.

For instance, in an evaluation made in 27 EU countries, it has been estimated that an average of 22.6 kg of CO_2 $^{-eq}$ equivalent greenhouse gas is emitted to the atmosphere for 1 kg of beef production, and this value is 3.5 kg CO_2 $^{-eq}$ for pork, 1.6 for chicken meat, 1.3 for milk production and 1.7 kg for egg production (Lesschen et al., 2011). It has been estimated that the C footprint for 1 kg of carcass beef production in Canada is 22 kg CO_2 $^{-eq}$, of which 63% is due to methane produced in the digestive tract, 27% from manure and N_2O passing into the atmosphere from the soil (Beauchemin et al., 2010). Methane and nitrous oxide released in production systems mean loss of production. In other words, as methane and nitrous oxide emissions increase, the efficiency of that production system decreases. Because the released methane and nitrous oxide mean energy that does not turn into production (meat, milk, etc.). The addition of legumes to the feed of

ruminants not only increases the yield potential, but also reduces the C footprint by reducing the release of ammonia and nitrous oxide in meat and milk production, and increases carbon sequestration (Undi et al., 2016).

By improving the roughage quality, methane release from the digestive system can be reduced (Chung et al., 2013). Legumes have a higher digestibility than grass and contribute to the improvement of animal production, as they are pass through the digestive system in a shorter time (Hristov et al., 2013). Due to the increased animal productivity, the feeding time is shortened. Thus, the same amount of production can be done with fewer animals, and the gas output from the digestive system is reduced. The decrease in gas release from the digestive system results from the production of more propionic acid in the rumen fermentation due to the decrease in structural carbohydrates (fiber) and the increase in the rate of digestion (Iwaasa and Lemke, 2014). Archimede et al. (2011), in a study on methane production in ruminants fed with C_3 and C_4 legume and grass fodder plants, found that ruminants fed with leguminous fodder plants produced 20% less methane. They also determined that the reason for this was due to the fiber structure of the plants and the time that leguminous forage plants stayed in the rumen. Likewise, the enteric methane emissions of beef cows grazing with birdsfoot trefoil and cicer milkvetch were reported to be approximately half that of those grazing with grass meadow brome (Pitcher, 2015).

Although it varies according to the composition of the feed, the harvesting stage and the storage type and conditions, in general, legumes reduce the amount of methane formed in the rumen from each unit of feed eaten (Martin et al., 2016). For example, it was determined that the methane formation in the digestive system of sheep fed with sole red clover decreased (Niderkorn et al., 2014) and meat cattle fed with alfalfa produced 70% less methane (McCaughey et al., 1999). Meat cattle grazing on an alfalfa+grass mixed pasture produced 25% less gastrointestinal gas than those grazing on grasses alone (McCaughey et al., 1997). It has been stated that by choosing suitable plants according to the regions and harvesting them at the most appropriate time, the gas output from the digestive system can be reduced by 5-10%, and it can also be reduced by 5% by adding legumes to the rations (Undi et al., 2016). This can be explained by the fact that the amount consumed is higher in legumes than in grasses, and legumes are digested in a shorter time in the rumen compared to grasses (Martin et al., 2016). However, studies have shown that methane release is different among leguminous plants. In some studies, it was concluded that sainfoin reduces urinary N losses in ruminants (Theodoridou et al., 2010; Aufrere et al., 2008). Condensed tannins in sainfoin may contribute more to the nutrition of ruminants compared to other perennial legumes such as alfalfa (Wang et al., 2015). According to the results obtained by Williams et al. (2011), it was determined that the NH_3 concentrations obtained from ruminants fed with sainfoin were lower than those fed with alfalfa. According to the study of Grose Brinkhaus et al. (2016), which had similar results, it was determined

that blood urea nitrogen obtained from dairy cows fed with alfalfa pellets was 21% higher than those fed with sainfoin, and there was a 38% increase in N in the urine. In another study by Lagrange et al. (2020), they found that one-year-old heifers grazing with sainfoin or birdsfoot trefoil produced about 40% less urea in their urine than those grazing with alfalfa.

In fact, single-species roughage is generally higher in nitrogen lost from the rumen due to the imbalance between degradable nitrogen and fermentable energy in these plants (Martin et al., 2016). In animals fed with pure forage, there is excessive ammonium absorption from the rumen wall, and some of the absorbed ammonium is converted to urea in the liver and excreted through the urine. Especially since the nitrogen in legumes has a higher degradability in the rumen, the loss through urine increases and the efficiency of N use decreases. Therefore, efforts to improve nitrogen utilization efficiency have focused on reducing the rate of degradation of crude protein in the rumen and prolonging the time (Südekum et al., 2016). The rations prepared for ruminants must be formed from mixtures that also include legumes, and protein-energy balance must be ensured and thus N utilization efficiency must be increased. Thus, not only the amount of N excreted in the feces is reduced, but also the rate of volatile urea nitrogen in the urine is reduced (Reynolds and Kristensen, 2008).

There is a soluble enzyme called “Polyphenol Oxidase” (PPO) in the structure of some legumes such as red clover. PPO reacts with caffeic acid to form o-quinones. This substance also combines with both

proteases and substrate proteins, resulting in a slowly degrading structure in the rumen (Südekum et al., 2016). Also, some legumes, such as sainfoin, birdsfoot trefoil, sulla, purple crownvetch, and cicer milkvetch contain a secondary compound called condensed tannin. Condensed tannins bind to protease and substrate proteins, forming a hard-to-degrade structure in the rumen (pH=6-7). This structure, which passes through the rumen is being less degraded, is completely broken down when it comes to the obamasum, where the pH drops to the range of 2.3-3.5, thus increasing the digestion and absorption of essential amino acids in the small intestine (Barry and McNabb, 1999; Archimede et al., 2011). Thus, secondary compounds such as PPO and condensed tannin reduce the amount of methane produced in the digestive tract. In New Zealand, cattle grazing on pastures with perennial grass combined with condensed tannin-containing plants such as sulla and birdsfoot trefoil have been found to extract 13-25% less methane per kg of dry matter consumed, compared with those grazing on pasture formed with perennial grass alone. Nitrogen, which passes through the small intestines without being broken down, is directed to feces, not urine, with the effect of tannins. N in the urine quickly turns into greenhouse gases ammonia and nitrous oxide and passes into the atmosphere. N in the feces turns into soil organic matter. Tannins are also predicted to inhibit some hydrogen-producing protozoans and/or methane-producing organisms that use hydrogen directly in the rumen (Martin et al., 2016). Thus, both greenhouse gas emissions decrease and animal productivity increase. Condensed tannins show anti-helminthic effect, reduce animal internal parasites and increase yield (Lüscher et

al., 2016). However, since the condensed tannin concentrations of forage legumes containing tannins in temperate climates are lower, it has been determined that they have less effect on reducing enteric methane emissions (Bouchard et al., 2013; Chung et al., 2013). In addition, the low cultivation of forage legumes containing tannins in the world livestock sector may limit the objectives of reducing methane gas (Lascano and Cárdena, 2010).

3. REDUCING POLLUTION

The inclusion of leguminous forage crops in the agricultural system makes very important contributions to the protection of natural resources and reduction of environmental pollution. Legumes provide nitrogen to the soil through symbiosis (symbiosis) with *Rhizobium* sp. bacteria, significantly reducing the amount of industrial nitrogen used in the agricultural system. In this way, since the fossil fuels to be used in the production of industrial nitrogen will decrease, less greenhouse gas emissions into the atmosphere as well as underground and aboveground resources and the environment are less polluted. In particular, it has been demonstrated by many studies that the nitrogen fixing efficiency of legumes is increased in areas where legumes are grown mixed with grasses (Lüscher et al., 2016). It has been determined that the amount of N_2 bound in a year in the grasslands where legumes and grasses are grown varies between 100-380 kg ha^{-1} and 10-70 kg ha^{-1} of this is transferred to grasses (Nyfeler et al., 2011). Deep-rooted legumes such as alfalfa and sainfoin, in addition to reducing nitrogen fertilizer requirement by fixing nitrogen, also significantly reduce the

amount of nitrogen that is washed in nitrate form and/or lost in the form of N_2O (Eckard et al., 2010; Iwaasa and Lemke, 2014). These deep-rooted legumes also take the nutrients that have gone deep by washing and take them to the upper layers of the soil. Thus, the loss of nutrients from the soil is reduced and the pollution of underground water resources and the ecosystem is prevented. It is known that clover and sainfoin roots can go down to 7-10 m depth (Acar and Ayan, 2012). Perennial legumes significantly increase the amount of carbon in the soil (Iwaasa and Lemke, 2014). Carbon capture means that the C_2O in the atmosphere passes into the soil in the form of plant residues and stable humus (Lal, 2006). Increasing the amount of C in the soil increases plant productivity as well as decreasing atmospheric C_2O. It reduces erosion and sedimentation by rehabilitating crumbly degraded soils and thus improves water quality (Undi et al., 2016).

4. CONCLUSION

It should not be forgotten that plants grown with legumes increase the efficiency of the plants they grow together and the N use efficiency of ruminants consuming roughage ruminants consuming roughage increase the efficiency of N use, thus providing significant yield increases on the one hand, and making a serious contribution to reducing pollution on the other hand. It should not be forgotten that legumes, which are the insurance of agricultural production, improve aeration, water retention and permeability by increasing the amount of organic matter in the soil, increase the fertility of the soil, reduce the use of chemicals by suppressing diseases, pests and weeds, in short,

enable an environmentally friendly sustainable agriculture. As a result, by including the most suitable legumes for the region in the system and harvesting them at the most appropriate maturity and the highest digestible energy, greenhouse gas emissions from the livestock sector and pollution from agricultural activities can be significantly reduced. By developing new varieties suitable for changing conditions and/or increasing the prevalence of existing varieties, it may be possible for producers to increase their production under changing conditions and reduce greenhouse gas emissions and environmental pollution (Undi et al., 2016).

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CHAPTER 6

TILLAGE EFFECTS ON SOIL AND PRODUCTION MANAGEMENT FOR WINTER WHEAT IN SOUTHWEST TÜRKİYE

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INTRODUCTION

Wheat is one of the essential crops which have the highest rate of the crop production in Turkey. Approximately 50% of the cropland in Turkey is for cereals and one third of this cropland is wheat production area. In the last 20 years, the cultivated area of the wheat is differs between 8.1-9.5 million ha and wheat production is differs between 17.6-21.5 million tones in Turkey. According to year 2008 data's Mediterranean Part is the third in wheat production of Turkey (Anonymous, 2008). Çukurova region is situated in the east part of the Mediterranean Part. Farmers generally burn stubble both for wheat production after harvesting the second crop or second crop production after harvesting the wheat. After burning the stubble, they first use a moldboard plough and discharrow to till the soil and than use a float to break the clods.

It becomes important problem burning stubble before soil tillage in wheat growing as conventional tillage method in Çukurova region. Unsuitable soil tillage methods minimize the sustainability of the agriculture canalized the researchers to apply the conservation tillage methods.

In this study conservation tillage systems were analyzed in terms of the soil moisture content, porosity, bulk density, working efficiency, the percentage of emergence, yield, biological yield, harvest index and fuel and time consumption for wheat production in Çukurova region.

MATERIALS and METHODS

The study was carried out on randomized blocks of 10 m x 2.8 m with four replications on lands of Çukurova Agricultural Research Institute Directorate Hacıali Undertaking between the years 2007-2008. For this research, a standard farm tractor that has 63 kW of drawbar power was used for tillage equipment. The technical properties of tillage equipment used in this study were given in Table 1. Ceyhan-99 variety as wheat seed was used in the study.

Table 1. The technical properties of the tillage machines used in the study

Equipments and machines	Unit number	Working width (m)	Working depth (cm)	Working speed (km h^{-1})
Disc-harrow	20 discs	2.10	10-15	7.75
Lister plow	4 (70 cm) ridges	2.80	15-25	3.34
Ridge float	4 (70 cm) ridges	2.80	15-20	5.02
Ridge drill	4 (70 cm) ridges	2.80	-	
No-tillage drill	8 rows	1.40	-	
Drill	12 rows	2.20	-	4.55

In order to determine moisture content, bulk density and porosity in the soil depth of 0-30 cm, from different points selected randomly, the soil core sampler equipment such as density rings with 100 cm^3, soil cups, hammer, spatula etc., was used.

The study carried out on trial plots of 10 m x 2.8 m, was planned in completely randomised block design with four replications for one year. The main plot treatments were minimum tillage without stubble (MT), ridge tillage with two rows with stubble (RTS2) and ridge tillage with

three rows with stubble (RTS3) and direct seeding with stubble (DS). The methods were:

1. MT : Burn stubble + Disc-harrow + Seeding
2. RTS2: Stubble + Disc-harrow (two times) + Lister + Ridge float + Seeding with two rows on ridge
3. RTS3: Stubble + Disc-harrow (two times) + Lister + Ridge float + Seeding with three rows on ridge
4. DS : Stubble + Direct seeding

Stubble on MT plots was burned while that on RTS and DS plots was retained. Herbicide (480 g l^{-1} glyphosate) was applied at 5 l ha^{-1} to DS plots before sowing. Fertilizer was applied at rates of 350 kg ha^{-1} for wheat (20% N, 20% P) using a spinning disc distributor. Wheat was sown into dry soil in November and the seeding rate was 171 kg ha^{-1} for wheat After seeding, top dressing (26% N, 350 kg ha^{-1}) chemical weed control (herbicides with 40 g ha^{-1} clodinafop-propargyl for wheat) and plant protection treatments (fungicide with 1.25 kg ha^{-1} tebuconazole for wheat) were applied uniformly over the entire area at the same time and rate.

The soil physical parameters such as moisture content, bulk density and soil porosity were measured in order to determine effect of tillage systems on soil properties. For this reason, soil samples were taken from depths of 0-10cm, 10-20cm and 20-30cm. Soil samples were tested in the laboratory for bulk density and total porosity using methods described by Blake and Hartge (1986), and Danielson and Sutherland (1986), respectively. One part of soil samples taken from

each plot was dried in the oven at 105 °C to determine soil gravimetric moisture content.

The plant numbers of the wheat were recorded about four weeks after sowing by counting the plants in 5 m rows per plot three times after the emergence period. From these counts, the percentage of emerged seedlings was calculated for each treatment to evaluate the effect of the tillage system, as reported by Bilbro and Wanjura (1982). The percentage of plant emergence is the ratio of number of germinated plant to number of seed sowed per meter. Winter wheat was harvested manually in late May to determine grain yield (at approximately 0.115 kg kg^{-1} grain moisture content). The yield was obtained from an area equivalent to 1 m^2 within each treatment with three replicates. Harvest index that is defined as grain yield divided by the biological yield (stalk+grain) was used to evaluate the effect of the tillage system on total yield of plant.

The task times for the tillage systems were measured by using a chronometer in order to obtain management data of farm machineries used seedbed preparation for wheat. Fuel tank of tractor at the beginning of each application was filled fully to determined fuel consumption. At the end of each application, the measured fuel was added up to fill in the fuel tank of the tractor. The added fuel was consumed fuel based on area for plots (Barut et al, 1996).

The experiment data was analyzed by SPSS statistics packet programme. The ANOVA procedure was used to evaluate the

significance of each treatment on soil properties and yield in a randomized complete block design with three replications. Treatment means were separated by the least significance difference (LSD) test to prove statistical significance of the results. All significant differences were reported at the %1 and 5% level.

RESULTS and DISCUSSION

The results of the soil sample analyses measured in the stem elongation period according to soil depth for different tillage methods were presented in Table 2, 3 and 4.

Soil moisture Considering all samplings in the study, the tillage systems had a significant effect ($P < 0.05$) on moisture content of the soil (Table 2). The highest soil moisture contents were in RTS plots while the lowest in DS plots. The moisture contents of tillage systems varied between 16.88 % and 19.69 % based on soil depth.

Table 2. Soil moisture (%) according to soil depth for tillage methods; RT: Ridge Tillage, MT: Minimum Tillage, DE: Direct Seeding

Soil Depth	Tillage Methods					
	RTS [b]		MT		DS	
0-10 [a]	**19,69 a**	**A**	17,02 c	B	16,88 c	B
10-20	**19,87 a**	**A**	18,20 b	B	17,92 b	C
20-30	19,24 a	A	**18,78 a**	AB	**18,92 a**	AB
Average	**19.60**		**18.00**		**17.90**	
CV (%)	2,3		1,74		1,84	

[a]: Means with different upper case letters in each line are significantly different at **0.05 level.

[b]: Means with different lower case letters in each column are significantly different at **0.05 level

There were no statistical differences between soil moisture contents in RTS based on soil depth. Means of moisture contents of tillage systems were 19.60%, 18.00% and 17.90% for RTS, MT and DS, respectively.

Soil bulk density The tillage systems had a significant effect ($P<0.05$) on bulk density (Table 3). Soil bulk density of 0-10 cm layer was significantly lower (1.32 g cm^{-3}) in DS than in the other plots. But, there was no statistical difference among tillage systems in the 20-30 cm soil layers as is seen in the Table. Crop residue has been reported to improve soil bulk density and cause lower bulk density (Valzano et al, 1997; Arshad et al, 1999; Ghuman and Sur, 2001).

Table 3. Soil bulk density (g cm^{-3}) according to soil depth for tillage methods; RT: Ridge Tillage, MT: Minimum Tillage, DE: Direct Seeding

Soil Depth	Tillage Methods					
	RTS [b]		MT		DS	
0-10 [a]	1.36 c	AB	1.37 b	A	1.32 b	B
10-20	1.48 b	AB	1.49 a	AB	**1.56 a**	A
20-30	1.64 a	A	1.53 a	A	**1.55 a**	A
Average	**1.49**		**1.46**		**1.47**	
CV (%)	4.19		4.82		2.83	

[a]: Means with different upper case letters in each line are significantly different at * 0.05 level.
[b]: Means with different lower case letters in each column are significantly different at * 0.05 level.

Soil porosity Total porosity in 0-10 cm soil layer was significantly affected by tillage systems as is seen in Table 4 ($P<0.05$). DS had higher porosity (50.70%) than other tillage systems. Total porosity in the same tillage system was significantly higher in the 0-10 cm depth than other both depths. The highest means of soil porosity of tillage systems was 45.43% in MT. However a study performed by Azooz and Arshad

(1996) indicated that total porosity was not affected by no-tillage compared with conventional tillage in the silt loam, distribution of pore sizes was affected (Arshad et al., 1999).

Table 4. Soil porosity (%) according to soil depth for tillage methods; RT: Ridge Tillage, MT: Minimum Tillage, DE: Direct Seeding

Soil Depth	Tillage Methods					
	RTS [b]		MT		DS	
0-10 [a]	**49.28 a**	AB	**48.94 a**	AB	**50.70 a**	A
10-20	**44.62 b**	**A**	44.33 b	AB	41.73 b	B
20-30	38.92 c	A	43.03 b	A	42.25 b	A
Average	**44.27**		**45.43**		**44.89**	
CV (%)	5.16		5.79		3.38	

[a]: Means with different upper case letters in each line are significantly different at * 0.05 level.

[b]: Means with different lower case letters in each column are significantly different at * 0.05 level

Grain yield The average grain yield for tillage systems was given in Table 5.

Table 5. Average plant density and grain yield for tillage systems; RT: Ridge Tillage, MT: Minimum Tillage, DE: Direct Seeding

Tillage systems	Plant density (plants m^{-2})	Grain yield (kg ha^{-1})
RTS2	384	6419.8 d
RTS3	398	7197.0 c
MT	418	8587.4 a
DS	406	8103.9 b
CV (%)	-	3.51
$LSD_{0.01}$	-	42.62

Means with different letters in each column are significantly different at 0.01 level.

The different tillage systems had a statistical ($P<0.01$) effect on grain yield of the wheat. The grain yield was the greatest (8587.4 kg ha^{-1}) in MT compared to other tillage treatments (Figure 1). The lowest grain yield was 6419.8 kg ha^{-1} in RTS2 (Figure 2). Ridge tillage with three rows with stubble (Figure 3) and direct seeding (Figure 4) methods were lower yield values obtained at the rates of 16.03% and 5.63% compared to the minimum tillage without stubble.

Figure 1. MT: Burn stubble + Disc-harrow + Seeding

Figure 2. RTS2: Stubble + Disc-harrow (two times) + Lister + Ridge float + Seeding with two rows on ridge

Figure 3. RTS3: Stubble + Disc-harrow (two times) + Lister + Ridge float + Seeding with three rows on ridge

Figure 4. DS : Stubble + Direct seeding

This result is pertinent to plant density. As the plant density increased, so did grain yield. While plant number per area on stubble plots was lower, weed population was higher. Even though expected that the yield

on plots with stubble would be higher, this result had not materialized. Due to the fact that weeds share water, nutrients and light by competing with crops, weeds and low plant density on stubble plots can be considered a factor which has decreased grain yield (Akbolat and Barut, 2001).

The harvest index It was determined there is not any statistical difference ($P<0.01$) between the different tillage systems on harvest index. The average harvest index values are seen in Table 6.

Table 6. The average harvest index values of the methods; RT: Ridge Tillage, MT: Minimum Tillage, DE: Direct Seeding

Methods	Harvest index (%)
RTS2	30.35
RTS3	31.18
MT	29.95
DS	30.32
CV (%)	12.88
LSD	6.28

As is seen in the table, the lowest harvest index was 29.95 % in MT, whereas the highest harvest index was 31.18 h ha^{-1} in RTS3. The high biological yield caused to low harvest index in spite of the greatest grain yield in MT.

The task time and fuel consumption The lowest time consumption was found 0.75 h ha^{-1} in DS, whereas the highest time consumption was found 3.94 h ha^{-1} in RTS (Table 7). Similar findings were observed for fuel consumption. This situation shows that task time for field tillage increases due to increasing the pass number. Owing to less the field traffic, the lowest fuel was consumed as 7.90 l ha^{-1} in DS. On contrary

to this, the highest fuel consumption was detected as 62.50 l ha^{-1} in RTS because of increment of pass number on the field as it was observed by Korucu and Kirişci, 2001.

Table 7. The task time and fuel consumption for tillage equipments

Methods	Equipment	Task Time (h ha^{-1})	Total Task Time (h ha^{-1})	Fuel Consumption (l ha^{-1})	Total Fuel Consumption (l ha^{-1})
MT	Goble	0.94	1.40	14.60	18.90
	Driller	0.46		4.30	
RTS	2 Goble	1.88	**3.94**	29.20	**62.50**
	Lister (70 cm)	0.85		10.50	
	Ridge Float (70 cm)	0.30		8.00	
	Ridge Seeder (70 cm)	0.92		14.80	
DS	No-tillage drill	0.75	0.75	7.90	7.90

Working efficiency The working efficiency (ha h^{-1}) determined by rating the measured total time consumption for soil tillage, preparing the seed bad and seeding with the working area in all blocks. The values were given in Table 8.

Table 8. The average working efficiency values of the tillage methods

Methods	ha h^{-1}
MT	0.72
RTS	0.26
DS	1.34

Among the tillage treatments, the highest average working efficiency value (1.34 ha h^{-1}) was obtained in DS. The MT (0.72 ha h^{-1}) and RT (0.26 ha h^{-1}) followed DS respectively. These values showed an inverse ratio between the time and efficiency of the methods. It was determined while the working time increased, working efficiency decreased.

CONCLUSIONS

The study has been performed to compare effects of different tillage and sowing systems on the plant growth, and to make technical analysis that is used for growing the wheat in 2007. The study has been carried out on the lands of Çukurova Agricultural Research Institute Directorate Haciali Undertaking.

The soil moisture content was increased in all the tillage systems except the ridge sowing in all soil depth. The ridge tillage method (RTS) has proved to be statistically unimportant on the soil moisture content according to soil depth. The highest soil moisture content in 0-10, 10-20 and 20-30 cm depths and the highest average value of the soil moisture content (19.60 %) were determined in the ridge tillage method (RTS).

The highest bulk density in 0-10, 10-20 and 20-30 cm depths and the highest average value of the bulk density (1.49 g cm^{-3}) were determined in the ridge tillage method (RTS). The highest bulk density (1.44 g cm^{-3}) was in minimum tillage method (MT).

The highest porosity in 0-10, 10-20 and 20-30 cm depths and the highest average value of the porosity (45.43%) were determined in the minimum tillage method (MT). The lowest porosity (44.27%) was in ridge tillage method (RTS).

The highest yield (8587.4 kg ha^{-1}) was obtained from minimum tillage system (MT) while the lowest yield (6419.8 kg ha^{-1}) was in the two

rows of the ridge tillage (RTS2). The maximum efficiency (1.34 ha h^{-1}) on wheat production was obtained from the direct seeding (DS) technique. The minimum fuel consumption (7.9 l ha^{-1}) and the minimum time consumption (0.75 h ha^{-1}) were in direct seeding (DS). The direct seeding system (DS) saved time on fuel consumption and working efficiency at the rate of 81-86 %.

It is essential that conservation tillage systems be used to preserve natural life and sustaining soil fertility. In this context selected tillage systems should be improved and new tillage systems functioning by mixing previous crop residue into soil should be investigated. So, further research is needed to overcome this problem in the tillage systems with residue. Improved soil properties, higher yield, and an economical tillage system indicate the MT and DS is an acceptable alternative practice for winter wheat production in this region.

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CHAPTER 7

ARE MEDICINAL AND AROMATIC PLANTS USEFUL FOR PLANT PROTECTION?

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INTRODUCTION

Medicinal and Aromatic Plants

Plants have been known as the primary raw material of different areas that consists food, energy and medicine since historical times (Shafi and Zahoor, 2021). They produce secondary metabolites to protect themselves from natural enemies and negative environmental conditions (Benderoth et al.,2006; Gajula and Nanjappan, 2021).

U. S. Forest Service (2023) declared that 40% of the drugs were obtained from the plants (Borkatulla et al., 2023). Medicinal and aromatic plants (MAPs) are identified as the plants of which leaves, flowers, fruits, seeds, stems, peels, roots, rhizomes, bulbs and stolons have bioactive materials (secondary metabolites) like alkaloids, terpenes, and phenolics at the ability of pharmacological activity. So MAPs are the plants mainly used in medicine, perfume, cosmetics, spice, and dye industries (Baydar, 2022).

The MAPs are classified according to the secondary metabolites they contain: Alcoloids are mainly used as drugs and pesticides, terpenes are the primary raw material of the perfume industry, and phenolics are the most valuable antioxidants. It is known that MAPS are natural insecticides and herbicides, too (Baydar, 2022).

The Importance of Plant Protection

Plant diseases, weeds and pests are important biological factors that cause significant yield losses in agricultural activities. This situation causes economic crisis and food security danger at household, national

and global levels (Savary et al., 2019). According to Alaoğlu et al. (2022), plant diseases reduce crop production 11.6%, the pests 13.8% and the weeds 9.5%. So, plant health is a primary issue in preserving food security, environment and biodiversity (Akbaş, 2019).

Plant protection is all the procedures to protect cultivated plants from diseases, weeds and pests that economically limit agriculture production (Kadıoğlu, 2021). Plant protection is the most important precaution to raise crop yield and quality.

FAO (2019) declared 220 billion USA dollars in commercial loss because of breaking down plant health. So controlling harmful organisms has enormous significance. There are five approaches in plant protection: Physical control (e.g. mechanical, pneumatic), human factors (e.g. expertise, laws, quarantine), biological control (e.g. parasites, predators), chemical control (e.g. herbicides, insecticides, fungicides) and biopesticides (e.g. plant extracts). The pesticides used in chemical control are easily used and effective. However, they adversely affect human, animal and plant health; and cause environmental pollution (Vincent et al.,2001). In the USA, 3.5 million tonnes of pesticide are used, and its financial value was calculated as 45 billion USA dollars.

The producers need to find alternative ways because of the disadvantages of pesticides. This study aims to explain the usability of MAPs in plant protection through scientific results.

Use of Medicinal and Aromatic Plants in Pest Control

One of the factors that bring about yield and quality losses in agricultural production is pests like insects, mites, nematodes, rodents, and birds. Pests can cause damage to agricultural products at all stages, from sowing to storage. Since the discovery of DDT in 1939 (Mellanby, 1992), pesticides have often been used to control pests. Agricultural production has risen considerably with the green revolution, especially in rapidly industrialised countries (Briggs, 2009). In order to meet the increasing nutritional needs of the increasing population, the use of pesticides has also increased in terms of the continuity of this increase in production. Pesticides are most commonly used in agriculture to control pests. Also, pesticides are used in public health activities (Philbert et al., 2014), unwanted plants and weeds and suppressing or avoiding the propagation of pests in human living spaces. The harmful effects of chemicals used in pesticide manufacturing on the environment and human health are known (WHO, 1990; Ansari, 2014; Costa et al., 2014; Bernardes et al., 2015). Therefore, consumers especially prefer organic farming products with fewer health risks. Biopesticides include different substances such as living microorganisms, botanical compounds and semiochemicals (e.g. pheromones) (Kiewnick, 2007). MAPs are also used to control insects and other pests. Essential oils (EOs) are effective pest control components (Ayvaz et al., 2010; Pérez et al., 2010). EO are secondary metabolites formed by MAPs and are volatile, natural, complex compounds. EOs are lipophilic, so it enters the insect, causing biochemical dysfunction and leading to death (Lee et al., 2004). The toxicity of EOs due to especially chemical compounds, but this effect

depends on many other factors. Factors such as the form of entry of the toxin, its molecular weight and its mechanism of action are the causes that reveal its toxic effects. AEOs obtained from the plant families, including Asteraceae, Myrtaceae, Apiaceae, Lamiaceae, Annonacea, and Rutaceae have insecticidal activity (Arun et al., 2009; Ebadollahi and Jalali Sendi, 2015). EOs of the plant families mentioned above can be used as a repellant or fumigant larvicidal and adulticidal in insects like Lepidoptera, Coleoptera, Diptera, Isoptera, and Hemiptera (Arun et al., 2009).

In the literature, the number of research about the pesticide effect of EOs extracted from members of the Lamiaceae family is quite high compared to the other plant families mentioned above. It has shown by studies that EOs obtained from members of the Lamiaceae family are effective for many pests (Heidary et al., 2020; Alloui-Griza et al., 2022; Plata-Rueda et al., 2022). Studies are showing that members of the Lamiaceae family are effective on the insect mentioned above orders Lepidoptera (Bibiano et al., 2022), Coleoptera (Alloui-Griza et al., 2022), Diptera (Khanikor and Bora, 2022), Isoptera (Aljedani, 2023) and Hemiptera (Sayed et al., 2022). EOs obtained from this plant family are very effective for many pests. This effect may be repellant or lethal. Also Asteraceae (Shahinfar et al., 2021), Myrtaceae (Ramachandran et al., 2023), Apiaceae (Sousa et al., 2021), Annonacea (Pares et al., 2021) and Rutaceae (Pei et al., 2023) has been tested as biopesticide in too many researchs and stated that they were influential on pests. Apart from the families mentioned, it has been investigated in terms of pesticide activities in plants belonging to other plant families such as Lauraceae (Çeliktaş et al., 2022), Fabaceae

(Zavala-Sánch et al., 2020; Zhang et al., 2020), Ranunculaceae (Tian et al., 2021), and it has been revealed that it has effective results.

Use of Medicinal and Aromatic Plants in Plant Diseases

Semiliquidambar chingii is an evergreen tree from the Hammalidaceae family. Zhang et al. (2023) researched this plant's effects to control the disease agent of Citrus canker, *Xanthomonas citri* subsp. *citri.* The ethyl asetate extract of *Semiliquidambar chingii* slim branches exhibited a significant antibacterial effect with a MIC (minimum inhibitory concentration) value of 0.25 mg/mL. This effect was associated with resveratrol, 3-oxo-oleanoic acid, lantanolic acid, and eriodictyol contents (Zhang et al.,2023).

Ali et al.(2022) investigated the six plant extracts (neem, mentha, and datura leaves; ginger and garlic rhizomes; and onion bulbs) against potato blackleg disease factor *Pectobacterium atrosepticum.* As a result of this research, neem, mint and ginger extracts are effective against the *Pectobacterium,* especially at the 700 ppm concentration. So, the researchers advised using plant extracts as alternatives to pesticides.

Monilinia laxa and *Monilinia fructigena* are economically harmful fungi that cause brown rot degradation in apple fruits. El Khetabi et al.(2023) used *Mentha pulegium, Citrus aurantium, Thymus vulgaris, Origanum compactum, Lavandula angustifolia, Syzygium aromaticum, Rosmarinus officinalis, Citrus sinensis* and *Eucalyptus radiate* plant extracts against these fungi and all of the extracts exhibited important antifungal activities. Especially *S. aromaticum* and *O. compactum* samples at 1.5 mg/ml concentrations showed the nearest effect to thiophanate-methyl

fungicide controlling the fungi. The authors stated these plant extracts' availability as biofungucide against brown rot.

Several researches stated that Methyl chavicol, that main compound of the *Ocimum ciliatum* plant extract, was antibacterial (Kordali et al., 2005; Jonghee, 2008). Moghaddam et al. (2014) studied the antibacterial effects of *Ocimum ciliatum* EO, which mainly contains methyl chavicol, against ten phytopathogen bacteria: *Ralstonia solanacearum, Pseudomonas syringae* pv. *lachrymans*, *P. syringae* pv. *syringae, P. tolaasii, Xanthomonas oryzae* pv. *oryzae, Xanthomonas citri, Brenneria nigrifluens, Pantoea stewartii* subsp. *indologenes, Agrobacterium vitis,* and *Rhodococcus fascians.* According to this research the EO had adverse effects on all bacteria. It is also stated that *X. citri* is the most affected bacteria strain by the *O. ciliatum* EO as it had minimum MBC (Minimum Bactericidal Concentrations).

Bacterial spot disease is a main danger that reduces tomato yield and is sourced from *Xanthomonas* species. *Satureja montana* L. is an important MAP plant with its terpene-rich (carvacrol, linalool) EO, and this EO was found effective against t *X. perforans* (Hajdari et al.,2016; Maccelli et al.,2020; Qiao et al.,2020). Oliveira-Pinto et al. (2020) investigated the effects of *Satureja montana* L EO on *Xanthomonas euvesicatoria* and declared the plant as a natural control agent of the bacteria; they also advised to produce new goods from this EO.

In the postharvest stage of the goods, fungi are important injurious phytopathogens. *Alternaria citri* (Penz.) is one of them and induces *Alternaria* black rot in citrus (Brown and McCornack, 1972).

Ramezanian et al. (2016) produced EOs from *Zataria multiflora* Boiss and *Thymus vulgaris* L. leaves and applied them to *Alternaria* black rot disease on 'Washington 'Navel' oranges. According to this research, 300 ml l^{-1} *Z. multiflora* and 400 ml l^{-1} *T. vulgaris* EOs inhibited *Alternaria citri* development and protected the fruit quality. The EOs contain mainly thymol, carvacrol and para-cymene, and these oils were recommended as an environmentally friendly choice to pesticides.

Use of Medicinal and Aromatic Plants in Weed Control

Weeds are described as plants that are undesirable in cultivated lands, and they decrease crop quality. Herbicides are chemicals to control weeds but harm the environment and organisms. So, bioherbicides obtained from plant extracts are used as alternative ways. The bioherbicides had damaging effects on weed physiology: Delaying germination, damaging the cell membrane and enzyme activity, altering mineral uptake etc. (Radhakrishnan et al.,2018).

Allelpathy term is used for identifying the negative or positive effects of 'plants' biochemical compounds or metabolic products on another plant, and these compounds are named allelochemicals. Plant EOs are rich in terms of secondary metabolites, and they have known to be allelochemical. Seconder metabolites, especially terpenes, have successful herbicidal effects and may be tried to control weeds against field conditions (Özen et al., 2017).

Thymol-rich *Thymus vulgaris* L. and carvacrol-rich *Origanum onites* L. EOs were studied to investigate the herbicidal effects on common purslane (*Portulaca oleracea* L.), redroot pigweed *(Amaranthus*

retroflexus L.) black nightshade (*Solanum nigrum* L.), and ground cherry (*Physalis angustifolia* L.) germination. The germination of the weeds was inhibited at a higher concentration of the EOs than 80 L/ha. As a result of the study, *Origanum* EOs were found to be more effective and advised to use in organic farming to control weeds (Arslan and Uremiş, 2015).

Tworkoski (2002) investigated the herbicidal activity of twenty-five plant EOs on dandelion at laboratory conditions and on common lambsquarters, ragweed, and johnsongrass in the greenhouse. The study results showed that eugenol, the main constituent of the cinnamon EO, had the most successful herbicidal effect in both treatments.

Bencha et al. (2019) examined the phytotoxic effects of two plants from Lamiaceae family, extraxts of *Thymus fontanesii* Boiss. et Reut. and *Satureja calamintha* subsp. nepeta Briq. on *Sinapis arvensis, Avena fatua, Sonchus oleraceus, Xanthium strumarium* and *Cyperus rotundus* seeds. Finally, the EOs (mainly carvacrol in *Thymus* and 1,8-cineole in *Satureja*) adversely affected weed physiology. They inhibited germination, reduced pigment content and caused electrolytic leakage of whole weeds.

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CHAPTER 8

SINGLE MOLECULE SEQUENCING TOOLS (THIRD GENERATION GENE SEQUENCING TECHNIQUES)

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1.SINGLE MOLECULE SEQUENCING TOOLS (THIRD GENERATION GENE SEQUENCING TECHNIQUES)

The second-generation sequencing (SGS) technologies discussed earlier have had a significant impact on DNA analysis and are more commonly used compared to first generation sequencing technologies. However, SGS technologies often require a time-consuming PCR amplification step, which adds to the execution time and sequencing cost. Additionally, SGS technologies struggle with the complexity of genomes, particularly with repetitive regions, and the relatively short reads make genome assembly challenging. To address these issues, scientists have developed a new generation of sequencing known as "third generation sequencing" (TGS). TGS offers advantages such as lower sequencing cost, easier sample preparation without the need for PCR amplification, and significantly faster execution time compared to SGS technologies. TGS technologies, particularly those that read long sequences, enable the identification and characterization of unknown regions in genome sequences. Long-read sequencing, also referred to as long-strand reading or single molecule sequencing, is widely used in transcriptomics research as it can capture entire mRNA transcripts. Furthermore, TGS is capable of assembling long genome sequences and resolving sequence reads in repetitive regions of complex genomes, including long reads that exceed several kilobases (Wu et al.2014; Derrington et al. 2010; Luan et al.2010; Goodwin et al.2016; Wee et al. 2019). Currently, there are two main types of long-read technologies: single-molecule real-time sequencing approaches and synthetic

approaches that utilize existing short-read technologies to generate long reads through computational methods (Robert et al.2013).

1.1. Single molecule real time sequencing (SMRT)

Pacific Biosciences (http://www.pacificbiosciences.com/) is a prominent company in the field of genomic sequencing, having pioneered the use of the Single Molecule Real-Time (SMRT) approach. This technology, which is considered the most widely utilized third generation sequencing method, differs from other sequencing technologies in its real-time detection of fluorescent signals emitted by the fluorescent dyes at the junctions, rather than relying on amplification nucleotide cycles. In 2011, Pacific Biosciences acquired LI-COR Biosciences Technology, which is currently working on the development of dye-repelling nucleotides. These nucleotides, when in their natural state, produce low signals due to the presence of a dye-repelling group attached to the base. The SMRT technology employs the highly activated φ29 DNA polymerase enzyme, which efficiently incorporates phosphorylated nucleotides and enables the re-sequencing of closed circular templates (Metzker 2010; Goodwin et al. 2016; Yuan et al.2021).

The Zero Mode Waveguide (ZMW) is implemented through the utilization of micro-pores that are specifically designed in the Single Molecule Real Time (SMRT) technique. These micro-pores are incorporated into metal ZMW templates, which are fabricated using positive tone electron beam lithography, reactive ion etching, and negative tone electron beam lithography methods. The resulting

templates consist of either aluminium or gold and possess a diameter of 70 nm and a depth of 100 nm (Miyake et al.2008; Korlach et al.2010). The metal pores in question consist of microfabricated nanostructures, specifically wells with a diameter of tens of nanometers, that have been created in a metal film deposited on a glass substrate. Each pore has the capacity to produce approximately 75,000 Zero Mode Waveguides (ZMW), which has the potential to facilitate the detection of approximately 75,000 individual molecule sequencing reactions simultaneously (Schadt et al. 2010; Gupta and Gupta 2020). The diminutive dimensions of the zero-mode waveguide (ZMW) impede the transmission of visible laser light with a wavelength of 600 nm, as the laser beams experience an exponential decay while traversing the metallic nano ZMWs. Consequently, when laser illumination is directed through the glass towards the ZMW, only the lower 30 nm of the ZMW is effectively illuminated. These particular ZMWs exploit the characteristics of light passing through apertures with diameters smaller than the wavelength, resulting in the confinement of light propagation. Owing to their minute diameter, the intensity of light diminishes along the wells, ultimately illuminating the bottom of the wells (Coy et al.2014; Rhoads et al.2015; Chin et al.2016; Mehdi et al.2017).

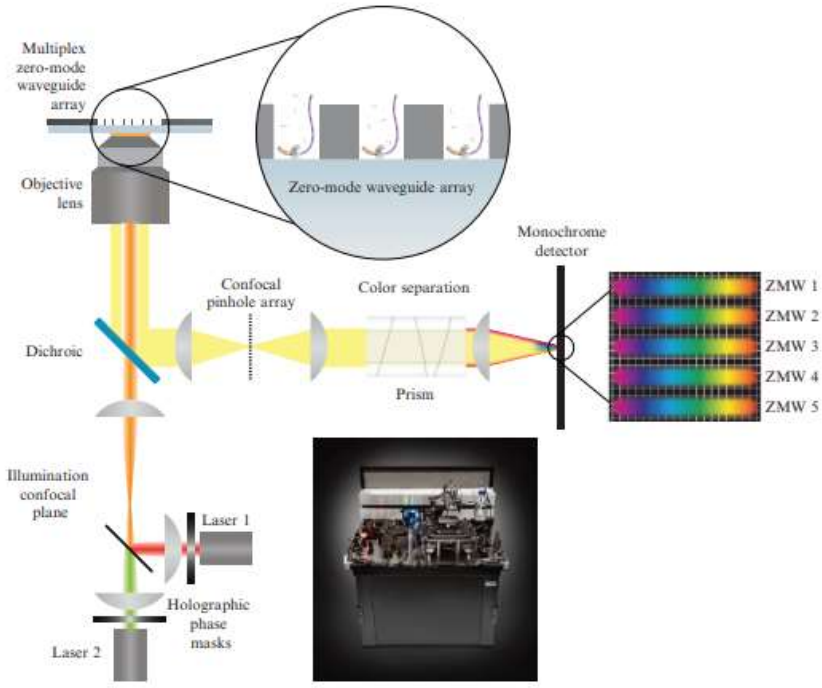

Figure 1. Schematics of an optical system for SMRT DNA Sequencing. This instrument provides simultaneous illumination of 3000 ZMWs with two different lasers, and wavelength-specific real-time detection of fluorescence from phospholinked nucleotides processed by DNA polymerase immobilized in the ZMWs. A photograph of an instrument is shown on the bottom (Korlach et al.2010)

In the SMRT (Single Molecule Real-Time) technique, the SMRTbell template is affixed to the hairpin adapters on both ends of the double-stranded DNA (dsDNA) template molecule. This process creates a circular DNA molecule with a dsDNA template in the middle and stable single-stranded DNA (ssDNA) regions at the ends. To facilitate the sequencing process, primers and the φ29 DNA polymerase molecule

are immobilized on the bottom glass surface through the biotin/streptavidin interaction (Korlach et al. 2010).

The prepared library is introduced into a zero-mode wavelength (ZMW) SMRT cell for the purpose of sequencing. In order to visualize the sequencing process, a mixture of fluorescently labeled nucleotides (A, T, G, and C) with distinct emission spectra is added. These nucleotides, each labeled with a different colored fluorophore, are prepared at the appropriate concentration and introduced into the pores of the ZMW. Due to nanoscale diffusion, the labeled nucleotides quickly enter the ZMW, surround the DNA polymerase, and then diffuse back out of the pore. Since the ZMWs do not allow laser light to pass through and excite the fluorescent labels, the labeled nucleotides within the ZMWs do not contribute to the measured signals. Only the bottom 30 nm of the ZMW emits fluorescence. When a nucleotide binds to the polymerase, it emits a distinct pulse of light, and the emitted rays from all ZMWs in the SMRT cell are captured in a "movie form" to record the replication process (Eid et al.2009; Quail et al.2012; Roberts et al.2013; Dorado et al.2019; Pavlovic et al.2020; Jain et al.2021).

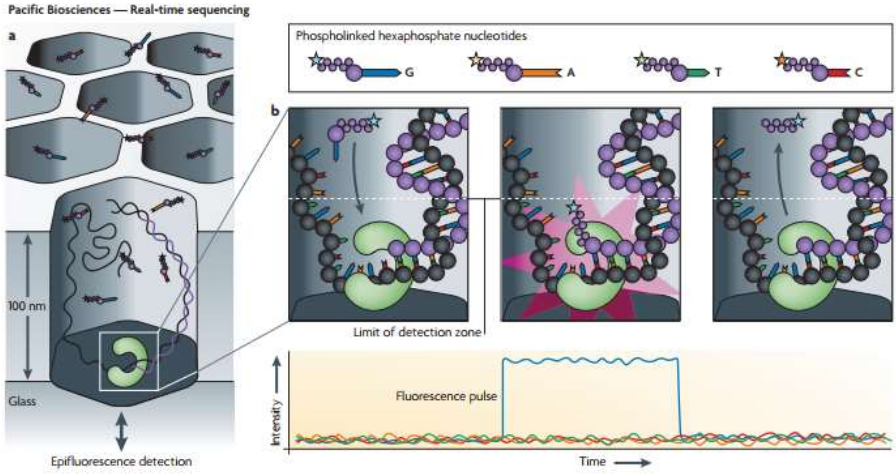

Figure 2: The SMRTbell, which is gray in color, enters a ZMW (zero-mode waveguide) and the adapter attaches to a polymerase that is immobilized at the bottom. Each of the four nucleotides is labeled with a different fluorescent dye. When a nucleotide is held in the detection volume by the polymerase, it emits a light pulse that identifies the base. First, a fluorescently-labeled nucleotide binds to the template in the active site of the polymerase. Then, the fluorescence output increases for the color that corresponds to the incorporated base. The dyelinker-pyrophosphate product is then separated from the nucleotide and exits the ZMW, causing the fluorescence pulse to end. The polymerase then moves to the next position. The next nucleotide binds to the template in the active site of the polymerase, initiating the next fluorescence pulse that corresponds to the base. (Metzker 2009).

The primary advantage of SMRT sequencing is its capability to generate long, uninterrupted reads, with an average length of 15 kb and a maximum length of 60 kb using newer systems. Additionally, this sequencing technology offers a faster base calling process as it does not require PCR amplification, enabling real-time sequencing. Moreover, the determination of base modifications, such as deletion, methylation, and epigenetic research, particularly in the diagnosis of novel diseases and cancer research, heavily relies on the analysis of tandem repeat lengths. However, SMRT sequencing does have several drawbacks,

including the production of approximately 70 to 140 MB of data per SMRT cell, which varies depending on the GC content of the DNA. Furthermore, this technology exhibits high reading errors in regions with random insertion-deletion events and is comparatively expensive when compared to other sequencing Technologies (Mardis et al.2013; Rhoads and Au, 2015; Sakai et al.2015; Reuter et al.2015; Ardui et al.2018; Pavlovic et al.2020).

1.2. Helicos Genetic Analysis Platform HeliScope™ Single Molecule Sequencer

PCR amplification has had a significant impact on DNA analysis, but it is not without its limitations. One of these limitations is the potential introduction of errors in the base sequence of the copied DNA strands. Additionally, PCR amplification can also favor certain sequences over others, leading to alterations in the relative frequency and length of different DNA fragments that were present before amplification. To overcome these challenges, researchers have sought to develop methods that allow for the direct determination of DNA sequences from a single molecule, eliminating the need for PCR amplification and base length quantification. This approach aims to achieve nanoscale miniaturization and minimize the use of biochemicals. One of the pioneering techniques in this field was developed by the S. Quake team and subsequently licensed by Helicos Biosciences (http://www.helicosbio.com/) (Quake 2008). The first single-stranded DNA, cDNA and direct RNA sequencing system was the Helikos

(http://www.helicosbio.com) sequencing system developed by HeliScope.

In the Helicos sequencing technology, the sequencing process is conducted using a fluorescent detection system of high sensitivity, eliminating the need for cloning, ligation, amplification, and cDNA synthesis. Notably, this platform distinguishes itself by not necessitating clonal amplification. Consequently, it relies on an exceptionally sensitive light detection system and a physical sequencing mechanism capable of detecting and discerning light emitted by a solitary dye molecule (Braslavsky et al 2003; Harris et al 2008).

The sample preparation procedure of this technology involves fragmenting genomic DNA into smaller pieces, followed by the addition of a poly(A) tail to the fragments. These fragments are then labeled and blocked using terminal transferase. Subsequently, these labeled templates are immobilized on a surface through hybridization with covalently linked oligonucleotides containing a 5' dT(50 bp) sequence. The surface is then examined using charge-coupled device (CCD) sensors, which allow for the identification and monitoring of the appropriately captured templates during the sequencing-by-synthesis (SBS) process. The subsequent steps of the process resemble the "wash and scan" stages of second-generation sequencing (SGS). A mixture of labeled nucleotides and polymerase is introduced into the system and incubated for a specific period of time. Following this, the surface is washed to remove the synthesis mixture, and a scan is performed to

detect the fluorescent label. The dye-nucleotide linker is then cleaved to release the dye, and this entire process is repeated (Wang 2021; Chen 2023).

The technique involves fragmenting genomic DNA, adding a poly(A) tail to the fragments, labeling them with terminal transferase, and blocking transcribed sequences. These nucleic acid fragments are then hybridized to primers, which are single molecule sequencing patterns irregularly labeled by capturing template libraries. These libraries are prepared with covalently attached poly-A tails, without the use of PCR amplification, and are randomly positioned on a glass cover slip in a flow cell. The hybridization occurs between the nucleic acid fragments and surface-bound poly-T oligomers. Using primers that are hybridized to these poly-T oligomers, the primers bind to single molecule sequences present in the template fragments. The primer-template pairs that are immobilized to the glass surface are then elongated independently of the template library surface (Shendure and Ji 2008; Harris et al. 2008; Hart et al. 2010).

In the sequencing-by-synthesis technique, DNA polymerase and a single nucleotide species that is fluorescently labelled are introduced to the glass support that is bound to the surface. The incorporation of the next base into the synthesized strand is determined by the emission of light from the base in question. This technique is performed on a DNA fragment without any amplification. The surface is then examined using charge-coupled device (CCD) sensors, which allow for the identification and subsequent monitoring of appropriately captured

templates for sequencing-by-synthesis. The process is similar to the "wash and scan" steps of the sequencing-by-genesis technique. A mixture of labelled nucleotides and polymerase is introduced into the system and incubated for a specific period of time. The surface is then washed to remove the synthesis mixture, and a scan is conducted to detect the presence of the fluorescent label (Alexander et al.2010; Goren et al.2010; Ozsolak et al.2009). After capturing a comprehensive set of images encompassing the entire sequence, the fluorescent label is chemically terminated and released, facilitating the subsequent cycle of extension and imaging. This system possesses the capability to analyze millions of individual DNA fragments concurrently, thereby enabling sequence reads in the Gigabase range with an average length of 25bp or larger (Shendure and Ji 2008; Ansorge 2009; Schadt et al.2010; Ozsolak 2012).

This technology has the capability to perform DNA sequencing by utilizing the reverse transcriptase enzyme. Additionally, it allows for direct RNA sequencing without the necessity of DNA polymerase, conversion of RNA to cDNA, or the ligation/amplification processes that are typically required for RNA sequencing using current second-generation sequencing (SGS) technologies. In this method, similar to DNA sequencing, each RNA molecule undergoes polyadenylation, and the 3'- PolA tails are obstructed. Fluorescently labeled nucleotides are then introduced, followed by hybridization with dT(50bp) oligonucleotides to capture light signals and sequence reads.

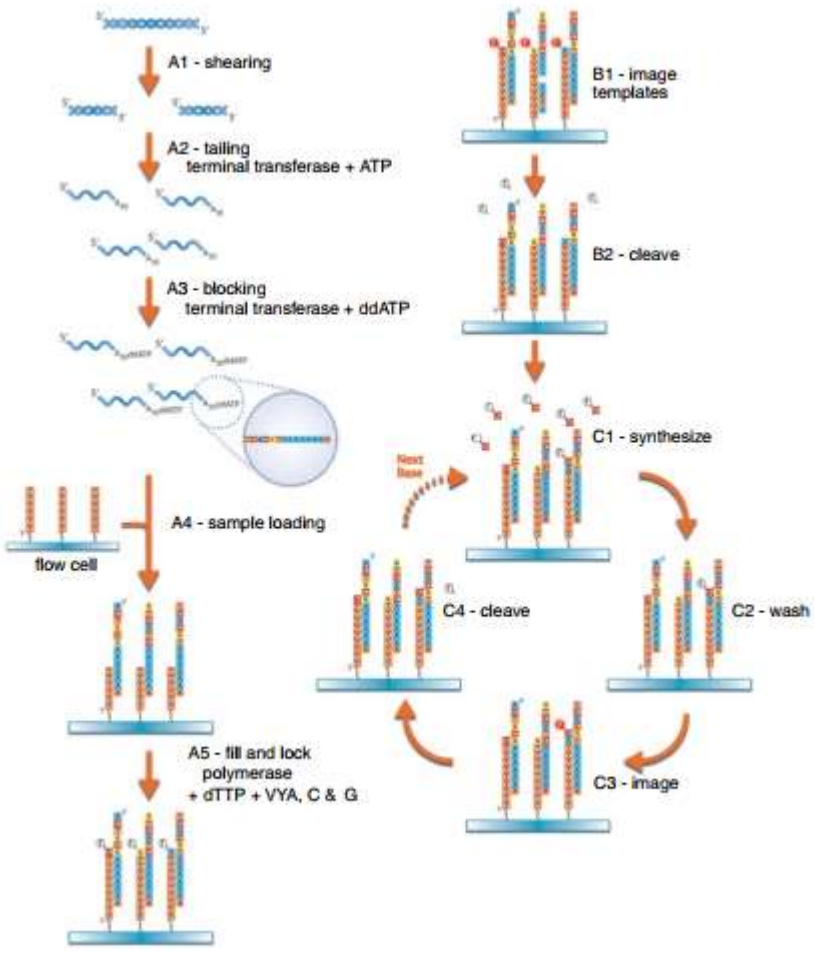

Figure 3. a) Helicos Sample preparation: In the process of DNA sequencing, the DNA samples undergo fragmentation, denaturation into single strands, and subsequent labeling with a 3' poly(A) tail and a terminal fluorescent adenosine. These modified DNA strands are then bound to the surface of a flow cell through poly(T) capture sites, which also serve as the starting point for the sequencing reaction. b) Captured templates are imaged to map their position and fluorescent labels are removed. c) Sequencing by synthesis: fluorescent nucleotides (C, G, T or A) are added one base per cycle and incorporated into the complementary strand in a template-dependent manner. In the process, unincorporated nucleotides are removed through a washing step, and subsequently, the strands are subjected to illumination and visualization techniques to ascertain the addition of bases and the sequence of DNA. Following this, fluorescent labels are detached, enabling the addition of the next base to proceed in a continuous cycle (Thompson and Steinmann 2010).

1.3. Oxford nanopore technology (ONT)

During the late 1980s, Deamer conducted research on liposomes and demonstrated their potential for DNA sequence screening (Deamer et al., 2016). Building upon this work, John Kasianowicz and his team proposed the idea that DNA sequences could be read through nanopores. They observed that α-hemolysis (α-HL) protein channels, with a molecular weight of 33 kD, derived from Staphylococcus aureus, exhibited a unique property of remaining open under neutral pH conditions and in high ionic environments when self-assembled in lipid bilayers (Kasianowicz et al. 1996; Deamer and Akeson 2000; Branton et al.2008).

Dan Branton from Harvard University demonstrated the potential use of alpha-haemolysis pores in high ionic media or electric current for the reaction of nucleic acids. In this study, attempts were made to pass single-stranded (ssDNA) and double-stranded (dsDNA) molecules through the pore using RNA homopolymers. Positive voltage exceeding 80 mV was applied to the trans compartment immediately after the addition of polymers such as polyadenylic acid (polyA) or polyuridylic acid (polyU) to the cis side of a lipid bilayer containing a single α-haemolysin channel. It was observed that the ionic current passing through the α-haemolysin channel resulted in numerous blockages within milliseconds, while no blockages occurred when negative electric current was applied in the trans tail tail (Akeson et al. 1999; Wang et al.2015; Jain et al.2016). The structural arrangement of α-hemolysin is well-documented as a heptamer with a mushroom-like

shape, featuring a stalk measuring approximately 5 nm in length. The inner channel of the protein has a diameter that varies between approximately 1.4 nm and 2.4 nm, depending on the size of the amino acid side chains that extend into the cylindrical channel, which is responsible for facilitating ionic current (Song et al.1996). In later investigations on the utilization of α-hemolysis ion nanopores for the sequencing of individual DNA and RNA molecules, diverse polymerase enzymes were employed to modulate the rate of DNA translocation within the nanopores by manipulating the catalytic efficiency of the enzymes. Specifically, the bacteriophage phi29 DNA polymerase (DNAP) enzyme, known for its strong affinity towards DNA substrates and ability to facilitate continuous synthesis, was employed to regulate the reactions (Gyarfas et al.2009; Lieberman et al. 2010; Manrao et al.2012; Chen et al.2023).

The inability of α-HL protein pores attached to double-layered lipid membranes to withstand high voltage, their ion permeability being affected by salt concentration and pH changes, the fact that the inner lumen of α-hemolysis protein can hold more than one nucleotide at the same time and cause different blockages in the channel in different ion currents made the applicability of the method difficult (Yang et al.2013). For this reason, Mycobacterium smegmatis Porin A (MspA) porin potein form (>1.2 nm), whose pores are smaller in size and more stable in structure, has started to be used instead of α-HL protein in nanopore sequencing (Stahl et al.2001). MspA has been preferred in nanopore technologies because it can bind metal ions better than α-

hemolysin to stabilise the protein structure and can be used in the detection of very small size analytes (Wang et al., 2021). As a result of scientific research on nanopore technology, phi29 DNA polymerase and a nanopore (α- haemolysin and MspA) combined with the operational recording of ionic currents for single-stranded DNA molecules that can be decomposed into signals from individual nucleotides. In nanopore sequencing technology, two different devices have been developed based on the NanoTag sequencing technique (GridION), based on the excision of monomers from the DNA strand and their individual orientation through a nanopore, introduced by Genia et al. (Oxford Nanopore MinION), which is based on strand-by-strand sequencing of single-stranded DNA passed base-by-base through nanopores. The nanopore sequencing technique is performed with the device released in 2014 by Oxford Nanopore Technologies in the form of MinION, a handheld sequencer that uses a grid of biological nanopores embedded in the membrane.

Nanopore sequencing technology utilizes a protein pore system at the nanoscale, referred to as a 'nanopore', which functions as a biosensor and is integrated within a polymer membrane with electrical resistance. To facilitate the sequencing process, lengthy DNA molecules are modified by attaching a hairpin adaptor to one end of the double-stranded molecule before being subjected to a helicase. The a-HL or MspA proteins, serving as template-bound motor proteins, then unwind and guide the single-stranded DNA through the nanopore channel (Ying et al.2022). The membrane in which the nanopores are located

contains an electrolytic solution that allows electric current to flow through the nanopores by separating the two ionic solutions, into which a constant voltage is applied to produce an ionic current through the nanopore, so that negatively charged single-stranded DNA or RNA molecules are guided through the nanopore from the negatively charged 'cis' side to the positively charged 'trans' side. The rate at which translocation occurs is regulated by a motor protein that incrementally advances the nucleic acid molecule through the nanopore. Variations in ionic current observed during translocation are indicative of the specific sequence of nucleotides within the detection region. These variations are subsequently analyzed using computational algorithms, enabling the real-time sequencing of individual molecules. By passing single-stranded DNA or RNA-DNA duplexes through the nanopore in a sequential manner, the nucleotide bases can be accurately determined by measuring the electric current flowing through the nanopore. The MinION device then translates these current measurements into corresponding base calls (Schadt et al. 2010; Jain et al. 2016).

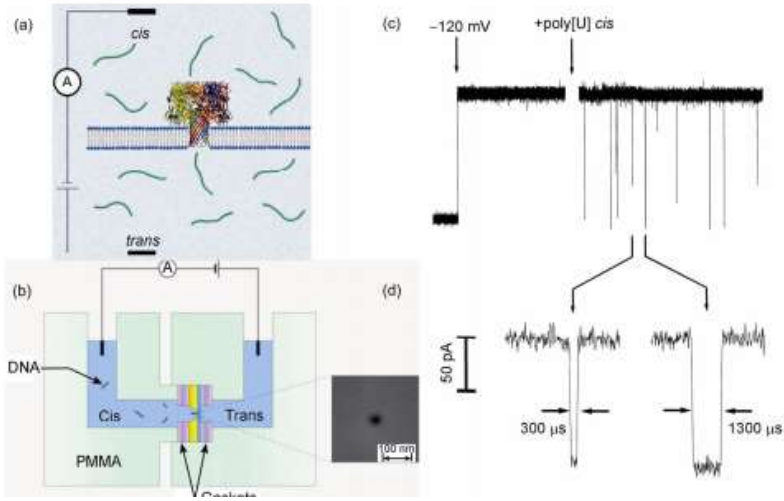

Figure 4. The characterization of ionic current during DNA translocation. (a) Schematic of α-hemolysin nanopore setup; (b) diagram of nanopore measurement circuit; (c) oligomers of poly [U] caused transient blockades in the α -hemolysin single channel current; (d) magnified view of single translocation event (Wu et al.2014).

Nanopore sequencing technologies mainly consist of biological nanopores forming α-Hemolysin, Mycobacterium smegmatis porin A (MspA), Bacteriophage phi29 and Aerolysin nanopore techniques, and solid state nanopore technologies, which were developed by Li et al. in 2001 as an insulating solid state membrane for the displacement of ssDNA molecules (Yang et al.2013; Chen et al.2023). Solid state nanopores have much better chemical and thermal stability, higher durability, and are difficult to denature; pores of biological nanopores range from 1~2 nm, while solid state nanopore pores range from 3~1,000 nm (Feng et al.2015). Boron nitride nanopores (BN), graphene, polymer films, alumina (Al2O3), silicon nitride (Si3N4), silicon dioxide (SiO2) and hybrid materials are used as solid state materials

(Tsutsui et al.2019; Acharya et al.2020; Fragasso et al.2020; Goto et al.2020).

The MinION device is a portable device weighing 90 g and carrying approximately 2048 nanopores in 512 different channels. In addition to MinION, Oxford Nanopore Technologies uses the GridION X5 and PromethION platforms in its sequencing technologies.

Figure 5. portable nanoporous devices

The potential advantages of the nanopore system are real-time sequencing of single molecules using massively parallel sequencing systems that can determine the sequences of different DNA strands in a single run at low cost. Real-time data collection can be performed rapidly in a short time, allowing for reproducibility of the application. Nanopore sequencing is advantageous as it is a low-cost and small-sized device; data is displayed on the screen and generated without completing the study. The lysate can be sequenced without PCR amplification or chemical labelling step. Nanopore DNA sequencing offers the possibility of a single-molecule and massively parallel sequencing approach that can be performed without the need to amplify samples (Gupta et al.2020; Hu et al.2021; Chen et al.2014). In very low quantities, long sequences of nucleic acid molecules can be directly analysed without any PCR step and sequence information can be

obtained directly from RNA or even modified nucleotides with high accuracy (Özsolak 2012). The disadvantages of nanopore technologies, which have many advantages, are high error rates in sequence readings, the pores are affected by high voltage currents, and the trapping of more than one base in some systems (Demkow 2015; Jain et al.2018; Kumar et al.2019).

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CHAPTER 9

DYE PLANTS

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Introduction

Vegetable dyeing has been done for many years in the world and in Turkey. The dyeing of raw materials such as wool, cotton and silk with extracts prepared by different methods from the vegetative and generative parts of plants cultivated in nature or self-grown, or from the whole plant, with different techniques is expressed as a dyestuff (Şanlı, 2011).

It is claimed that the first people primarily benefited from plants in dyeing. When the historical development of herbal dyeing is examined, it is stated that 2000 B.C. the Chinese dyed silk fabrics with special dyes called herbal indigo and Chinese green, the indigenous people in Mexico and Peru were engaged in herbal dyeing, and the African natives colored their daily lives with various herbal dyes (Uğur, 1988).

According to Herodotus, aerial, oak and walnut were ancient vegetable dyestuffs known to the Greeks. It is known that the ancient Egyptians used the flowers of the safflower (*Carthamus tinctorius*) plant for dyeing mummy wraps. In this respect, the art of herbal dyeing started with the art of weaving and developed in parallel with it (Uğur, 1988).

There are findings in India showing that basma dyeing is done with the help of specially carved wooden molds with dyes obtained from plants. The most important findings confirming the use of herbal dyeing in ancient times constitute the weaving pieces dyed with these dyes. With the unearthing of these weavings, the dyeing workshops, which house

all the tools and materials for dyeing, were also revealed (Barber, 1992; Karadağ, 2007).

The paint industry began to develop in England around 1600, followed by the Netherlands, France and Germany. The paint industry began to develop in England around 1600, followed by the Netherlands, France and Germany. The paint industry began to develop in England around 1600, followed by the Netherlands, France and Germany. In addition, it is stated that the Ottoman Empire alone met a large part of the world export of this plant in those years (Ceylan, 1991; Uğur, 1988).

In addition, it is stated that the Ottoman Empire alone met a large part of the world export of this plant in those years. In 1740, Bartın reported that they obtained indigo carmen by dissolving indigo in sulfur, aniline from William Perkin coal tar in 1850 with the help of oxidizing agents, Bayer indigo in 1860, Giraber and Liberman alizarini in 1868, Biottiger cotton in 1884, they chemically obtained Congo red (Feddersen-Fieler, 1982).

The discovery of these chemical dyes in the history of dyeing and their application to textiles and techniques have gradually reduced the demand for vegetable dyes both in the world and in Turkey. The discovery of these chemical dyes in the history of dyeing and their application to textiles and techniques have gradually decreased the demand for vegetable dyes both in the world and in Turkey. Due to these advantages, the use of natural dyestuffs in recent years; It has been

instrumental in gaining momentum in the fields of food, cosmetics and textile dyeing industry (Ali et al., 2007).

The fact that carpets and kilims woven with yarns dyed with herbal dyes are important in terms of tourism and that they are appreciated in the domestic and foreign markets have positively affected the development of this art and in recent years, dyeing carpets and kilim yarns with herbal dyes has come to the fore again in our country. Many public institutions, universities and the private sector have started and are carrying out various studies in order to keep this ancestral art, which has been engaged in for many years, alive, and to make it a source of income (Karadağ, 2007; Şanlı, 2011).

2. THEORETICAL INFORMATION AND RESOURCE SCREENINGS

2.1. Dye Plants ın TURKEY

There are more than 10.000 natural plant species in our country, and it is one of the richest countries in Europe and the Middle East in terms of vegetation. In addition, in parallel with having this rich flora, the number of plants used in natural dyeing is quite high (Yaşar et al., 2009). Dye plants have many uses such as food, textiles, cosmetics and pharmaceuticals (Piccaglia and Venturi, 1998). In our country, the most widely known dye plants are indigo (*Isatis tinctoria*), madder (*Rubia tinctoria*), daisy (*Anthemis tinctoria*), budgerigar (*Reseda lutea*). Although most of the natural dyestuffs are obtained from plants, the

cultivation of dye plants is not done in our country. Demand is met by collecting from nature (Anonim, 2003; Mert, 1992).

Herbal dyestuffs, which have a wide area of use in dyeing carpet and kilim yarns, which are one of the traditional arts of Turkish society, but have another usage area such as dyeing foodstuffs, have gained importance again today (Ceylan, 1991; Gönuz et al., 2006).

When we look at the literature review about dye plants and dyeing properties, it is seen that many studies have been done.

Aksoy and Aytaç (2004), in their study, emphasized the importance of natural dyeing for the region and summary information on the definitions of dye plants used by the public in Çanakkale-Ayvacık region.

Deniz (2005), Thompson (1986), it is seen that people in Ayvacık district of Çanakkale province talk about the dye plants and dyeing techniques used in dyeing carpets and other flat woven fabric yarns.

Uysal (1991), in his study, examined some dye plants in Çanakkale and mentioned their morphology, chronology and their use in dyeing.

Aksoy and Öztürk (2000) reported that they determined the dyestuff properties and light washing fastness of the Hypericum triquetrifolium taxon collected from Çanakkale province by using wool yarns belonging to the region.

Mert et al., (1992) investigated the botanical properties of some dye plants used in natural dyeing in their studies.

Adrosko, (1971), Grierson (1989) gave information about natural dyes and house dyeing in their studies.

Since the 1930s, most researchers have studied various natural dyestuffs used in dyeing local fabric and carpet yarns in Turkey and dyeing techniques applied by the public, and have presented them with reports (Anonim, (1947); Anonymous, (1991); Bayatlı. , (1957); Baylav, (1963); Baykara, (1964); Baykara, (1998); Bodur, (1984); Eren, (1977); Eyüboğlu et al., (1983); Gündüz, (1993); Öztürk, (1982); Öztürk, (1988); Öztürk, (1997); Uğur, (1988); Uslu, (1982)).

Studies on natural dyeing have been carried out under laboratory conditions by various researchers in recent years, and the fastness values of plants and their effects on different materials have been investigated (Arlı et al., (1995); Aytaç, (1999); Dedhia and Khanna, (1999); Duran. , (1992); Erdoğan and Yazıcıoğlu, (1995); Eşberk, (1939); Eşberk, (1945); Eşberk, (1947); Eşberk et al., (1952a); Eşberk et al. , (1952b); yusufr and Seventekin, (1987); Harmancıoğlu, (1955); Karadağ, (1997); Kayabaşı et al., (2000); Korur, (1937); Köşker, (1945a); Köşker, (1945b)); Köşker, (1945c); Seventekin, (1989); Yazıcıoğlu et al., (1999).

2.2. METHODS OF MORDANTİNG AND DYEING IN DYE PLANTS

2.2.1. Mordanting

Mordants are metal salts, natural compounds containing metal ions or other complexing agents that improve the uptake and fixation of the dye, determine the obtained color and fastness properties. The yield and use of color is highly dependent on the type of mordant and the mordanting method. By forming a different dyestuff complex from each purple, it allows to obtain completely different colors and fastness properties (İşmal and Yıldırım, 2019).

2.2.2. Dyeing

Natural dyeing is done by mordant dyeing, cube dyeing and direct dyeing methods (Karadağ, 2007). In addition to being an auxiliary substance that allows the mordant dye to bond with the fiber, it is possible to obtain hundreds of color nuances by using different mordants from the same plant (Mert et al., 1992). In the dyeing process, all parts of a plant are a dye product, but different colors and densities can be obtained depending on the age, location, growing conditions, time and parts of the same plant species (Kedzie-web et al., 2000).

Brief information is given about the botanical characteristics, history, growing places, dyestuffs and dyeing processes of some dye plants grown in our country's natural flora (Karadağ, 2007).

Madder *(Rubia tinctorum* L.*)*

Figure 1: Madder plant and its distribution in our country

Madder is a perennial plant that can grow up to 1-2 m in length, has a rhizome structure, and grows in fertile soils. Growing in summer, the plant has pale yellow flowers. In winter, the flowers of the plant fall, but it blooms again in the spring. Its leaves are circular, from the same node, to 4 to 6 leaves.

The homeland of madder is Anatolia. However, it naturally spreads as far as the Caucasus, Iran, Central West Asia and the Himalayas. Although it grows wild in provinces such as Manisa, Demirci, Gördes, Konya, Aksaray, Niğde, Kayseri, Kırşehir, Çorum, Yozgat, Malatya, Elazığ, Adıyaman, Amasya, Ankara, Tokat, Kahramanmaraş, Çanakkale, Muğla in Turkey, it is cultivated in some regions.

From the past to the present, it has been determined in the dyestuff analyzes that the red color of many Eastern carpets, Ottoman carpets, Hereke carpets and many fabrics belonging to different periods were dyed with madder. Today, the production and use of madder is supported by a number of European Union Projects.

The effect of the concentration, mordant type, mordanting method, fixation temperature and durations has been examined and presented as an ecological alternative, by using madder in the printing of various natural fibers (Özgüney et al., 2015)

Dyestuffs Contained: *Alizarin, pseudopurpurin, purpurin, munjistin, rubiadin, xanthopurpurin, purpuroxanthin, lucidin, chinizarin, christofin, antrhagallol.*

Dyeing Process: It is made with the dried and ground shoots (roots) of the plant and the method of dyeing with mordant.

Buckthom *(Rhamnus petiolaris*)

Figure 2: Buckthorn plant and its distribution in our country

Buckthorn is a thorny bush or shrub that can grow up to 3 meters tall. It grows in mountainous, hilly, rocky places at altitudes of 1000-1300 m, on sunny slopes, in forest clearings, under or near sparse forests. Buckthorn has 22 species. Also known as the golden tree, Rhamnus petiolaris grows endemic in Central Anatolia. As a result of the dyestuff analysis, it was determined that buckthorn was used as a source of dyestuff in the yellow colors of many Anatolian carpets woven in the

15th and 17th centuries. In the first examples of Hereke carpets, buckthorn was used in the yellow parts.

Dyestuffs Contained: *Rhamnetin, rhamnezin, quercetin, kempferol.*

Dyeing Process: It is made with the dried and ground fruits of the plant by dyeing with mordant.

Sage (*Salvia sp*.)

Figure 3: The sage plant and its distribution in our country

Sage is the general name given to the Salvia species. It is a perennial shrub or herbaceous plant that can grow up to 60-100 cm in height. Between June and July, blue, purple, or white blooms are blooming at the tip of the plant. The stems of the above-ground parts of the plant are highly branched. The most important active ingredient of sage are essential oils.

There are nearly 500 species of the sage plant. Many species of the plant are found in Mediterranean countries; Commonly found in Greece and the Greek islands, Southern Italy and Sicily. In some species, it grows in Central Europe and also in tropical regions of the northern and

southern hemispheres. It grows in the western and southern parts of Turkey.

Dyestuffs Contained: *Luteolin.*

Dyeing Process: The dried and ground flowers and leaves of the plant are used in dyeing. The dyeing process is done first by mordanting and then by dyeing method.

Barberry *(Berberis vulgaris* L.*)*

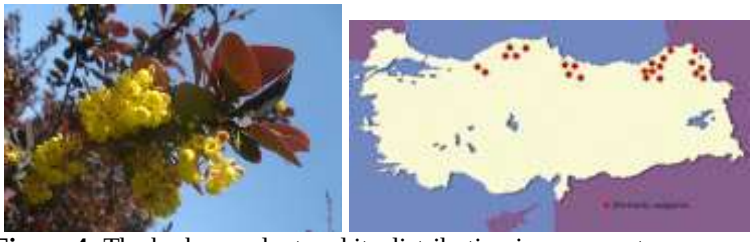

Figure 4: The barberry plant and its distribution in our country.

It is also taken as a female salt shaker and is a perennial, very thorny, bushy plant with red berry fruits that can grow up to 2 meters. The use of barberry roots goes back to the 14th century. It is a plant that can dye different species easily with a very simple and quick dyeing method. However, the dyed fiber turns brown over time. For this reason, it was used in the painting of tents of the Ottoman Armies in the First World War. The yellow roots of the plant are still used in wool dyeing in Anatolia today.

Dyestuffs Contained: *Berberin*.

Dyeing Process: The dried and ground roots of the plant are made with the direct dyeing method.

Alder *(Alnus glutinosa* L.*)*

Figure 5: Adi kızılağaç bitkisi ve ülkemizdeki dağılımı

Alder is a tree with a smooth dark brown trunk that can grow up to 20-25 meters in height and shed its leaves in winter. The young shoots are sticky, hairy or glabrous, and the flowers are unshown.

Until about 1940, the dyestuff obtained by boiling the bark of alder alder in salt water in Malatya was used in the dyeing of sandal leather and yarn. The dye obtained by this method is called “Afku” or “Agku”.

Dyestuffs Contained: *Tanin, querceti 3-glikozit, emodin.*

Dyeing Process: It is made with the dried and ground branches and bark of the plant and the mordant dyeing method.

Mediterranean laurel *(Laurus nobilis* L.*)*

Figure 6: Mediterranean laurel and its distribution in our country

Mediterranean laurel is an evergreen tree with a broad crown, often up to 10 meters tall. The oil obtained from its leaves and fruits is used in the soap industry, and the dried leaves are used as a spice. It is not known whether the plant, which is very rich in dyestuff it contains, was used in the field of dyeing in the past.

Dyestuffs Contained: *Quercetin, rutin, kempferol, leucocyanindin.*

Dyeing Process: It is made with the dried and ground flowers and leaves of the plant with the mordant dyeing method.

Grape *(Vitis vinifera* L.*)*

Figure 7: Grape plant and its distribution in our country

The grape belongs to the genus Vitis of the vitaceae family. It has a climbing and cuddling feature and grows by hugging the trees or bushes

nearby. The fresh shoots of the grape, which can reach up to 30 meters in length, are bare or softly hairy.

It was not found in the dyestuff analyzes of historical and archaeological textiles due to its low light fastness dyestuff content. From this, it is understood that this plant is not used in such textiles or has not preserved its color until today.

Dyeing Process: It is made with the dried and ground flowers and leaves of the plant with the mordant dyeing method.

Safflower *(Carthamus tinctorius* L.*)*

Figure 8: The safflower plant and its distribution in our country

Safflower is a herbaceous plant belonging to the genus Carthamus. There are single or biennial species. Safflower, which can grow to an average of 60-70 cm, blooms yellow, red and orange flowers in July-September, depending on the variety. It is known that the safflower plant originated in South Asia and was first cultivated in the south of the Asian continent, in the Middle East region and in the Mediterranean countries.

The light fastness of dyes made using safflower plant is low. However, it was used extensively in both yellow and red dyeing of textiles in the

past. Safflower was used in silk carpets belonging to the period of Shah Abbas in Iran between 1557 and 1628. Although many of these carpets have survived to the present day, the low light fastness of the dye plant caused the red colors to fade. However, at the nodal points of the loops, which were not exposed to light, colored parts were encountered. In the past, there are sources that indicate that this plant was used in dyeing in Anatolia.

Dyestuffs Contained: *Carthamin, corocetin.*

Dyeing Process: It is made with the dried flowers of the plant by mordant dyeing method.

Cancer weed *(Inula viscosa (*L.*) Aiton)*

Figure 9: Cancer weed and its distribution in our country

Cancer weed is also known as andız grass among the public. It is a perennial, yellow-flowered and herbaceous plant with a pile root, 1-2 meters tall. It is extremely suitable to be used as a dye plant because of the high fastness of the dyestuffs it contains and the ability to grow easily in many places. It has been determined in the studies that it was used especially in wool dyeing in the weaving villages of Anatolia.

Dyestuffs Contained: *Quercetin, datiscetin, az luteolin (x1, x2, x3). (x1, x2 ve x3 'ün yapısı aydınlatılamamış).*

Dyeing Process: It is made with the dried and ground flowers, leaves and stems of the plant with the mordant dyeing method.

Genista tinctoria *(Genista tinctoria* L.*)*

Figure 10: Genista tinctoria and its distribution in our country

Genista tinctoria is a perennial and shrub-like plant with yellow flowers, sparsely branched and thornless, which can grow up to 1-1.5 meters.

Genista tinctoria is native to Southern and Central Europe, Caucasus, Anatolia and Northern Iran. It is common in the north of Anatolia in Turkey, and is also common in Thrace and the Black Sea Region.

Genista tinctoria was used as a dye plant in medieval Europe. In 1312-1377 it was used in England in combination with indigo for green dyeing. This green color obtained has gained a reputation as "Kendal green". The stems, leaves, seeds and flowers of this plant, which was very popular with old English painters, contain a large amount of dyestuff.

Dyestuffs Contained: *Luteolin, genistein.*

Dyeing Process: It is made with the dried and ground flowers, leaves and stems of the plant with the mordant dyeing method.

Dyer sumac *(Cotinus coggygria* SCOP = *Rhus cotinus)*

Figure 11: Dyer sumac and its distribution in our country

This plant, also known as the smoke tree and wig bush, is mostly seen in the regions where there are maquis areas on the Mediterranean coasts in our country of origin from Southern Europe and China.

Dyer sumac has been known as a dyestuff source since the Roman Empire. It was widely used in Europe in the Middle Ages and gained an important place economically. In the 19th century, it is stated that this plant was widely used in yellow dyeing, especially in silk dyeing. It is mentioned that in Anatolia in the 19th century, it was used for dyeing yellow yarns on carpets. Especially in the yellow colors of Taşpınar carpets, it is seen that this plant is used.

Dyestuffs Contained: *Fisetin, sulfurein, sulfuretin.*

Dyeing Process: The dried and ground leaves, sprouts and bark of the plant were dyed with mordant dyeing method.

Walnut *(Juglans regia* L.*)*

Figure12: Walnuts and their distribution in our country

Balkans, Anatolia, Middle East is the homeland of walnut tree. It does not grow in areas with a harsh winter climate.

The green peels and leaves of the outermost fruit are used for dyeing. It is an important dye plant used for brown dyeing in almost every country in America, Europe and Asia and in Turkey. Although walnut shell is not used for brown in Turkish carpets of the 15th and 17th centuries, it is used in Persian carpets of the same period. Today, walnut shell is still used for brown dyeing in Türkiye and Iran.

Dyestuffs Contained: *Juglon.*

Dyeing Process: It is dyed by direct painting method with the dried and ground leaves of the plant and the outer shell of the fruit.

Yarrow *(Achillea sp.)*

Figure 13: Yarrow and its distribution in our country

Yarrow is the general name for the Compositea species. It is a perennial, herbaceous, hairy white or yellow flowering plant with a strong smell, which can grow up to 100 cm in height.

It grows in many regions in Turkey and on rocky and unproductive soils in Southeast, Southwest and Central Asia in Europe. For dyeing, the flowers and stems of the plant are used. In terms of dyestuff, it is especially used in wool dyeing. It is claimed that the yellow colors of the 17th century Konya carpets and the yellow components of the green colors were used.

Dyestuffs Contained: *Luteolin, quercetin, isorhamnetin, apigenin.*

Dyeing Process: It is made with the dried and ground flowers of the plant and the mordant dyeing method.

Indigo *(Isatis tinctoria* L.*)*

Figure 14: Indigo and its distribution in our country

Indigo is a biennial, bright yellow flowered and herbaceous plant that can grow up to 40-90 cm. The homeland of indigo is the slopes of the Caucasus, and it has spread from the far east to the Himalayas. But it is not known when she came to Turkey.

It is stated that indigo was used in blue color dyeing in Mesopotamia in the periods BC. It is also stated that in Ancient Greece and the Roman Empire, indigo was known as the source of indigo dyestuff.

Dyestuffs Contained: *İndikan, isatin B.*

Dyeing Process: *Indigestion (indigo),* which is obtained as a result of fermentation of the leaves of the Isatis tinctoria plant, is made by using the cube dyeing method using auxiliary chemicals.

Daphne *(Daphne oleoides* Schreber*)*

Figure 15: Daphne plant and its distribution in our country

Daphne, also known as havana or camelicot among the people, is a perennial herb that can grow 20-60 cm in length, with hairy young branches. Its white flowers in spring have a sharp odor, and its fruits are egg-shaped, 4-6 mm in length, orange-red in color. It is a common plant of the Anatolian mountains, and its leaves are used for dyeing yellow.

It was used in the yellow color dyeing of Antalya underfloor carpets and it is still being used today.

Dyestuffs Contained: *Luteolin.*

Dyeing Process: It is made with the dried and ground leaves of the plant with the mordant dyeing method.

Dock *(Rumex sp.)*

Figure 16: Dock plant and its distribution in our country

Dock, also known as Labada, is a perennial herbaceous plant belonging to the Rumex genus, and its green seeds later turn brown. There are 25 species of efelek in our country, and some types are also consumed as a vegetable.

The yellow roots of the plant contain more dyestuffs than the leaves and stems. For this reason, its roots are used as a dyestuff source. The light fastness of this plant, which is used to obtain yellow and orange colors in dyeing, is low. For this reason, the feathers dyed yellow and orange with dock turn from yellow to brown in time when exposed to sunlight.

According to the results of the dyestuff analyzes carried out on 19th century Turkish carpets and rugs, it is stated that labada roots are frequently used.

In Western Anatolian villages, it is stated that the leaves of dock are also used as a mordant agent to obtain brown-red together with root dye (Rubia tinctorum). It is still in use today.

Dyestuffs Contained: *Emodin, emodin 8-O-glukozit, physicon glukozit, chrysophanol, physican.*

Dyeing Process: It is made with the dried and ground leaves of the plant with the mordant dyeing method.

Alkanet *(Alkanna tinctoria* Tausch *ve Arnebia densiflora)*

Figure 17: Alkanet plant and its distribution in our country

Alkanet is a perennial, hairy, herbaceous plant with red roots and blue flowers in April - July, which can grow to 10-30 cm in height. It grows comfortably in places where dry soil wastes are found and on the roadsides. It is common in Central and Eastern Anatolia.

The roots of the plant are mostly used in dyeing.

Alkanet has been in cultivation for a long time. The use of textile fiber in dyeing goes back to the 2nd century BC. In Europe and South America, it is used in food coloring and cosmetics.

Dyestuffs Contained: *Alkannin.*

Dyeing Process: It is made with the dried and ground leaves of the plant with the mordant dyeing method.

Chaste Tree *(Vitex agnus castus* L.*)*

Figure 18: Chaste Tree and its distribution in our country

It is an aromatic bushy plant that usually grows up to 1-3 meters and sometimes up to 5 meters in height. The plant, which produces pale pink, purple or blue flowers between June and September, sheds its leaves in winter. Its red spherical fruits with a diameter of 3-4 mm are fragrant and bitter.

The chaste tree plant is widely grown in the warm regions of Anatolia, especially in the coastal areas of the Mediterranean, Black Sea, Southern Anatolia and Western Anatolia, in river and stream beds. It is a plant with high light fastness and a dyestuff content.

It was used as a dye plant in the past in Anatolia, and it is frequently used in wool dyeing in the regions where it grows today.

Dyestuffs Contained: *Luteolin.*

Dyeing Process: It is made with the dried and ground flowers and fresh leaves of the plant by dyeing with mordant.

Nettle *(Urtica diocica* L.*)*

Figure 19: Nettle and its distribution in our country

Stinging nettle is the general name given to the Urticaceae species. Nettle (Urtica diocica L.) is a perennial herbaceous plant in Turkey.

In China, it was formerly used for dyeing black. In Turkey, it is stated that it was not used for dyeing in the past.

Dyestuffs Contained: *Tanin.*

Dyeing Process: It is made with the dried and ground leaves of the plant by mordanting and direct painting methods.

Weld *(Reseda luteola* L.*)*

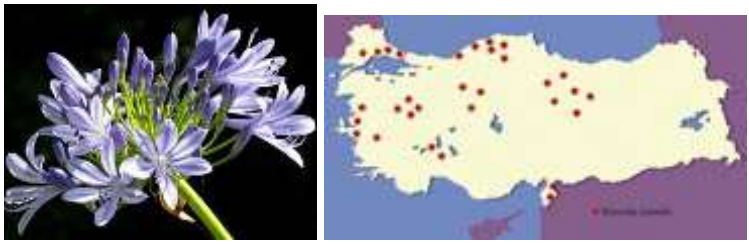

Figure 20: Weld and its distribution in our country

Weld is a biennial plant that can grow up to 150 cm, and only leaves are formed in the first year of the plant, and its development is completed in the second year. It is a plant species that is widely cultivated in Western Asian and Mediterranean countries.

Many old textiles, which were determined to be dyed with luteolin dyestuff as a result of dyestuff analysis, have survived to the present day without fading or with very little fading.

Dyestuffs Contained: *Luteolin, apigenin.*

Dyeing Process: It can be made with the dried and ground stem, leaves and flowers of the plant with the mordant dyeing method.

2.3. Collectıon of Plants

Some dyes use all parts of plants for dyeing, while others use a certain part (flower, root, leaf, bark, seed). When it is necessary to benefit from the whole or certain parts of the plant, the point to be considered is the collection time must be calculated very well. Generally, it is understood that the most mature period of the part to be collected is suitable for the collection time. Such as; It is collected when the flowers bloom, the leaf takes its full size, the seed is ripe, the dyestuff in the roots reaches maturity (for example, in spring). Care should be taken when collecting plant organs (rhizome, stolon, tuber, bulb, root, stem, etc.), which take a long time to grow, and these organs should be collected in the spring and autumn months. In addition, the collection of dye plants from the region where they are best adapted is important in terms of dye quality. Because ecological conditions are the most important factors that affect the amount and quality of dyestuff in a plant (Ceylan, 1991; Şanlı, 2011).

2.4. Dryıng and Usıng Plants

Dye plants are used both fresh and dry. If it needs to be collected in the season and will not be used immediately, these plants should be dried in order to be able to store them for a while. It is recommended that the drying process be done in the shade and in a ventilated place or in an oven that is not very hot. The plants dried in this way should be kept in cloth bags or paper bags. Mold and rot in plants adversely affect the amount and quality of dyestuff. For this reason, the collected plants should be kept away from situations where mold will be possible. In addition, depending on the plant species, changes occur in the amount and properties of dyestuffs of plants stored for many years (Ceylan, 1991; Şanlı, 2011).

The desired situation in Turkish carpet weaving today is related to the presence of dye plants used in Anatolia centuries ago. There are very few documents on dyestuffs in Anatolia and especially in the carpet-weaving regions. This is an important reason why families dealing with dyeing keep it a secret and make it a tradition. Nowadays, it is possible to benefit from people in the 70-90 age group to obtain information on this subject (Ceylan, 1991).

As a result of the researches, different dyeing methods were encountered in different regions of Anatolia. For example; A plant called Tehil (Crawfish- Geranium sp.) by the local people, which is used to dye wool black, has been found in Van province. In dyeing with this plant; It is stated that different colors such as mustard color, brick

color, naphtha green, claret red, canary yellow are obtained with different mordant substances without using mordant (Ceylan, 1991).

The part of the important vegetable dyes grown in Turkey used in dyeing and the main colors taken are shown in Table 1.

Table 1. Some plants used in herbal dyeing and obtained colors in Turkey (Şanlı, 2011)

Plant Species	Parts Used in Dying	Obtained Colors
Madder (*Rubia tinctoria*)	Subsoil shoots of the plant	brown-red
Buckthom (Rhamnus petiolaris)	berries	Shades of yellow and brown
Safflower (*Carthamus tinctorius*)	the body of the plant	yellow-green yellow
Walnut (*Juglans regia*)	all parts of the plant	All shades of khaki and green
Onion (*Allium cepa*)	Tuber outer shells	dark brown-orange
Grape (*Vitis vinifera*)	leaves	shades of green
Red pine (*Pinus brutia*)	trunk shells	Yellow green
Euphorbia (Euphorbia tinctoria)	all parts of the plant	Yellow-green and its shades
Dock (*Rumex conglomeratus*)	seeds	Brown and its shades
Barberry (*Berberis crataegina*)	all parts of the plant	Yellow and its shades
pomegranate (*Punica granatum* L.)	fruit skins	yellow, brown, black
Mint (*Mentha pulequem*)	all parts of the plant	yellow, khaki, green
Thyme (*Thymus kotschyanus*)	stem-leaves	yellow-green-gray
Chaste Tree (*Vitex agnus*)	leaves	yellow, green
Dyer sumac (Rhus coriaria)	all parts of the plant	yellow, red

Daisy (*Anthemis tinctoria*)	all parts of the plant	Yellow and its shades
Mullein (*Verbascum phlomoides*)	stem-leaves	mustard, green, yellow
Sage (Salvia triloba)	all parts of the plant	Yellow and its shades
Pennyroyal (*Mentha tomentosa*)	all parts of the plant	Yellow and its shades
Loadwort (*Plumbago europeae*)	all parts of the plant	Yellow and its shades

3. CONCLUSION and SUGGESTIONS

From past to present, every society in the world has evaluated many cultural plants in dyeing and has revealed their own traditional dyeing methods. Natural dyeing, which started with the weaving culture and developed with it in the Turks, created a deep-rooted dyeing tradition after the migration to Anatolia, using the advantage of the rich vegetation of the region. For many years, this deep-rooted tradition has continued, and a special color such as Turkish Red, which emerged with the taste and skill of Turkish weaving, and its dyeing recipe has spread to the world. Today, as a result of the increasing place of chemical dyes in our daily life, vegetable dyes show that they have lost their former importance (Genç and Göçmen, 2007).

In order to ensure the continuity of herbal dyeing, the cultivation of dye plants is one of the most important precautions that can be taken. The continuation of the production is possible as long as the product has a market. For this reason, planning should be done correctly and

production should start by providing communication between the manufacturer and the user. For the planning and implementation activities to be carried out for this purpose, priority should be given to the regions where hand-woven carpets and kilims are made intensively, and the traditional dye plants of the region should be included in the scope of the study (Genç and Göçmen, 2007; Şanlı, 2011).

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CHAPTER 10

SOME PHYSICOCHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF THE MUSCLE, SKIN AND DEPOSIT TISSUES OF GEESE RAISED IN LOW WINTER TEMPERATURES

Running title: Fatty acid composition of geese tissues

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Practical Application

Goose is a bird that can be raised in almost every region of Türkiye, but some provinces such as Kars, Ardahan and Erzurum are famous for breeding goose. However, goose raising in Altıntaş (Kütahya, Türkiye) is important due to climatic and geographic conditions. Goose meat is particularly consumed in winter season. This study revealed fatty acid composition of different tissues of geese raised in winter conditions.

1. Introduction

Meat and meat products are among the most important foods of animal origin that need to be consumed by people of all ages to preserve their physical and psychological health (Schlumpberger, 2004). With its nutritional value and its peculiar taste and smell, meat is one of the food products indispensable to humans. Beside amino acids and essential fatty acids, meat with its vitamin and mineral content is a food that can meet one's need for almost all food products even in a small amount. Also, people prefer poultry products due to the negative health effects of red meat and meat products. The winged animals in the white meat group is increasingly consumed on the grounds that it has a low cholesterol level and higher levels of unsaturated fatty acid (Hui, 2001; Richardson &Mead 1999). Chicken meat is also known as a food product rich in such vitamins as B_2, B_6 and B_{12} that feed or support the nervous system in addition to its having higher unsaturated fatty acid and lower cholesterol levels than red meat. As its muscle fibers are relatively shorter, chicken meat is a source of protein recommended to

those who have problems with their digestion (Richardson&Mead 1999; Sams, 2001). Especially in Asia and Europe, consumption of goose meat is increasing rapidly (Muğlalı et al., 2002). It is a particular advantage that these animals can be raised in cold climates. The increasing prices of feed stuff and drugs have made chicken meat more expensive. In addition, factors such as chickens' low resistance to diseases and vulnerability to stress factors as well as their inability to consume roughage have contributed to the raising and consumption of goose meat. Geese do not need a special shelter like a coop, and they are not affected by cold weather conditions. They can increase performance and consume roughage in the best way. Geese are also valuable due to the high prices attached to their livers and feathers abroad (Maraşlı et al., 2000).

Goose is a bird that can be raised in almost every region of Türkiye, especially in cold provinces like Kars and Erzurum. It is raised without using any special technology or shelter and makes an important contribution to the needs of families (Anonymous, 2009a). It is extensively raised in regions where the climate is colder and there are many fresh river sources and wide grass fields (Kırmızıbayrak, 2002). Goose breeding in Altıntaş is positively affected by the following: The region has favorable climatic and geographic conditions; it costs little to raise geese; there are local storing techniques for goose meat such as salting and drying, which are also accompanied by a long winter season that makes it easier to store it; and finally the local people are largely accustomed to consuming goose meat. In addition, goose meat is a

traditional food source that meets the protein intake needs of local people. It should, however, be added that local people engage in goose-raising not only for goose meat but also for the grease and feathers of the goose (Anonymous, 2009b).

This study has aimed at determining the fatty acid composition and some physicochemical properties such as fat content, pH, color, ash and dry matter in various tissues of domestic male and female geese raised in Altıntaş in the Kütahya region of Türkiye.

2. Materials and Methods

2.1. Materials

The geese included in the present study were obtained from Altıntaş district in Kütahya province in January. The male and female geese weighed about 3 kg and were slaughtered by hand in accordance with hygienic conditions and under the supervision of a vet. After the feathers of the geese were removed using the wet plucking technique and the entrails were separated, each goose was given a number to avoid any contamination and immediately put in a fridge in proper pouches and transferred to the lab under refrigerator conditions. The geese were stored at 4 ºC overnight, and then underwent a standard process of chopping with the help of an expert butcher. Thus, the pectoral muscle meat, skin and deposit lipids (abdominal fatty parts) were set apart to be used in the analysis. Three geese were used for each group. The geese had been raised with natural feed in their natural setting in

Altıntaş. The pectoral muscle meat, skin and deposit lipids were removed from the geese and preserved in a pouch. The air in the pouches was vacuumed as much as possible.

2.2. Dry Matter Analysis

The % dry matter content of the samples was determined by using the drying cupboard method and the analysis was carried out at 125ºC (Gökalp et al., 1995).

2.3. Ash Analysis

After putting the samples into crucibles, they were dried in the oven at 105 ºC for 12 hours and the dried samples were then put in an ash furnace (Protherm PLP 120/5, Türkiye) and were burned at 525ºC for 18 hours. When the samples reached constant weight, their ash amounts were determined (Gökalp et al., 1995).

2.4. Total Fat Analysis

The total fat contents of the previously-dried samples were determined by using the Soxhlet extraction method (Gökalp et al., 1995).

2.5. pH

For determination of the pH values of the samples, 10g of homogenized sample was weighed and then 100 ml distilled water was incorporated into it. The pH value of the sample, which was homogenized for 1 minute by an Ultraturrax (IKA T18 Basic, Staufen, Germany) was

recorded with a pH meter calibrated with suitable buffers (Gökalp et al., 1995).

2.6. Color

An automatic colorimeter (Lovibond RT Series Reflectance Tintometer, U.K.) was used to determine the color properties of the samples and the device was calibrated before each measurement. The samples for the color measurement were firstly homogenized in a blender and then placed in the capsule of the device, and thus the values of L^*, a^* and b^* were determined. L^* value indicates the brightness-darkness from black (0) to white (100); a^* shows the color dimension of green-red, and b^* shows the color dimension of yellow-blue.

2.7. Determination of Fatty Acid Compositions

The fatty acid composition of the goose meat was determined with gas chromatography. The fat samples obtained after the extraction, which was conducted with ether in the Soxhlet extraction system were taken as 100 mg and then 3 mL of hexane and 100 µL of 2N KOH in methanol were added. The mixture was homogenized with vortex for one minute. Then the sample was centrifuged at 5000 rpm for 5 minutes. After that, 1 ml of supernatant of mixture prepared was added to the vials and injected into the gas chromatography (Yalcin et al., 2011). In this study, Agilent 6890 model GC was used and detection in the analyses was performed with FID detector. An HP-88 column 100 m x 0.25 mm with an ID, of 0.2 µm, inlet temperature of 250°C, split rate of 1/50 and 2 mL/min fixed flow rate were used. The carrying gas was helium.

2.8. Statistical Analysis

Physicochemical analysis of the goose meat within the scope of the project was conducted in three replications. In the study, the muscle meat, skins and deposit lipids of the male and female geese were taken as the basis. SAS statistics software (version 6.03) was used for the evaluation of the results (SAS, 1988).

3 RESULTS

3.1. Physicochemical Properties

In this study, the pH, ash, dry matter and total fat analyses of the muscular tissue, skin and deposit lipids of the female and male goose meat were determined and the results were evaluated statistically. The findings for these parameters are shown in Table 1. The pH values of the muscular tissues, skin and deposit lipids in the samples studied were determined as 5.64, 5.94 and 5.87 in female geese, respectively, and 5.68, 5.96 and 5.85 in male geese, respectively. When the pH values of the samples were examined, no significant difference between the tissues was observed ($p > 0.05$). According to the statistical analyses, the effect of sex on the pH values of goose meat was found to be insignificant ($p > 0.05$), while the effect of tissue difference on the pH values of the goose samples for the same species was found as significant ($p < 0.05$).

The ash content of the female goose meat varied between 0.13% and 1.24%, while that of the male goose meat ranged from 0.12% to 1.18%.

As seen in Table 1, the ash amount of the muscular tissue in both samples was higher than that of the skin and deposit lipids. The statistical analysis showed that the effect of sex and different tissues on the ash content of the goose meat was significant ($p<0.05$).

The dry matter contents of the different tissues of female and male goose meat were found to be between 27.4 and 92.61% and 27.61 and 95.46%, respectively. The dry matter contents of the skin and deposit lipids were found to be higher because these tissues had more fat content than that of muscular tissue. The statistical analysis showed that sex and different tissues have a significant effect ($p<0.05$) on the dry matter contents of goose meat.

According to the findings of the present study, the average fat content of female goose meat was 2.55% in the muscles, 73.3% in the skin and 94.3% in the deposit lipids. In male goose meat, however, the average fat content was found as 1.98%, 72.79% and 97.27%, in the muscles, skin and deposit lipids, respectively. When the study results were examined, it was seen that the skin and deposit lipids contained more fat compared to the muscular tissue, as expected. When the fat contents of the muscular tissues of the female and male goose meat were compared, the female samples contained more fat than the male ones. As a result of the statistical analysis, it was found that the effect of sex on the fat contents of the goose samples was insignificant ($p>0.05$), while the effect of tissue difference on the amount of fat contents in the same goose samples was significant ($p<0.05$).

3.2. Color

The color values of the male and female goose meat analyzed in the study are given in Table 1. When the results of the color parameters of the tissue samples of the female and male geese were considered, it was determined that the *L**, *a** and *b** color values of the muscular tissue, skin and deposit lipids were similar to each other.

However, when the color features of muscular tissue, skin and deposit lipids were examined for each species, it was seen that the *L** values that represent darkness-brightness quality yielded the highest value on the skin part of the goose samples, followed by the deposit lipids and muscle meat, respectively. The *a** color values that indicate the density of color red were found to be higher in the muscular tissue than in skin and deposit lipids in both samples. The *b** values that indicate the density of color yellow were found to be the highest in deposit lipids. When the results were examined statistically, the effect of sex on the *L**, *a** and *b** color features was found to be insignificant ($p>0.05$) and the tissue difference of the color features of the same species of goose samples was found to be significant ($p<0.05$).

3.3. Fatty acid composition of the goose tissue samples

The fatty acid composition of the goose meat was determined with gas chromatography in the study, and the amounts of total saturated and unsaturated fatty acid levels of muscle tissue, skin and deposit lipids of female and male goose meat are given in Figure 1. The amount of total saturated fatty acids was found to be between 31.42% and 33.76% in

female goose meat and between 29.17% and 33.07% in male goose meat. The saturated fatty acid content of female and male goose meat was found to be higher in muscular tissues than in skin and deposit lipids. The amount of total unsaturated fatty acids was found to be between 66.24% and 68.58% in female goose meat and between 66.93% and 70.83% in male goose meat. The saturated fatty acid content of female and male goose meat was found to be higher in the skin tissues than in the muscle and deposit tissues. A total of 15 different fatty acids were determined in the goose tissue samples in the study. The total saturated and unsaturated fatty acid compositions of the muscle tissues, skin and deposit lipids of female and male goose meat are given in Table 2.

It was determined that the most common fatty acid in the samples was palmitic acid (C16:0), one of the saturated fatty acids, and oleic acid (C18.1), one of the unsaturated fatty acids. The amount of palmitic acid was found to be 24.08, 23.90 and 23.90% in the muscle tissue, skin and deposit lipids of the female geese, respectively; the highest level was found in the deposit lipids of the female geese. On the other hand, the oleic acid contents of the same geese were found to be 41.89, 49.75 and 49.75% in the muscles, skin and depot lipids, respectively. The palmitic acid amount of the male goose samples in the muscle tissue, skin and deposit lipids varied at 24.80, 22.76 and 23.72%, while oleic acid content was found to be 44.34, 54.30 and 53.52%, respectively. Oleic acid (C18:1), a monounsaturated fatty acid, was found in the skin fat of the male geese at the highest level. The amounts of linoleic acid (C18:2)

and linolenic acid (C18:3), which are essential fatty acids, were found in the muscle tissue of the female goose meat at the highest level. The findings of the study revealed that the effect of sex and different tissues on the fatty acid compositions of the male and female goose meat was statistically significant ($p<0.05$).

4 DISCUSSION

In general, the body features of poultry are affected by some factors such as their age, sex and species, the amount and quality of food consumed, and the environments in which they live. In recent years, consumers' demand for the winged animals fed with organic food has increased rapidly (Fanatico et al., 2006; Kırmızıbayrak, 2002, Ponte et al., 2008).

In a study in which white and mottled geese were compared, it was reported that the pH value in the breast meat of the geese was around 5.7% and the amounts of dry matter, ash and fat were 28-30%, 2.3-2.7% and 3.07-4.55%, respectively (Yakan et al., 2012). In another study in which the effect of sex and age on the meat of the geese raised in free range farming conditions was examined, it was reported that the sex and age of the geese affected some physicochemical parameters and the color qualities of the breast and thigh meat of the geese (Kirmizibayrak et al., 2011).

The effects of season, years and sex on the fatty acid composition of the abdominal fat tissue of female and male winter Canadian geese were investigated and it was determined that the myristic acid (C14:0)

content of the abdominal fatty tissues of the female geese was higher than that of the male ones. Moreover, C14:0, C16:0, C16:1, C18:1 and C18:3 fatty acids were found to vary for different years (Austin, 1993).

Also, the fatty acid composition of the back muscles and livers of geese fed with different grains was examined and it was determined that feeding geese with different grains changed the fatty acid composition (Kalayci &Yilmaz, 2014). However, in a study in which the effect of sex, age and species on the fatty acid composition was examined, it was determined that sex and age did not significantly affect the fatty acid composition (Friend et al., 1983). Liu and Zhou (2013) examined the effect of feeding geese with grass on fatty acid composition and some meat quality characteristics and found that feeding them with grass caused changes in the L* and pH values of the breast meat of the geese, but there were significant increases in the linolenic (C18:3n-3) and eicosapentaenoic acid (C20:5n-3) amounts.

The above mentioned studies showed that factors such as sex, age, species, feeding type and season leed to some changes in some physicochemical, color and fatty acid compositions of geese. In the present study, too, it was determined that the sex of the geese did not affect the physicochemical parameters or the color qualities of the geese and oleic acid, a major fatty acid, was more prevalent in male geese.

In recent years, due to the increased living standards in many countries people have begun to be more sensitive and conscious about the effects of different foods on their health. Currently people not only consume

food, they also care about the harm or benefit of foods to their health. As conscious consumers try to avoid the consumption of red meat and meat products because of some of their negative effects, there appears to be an inclination towards white meat. The winged animals in the white meat group are increasingly, and should be, consumed for such reasons as the lower cholesterol level of their meat and the higher rates of unsaturated fatty acid. These studies are intended to make consumers aware of the fact that goose meat should take a greater place in their diets and it can be consumed much more.

5. CONCLUSION

As a result, the geese raised in the Altıntaş district increase the levels of animal food production and contribute to nutrition of the local residents. It could be said that the improvement initiatives to be undertaken will positively contribute to an increase in goose meat production and to meeting local people's need for food.

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Table 1. Some physicochemical and color properties of female and male geese

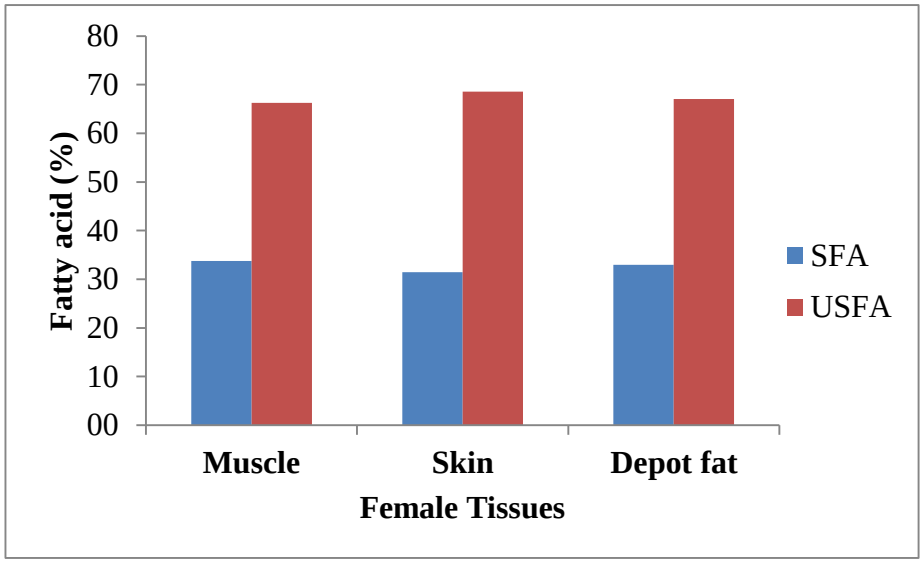
Parameters	Sex	Muscle	Skin	Deposit
pH	F	5.64^{Ac}±0.02	5.94^{Aa}±0.02	5.87^{Ab}±0.02
	M	5.68^{Ab}±0.07	5.96^{Aa}±0.02	5.85^{Aa}±0.03
Ash (%)	F	1.24^{Aa}±0.02	0.41^{Ab}±0.04	0.13^{Ac}±0.00
	M	1.18^{Aa}±0.05	0.30^{Bb}±0.02	0.12^{Ac}±0.01
Dry matter (%)	F	27.44^{Ac} ±0.63	79.07^{Ab}±1.47	92.61^{Ba} ±1.06
	M	27.61^{Ac} ±0.40	78.53^{Ab} ±0.92	95.46^{Aa}±1.59
Fat (%)	F	2.55^{Ac}± 0.21	73.30^{Ab}± 3.51	94.30^{Aa}±3.05
	M	1.98^{Ac}± 0.32	72.79^{Ab}±0.60	97.27^{Aa}±0.57
*L**	F	35.05^{Ac}±2.81	62.90^{Aa} ±2.27	56.47^{Ab} ±0.89
	M	36.30^{Ac}±2.30	61.14^{Aa}±1.57	54.26^{Ab}± 3.00
*a**	F	8.75^{Aa}± 0.49	1.62^{Ac}±0.40	3.37^{Ab}±0.15
	M	7.91^{Aa}±0.99	1.49^{Ac}±0.32	3 17^{Ab}±0.40
*b**	F	13.79^{Ab}±1.04	16.46^{Aa}±0.17	18.10^{Aa}±0.87
	M	13.79^{Ab}±1.04	15.32^{Ab}±1.60	18.76^{Aa}±0.87

F: Female, M: Male, $^{A-B:}$ The capital letters in the same column for the meat of each sex of goose are a comparison of female and male geese, and show that there are not statistical differences between the samples. $^{a-c}$ The lowercase letters in the same line are a comparison of different tissues, and the same letters show that there are not statistical differences between the samples.

Table 2. Fatty acid composition of the female and male geese (%).

	Fatty acid	Female			Male		
		Muscle	Skin	Deposit	Muscle	Skin	Deposit
Saturated	C14:0	0.66^{b}±0.02	0.80^{a}±0.01	0.80^{a}±0.01	0.61^{a}±0.05	0.67^{a}±0.02	0.62^{a}±0.01
	C15:0	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.11^{a}±0.01	0.09^{a}±0.00
	C16:0	24.08^{a}±0.56	23.90^{b}±1.20	23.90^{b}±1.20	24.08^{a}±0.67	22.76^{b}±0.43	23.72^{b}±0.85
	C17:0	-	0.13^{a}±0.01	0.13^{a}±0.01	-	0.13^{a}±0.01	0.14^{a}±0.03
	C18:0	9.02^{a}±0.37	6.48^{b}±0.60	6.48^{b}±0.60	8.38^{a}±0.15	5.50^{b}±0.33	7.27^{a}±0.33
Unsaturated	C14:1	-	0.08^{a}±0.01	0.08^{a}±0.01	-	0.06^{a}±0.00	0.07^{a}±0.01
	C16:1	3.71^{b}±0.03	4.79^{a}±0.07	4.79^{a}±0.07	4.01^{b}±0.12	4.69^{a}±0.04	3.52^{c}±0.02
	C17:1	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.12^{a}±0.02	0.10^{a}±0.01
	C18:1 *trans*	-	0.32^{a}±0.01	0.32^{a}±0.01	-	0.33^{a}±0.01	0.34^{a}±0.01
	C18:1 *cis*	41.89^{b}±0.98	49.75^{a}±0.10	49.75^{a}±0.10	44.34^{b}±0.10	54.3^{a}±0.25	53.52^{a}±0.50
	C18:2 *cis*	11.92^{a}±0.05	8.61^{b}±0.05	8.61^{b}±0.05	11.3^{a}±0.05	7.44^{b}±0.23	6.55^{c}±0.23
	C18:3n-6	-	0.11^{a}±0.01	0.11^{a}±0.01	-	0.10^{a}±0.00	0.10^{a}±0.01
	C18:3n-3	5.81^{b}±0.08	4.56^{a}±0.03	4.56^{a}±0.03	4.32^{a}±0.03	3.38^{b}±0.06	3.52^{b}±0.03
	C20:1	-	0.36^{a}±0.01	0.36^{a}±0.01	-	0.41^{a}±0.02	0.38^{a}±0.01
	C22:1	2.91±0.05	-	-	2.96±0.04	-	-

[a-c] The lowercase letters in the same line are a comparison of different tissues, and the same letters show that there are not statistical differences between the samples.



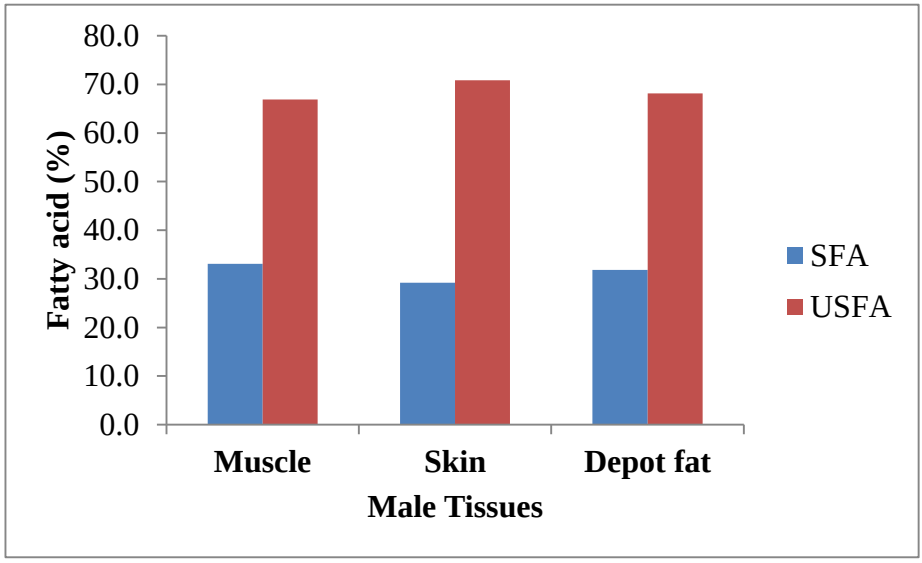


Figure 1. The amounts of SFA and USFA in various tissues of female and male geese

SFA: Saturated fatty acids, **UFSA:** Unsaturated fatty acids

CHAPTER 11

UTILISATION OF MICROALGAE AS FUNCTIONAL FOOD SUPPLEMENTS AND/OR ADDITIVES

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Introduction

Planktons with simple plant characteristics, which can perform photosynthesis with the help of chlorophyll in their cells and vary in size from a few microns to a few hundred microns, are called phytoplankton or microalgae. These creatures, which are the primary producers in the aquatic system, play an important role in feeding the living creatures in the sea and fresh waters, thanks to their high protein content. These microscopic creatures can be found in lakes, rivers, estuaries, oceans, glaciers or soils, as well as they are grown by different biotechnological methods. Microalgae can grow heterotrophic, myxotrophic, or cultivated in photobioreactors under photoautotrophic conditions. They take part in the biological CO_2/O_2 cycle and can produce about 50% of global oxygen. In the classification of microalgae, their characteristics such as cell morphology, cytology, pigment substances they contain and breeding types are used. While the most important structurally important species of microalgae are *Cyanophyta*, *Chlorophyta*, *Euglenophyta* and *Diatomophyceae* members in abundance in fresh waters; *Pyrrhophyta, Crysophyceae*, *Xanthophyceae* members are relatively rare. The most common microalgae in the sea are numerous *Diatomophyceae* species (Şahin and Akyurt 2010; Richmond and Hu 2013; Kim 2015; Patras et al. 2019).

Microalgae are used in different areas due to their rich nutrient content and health effects, and have been consumed by people for centuries as food and food additives. The nutritional composition of microalgae consists of different chemical and biological compounds such as

carbohydrates, proteins, vitamins, lipids, antioxidants and other trace elements. Thanks to the bioactive properties of the compounds contained in microalgae, the health benefits and effects; antiviral, anticancer, antidiabetic, antibiotic, antioxidant, prebiotic, probiotic, immune system booster, cardiovascular system protector, hypocholesterolomic and antiallergic. In addition to these, different numbers of products used as antibiotics and antibacterials are obtained from microalgae (Kavas and Kavas 2009; Yılmaz and Duru 2011; Sánchez and Vázquez 2017; Aksay and Arslan 2018; Barkia et al. 2019; Ramos-Romero et al. 2021). The general chemical composition of different microalgae is given and compared with the composition of traditional foods in Table 1.

Table 1. General composition of different foods and microalgae (% dry matter) (Richmond 2004; Spolaore et al. 2006; Gouveia et al. 2008a).

Commodity	**Protein**	**Carbohydrate**	**Lipid**
Baker's yeast	39	38	1
Meat	43	1	34
Egg	47	4	41
Milk	26	38	28
Rice	8	77	2
Soya	37	30	20
Anabaena cylindrica	43-56	25-30	2-7
Aphanizomenon flosaquae	62	23	3
Chlamydomonas rheinhardii	43	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechococcus sp.	63	15	11

Microalgae, which have a wide range of uses thanks to their rich composition, are used as additives or food support products for functional foods. Many industrially valuable compounds such as pigments could be obtained from microalgae. Furthermore microalgae are used in the food industry for health purposes, in the production of protein-rich and nutritious, low-calorie food. Microalgae are also used in several areas animal feed, pharmaceutical, biodiesel production, cosmetic industry, soil enrichment and wastewater treatment (Figure 1) (Yılmaz and Duru 2011; Eliçin et al. 2013; Gökpınar et al. 2013; Arslan, 2018).

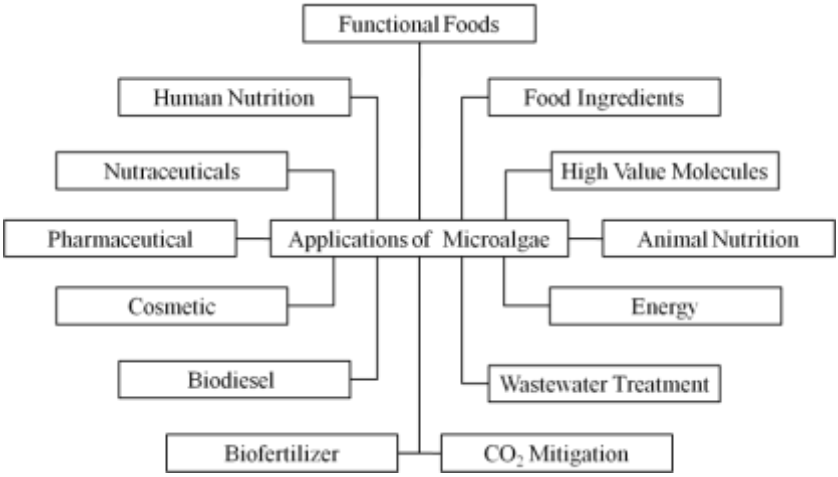

Figure 1. Applications of microalgae

In parallel with the widening of its usage areas, the production of microalgae is increasing day by day. In this review, bioactive effects of protein-amino acids, essential fatty acids, vitamins, natural pigments and other components obtained from microalgae on human health, their

use as functional food and food additives and their usage areas will be discussed.

Microalgal compounds and their bioactive properties

Food products with natural ingredients are increasingly attracting the attention of consumers. Recent research shows that Microalgae are the best resource for many areas, especially the food and feed industry (Patras et al. 2019). Microalgae are very rich in chemical compounds and are a source of raw materials for many natural products that can fight against various diseases and are used in human nutrition (Venugopal 2009). Also, the cell contents of microalgae consist of polyunsaturated fatty acids (PUFA), sterols, pigments, proteins and enzymes, vitamins and other bioactive substances, and these substances have the potential to be used in many areas as shown in Table 2.

Table 2. Bioactive properties and application areas of microalgal compounds (Samarakoon and Jeon 2012; Raposo et al. 2013; Koller et al. 2014).

Group of compounds	Components	Microalgae/cyanobacteria	Bioactivity
Proteins Peptides Enzymes	Proteins Peptides Superoxide dismutase (SOD) Carbonic anhydrase	*Amphidinium carterae* *Anabaena* *Chlorella vulgaris* *Dunaliella salina* *Isochrysis galbana* *Pavlova lutheri* *Phaeodactylum tricornutum* *Porphyra yezoensis* *Porphyridium cruentum* *Prorocentrum minimum* *Spirulina platensis* *Synechococcus* *Undaria pinnatifida*	Antihypertensive Antioxidant Anti-inflammatory

Pigments	Carotenoids Chlorophylls Phycobilins	*Botryococcus braunii* *Chlorella vulgaris* *Dunaliella salina* *Haematococcus pluvialis* *Porphyridium cruentum* *Spirulina platensis*	Antioxidant Anticancer Anti-inflammatory Immune-stimulatory
PUFA	Eicosapentaenoic acid (EPA) γ-Linolenic acid (GLA) Arachidonic acid (ARA) Docosahexaenoic acid (DHA)	*Crypthecodinium* *Dunaliella salina* *Isochrysis galbana* *Nannochloropsis* *Nitzschia laevis* *Pavlova lutheri* *Phaeodactylum tricornutum* *Porphyridium cruentum* *Schizochytrium* *Spirulina platensis*	Antimicrobial Anti-inflammatory Anti-aging Anticancer Immune system booster Cholesterol lowering Brain and eye development Preventive cardiovascular diseases Nutraceutical
Vitamins	A, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C, E and K vitamins	*Dunaliella salina* *Isochrysis galbana* *Pavlova lutheri* *Porphyridium cruentum* *Spirulina platensis* *Tetraselmis*	Antioxidant Immune system booster Blood cell formation Blood clotting mechanism
Other compounds	Brassicasterol Stigmasterol γ-Amino-butyric acid (GABA) Okadaic acid	*Chaetoceros* *Gambierdiscus toxicus* *Isochrysis galbana* *Pavlova lutheri* *Porphyridium* *Prorocentrum lima* *Skeletonema*	Antifungal Anti-inflammatory Antioxidant Hypocholesterolemic effect Immune system booster Neurotransmitter Regulation of the nervous system

Protein, peptide and amino acids

In Recent years, the interest in obtaining bio-functional proteins and certain peptides from microalgae has increased. Comprehensive analyzes and nutritional studies have shown that algal proteins are of high quality (Samarakoon and Jeon 2012; Becker 2007). As shown in Table 1, the high protein content of various microalgae species is one

of the main reasons for considering them as an unconventional protein source. Also, the amino acid composition of most microalgae is similar when compared to other foods used as protein source. Because they can synthesize all amino acids, they can provide essential amino acids to humans and animals (Spolaore et al. 2006; Vonshak 1997; Rasheed et al. 2020).

Proteins and peptides derived from microalgae; has antioxidant, anti-inflammatory, antioxidant and, antihypertensive effects (Arslan 2018; Samarakoon and Jeon 2012; Raposo et al. 2013). Phycobiliproteins are one of the most important protein groups obtained from microalgae. These proteins, which have a complex structure that dissolves in water, consists of phycocyanobilin and phycoerythrobilin proteins. Phycobiliproteins are partially responsible for hepatoprotective, anti-inflammatory and antioxidant activities (Richmond 2004; Ibañez et al. 2012).

Protein, peptides and amino acids have health benefits as well as nutritional benefits. Therefore, microalgae such as Spirulina and Chlorella can be used as nutraceuticals or incorporated into functional foods to prevent certain diseases and cell / tissue damage (Raposo et al. 2013). For example, although Spirulina, which contains about 50-70% protein, does not contain all of the essential amino acids, the amino acids it contains have excellent bioavailability. Dunaliella microalgae can produce 50-100 times more protein than traditional plants and animals grown for food (Spolaore et al. 2006; Lordan et al. 2011; Ejike et al. 2017; Chew et al. 2017).

Marine proteins can be used as functional ingredients in foods, as they have properties such as film and foaming capacity, gel-forming and antimicrobial activity (Lordan et al. 2011). In addition, the functional properties of microalgae containing food products (nitrogen solubility, water and oil absorption capacity, emulsification capacity, viscosity) were found to be comparable to those of a similar product containing soybean meal (Ejike et al. 2017).

Pigments

Microalgae (such as Chlorella, Dunaliella and Spirulina) are important creatures not only for food production but also for obtaining commercially important chemicals such as pigments. The most important pigments obtained from microalgae; chlorophyll, β-carotene, astaxanthin, phycocyanin, xanthophyll and phycoerythrin. These pigments can be used as natural pigments instead of synthetic pigments used in areas such as food, pharmacy, textile and cosmetics. In addition, these pigments are also used as nutraceutical and pharmaceutical thanks to their antioxidant, anticancer, anti-inflammatory and cholesterol-lowering effects. While the amount of pigmen varies according to the species, in general, microalgae cells can produce pigments such as chlorophyll, 0.1-0.2% carotenoids and 14-20% phycobiliproteins of their dry weight. However, the Dunaliella strain can produce up to about 14% β-carotene by dry weight. Commercially produced microalgae and pigments obtained from them can be listed as follows; β-carotene from *Dunaliella salina* and *Scenedesmus acutus*, phycocyanin from *Spirulina sp*., astaxanthin from *Haematococcus pluvialis*, xanthophylls

from *Nannochloropsis oculata*, lutein from *Muriellopsis sp*, and Phycoerythrin from *Porphyridium cruentum* (Patras et al. 2019; Spolaore et al. 2006; Arslan 2018; Rasheed et al. 2020; Akoğlu and Çakmakçı 2011;Duru and Yılmaz 2013).

Pigments have an important role in photosynthesis metabolism and pigmentation in microalgae. Major pigments in microalgae fall into three basic classes: chlorophyll, carotenoid, and phycobiliprotein. Chlorophyll is a low-polarity and fat-soluble pigment commonly found in fruits and vegetables. This green pigment is responsible for converting solar energy into chemical energy in photosynthesis. Most microalgae have chlorophyll-a, but microalgae like *Dinophyta* can have chlorophyll-b and chlorophyll-c. Microalgae contain 0.5-1.0% chlorophyll by dry weight. Chlorophyll is used as a food additive (E140) to give a green color to various foods and beverages such as pasta, pesto or wormwood drink. Carotenoids are oil-soluble pigments ranging in color from brown, red, orange and yellow. Carotenoids are pigments that support chlorophyll in capturing light energy. Carotenoids, which have antioxidant properties, are important components for functional food products as they prevent the negative effects caused by free radicals. Moreover, β-carotene is converted into vitamin A in the human body. The main carotenoids of microalgae are: β-carotene, lycopene, astaxanthin, zeaxanthin, violaxanthin and lutein. Phycobiliproteins are protein complexes that help gather light during photosynthesis in microalgae. Phycobiliproteins, which are water soluble and constitute a large part of the total cell protein, are formed

by the combination of non-protein compounds with proteins. Phycobiliproteins consist of phycocyanin, allophycocyanin, phycoerythrin. Phycocyanin is a blue pigment found in cyanobacteria. Phycoerythrin is the red pigment found in red microalgae (Arslan 2018; Koller et al. 2014; D'Alessandro and Filho 2016; Chew et al. 2017).

Thanks to the bioactive property of astaxanthin obtained from *Haematococcus pluvialis* microalgae, it plays an important role in the prevention of cancer, in the treatment of Alzheimer's, Parkinson's, brain diseases and cardiovascular diseases. Carotenoids (β-carotene and xanthophyll) obtained from Dunaliella species are pigments with high bioactivity and anticancer properties. carotenoids obtained from *Dunaliella* species are pigments with high bioactivity and anticancer properties. In addition, pigments obtained from this microalgae are used in margarine, dairy products, fruit juices, baked goods and breads. Blue colored phycocyanin pigment obtained from *Spirulina platensis* has an anti-inflammatory and immune system strengthening effect as an antioxidant. Phycocyanin is also used as a food color (Kavas and Kavas 2009; Arslan 2018; Raposo et al. 2013; Raja et al. 2018).

Lipids and Fatty Acids

Microalgae are rich in fatty acids, especially omega-3 and omega-6 fatty acids. It is also a valuable source of essential vitamins (A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid), many of which it can synthesize. Microalgae are capable of accumulating lipids up to 90% of cell dry weight, with an average lipid content

ranging from 1-70%. Microalgae such as *Chlorella*, *Dunaliella* and *Spirulina* accumulate significant amounts of fat (up to 60% of cell dry weight) when grown under certain environmental conditions such as high temperature and light intensity, increased salinity (Spolaore et al. 2006; El-Baky and El-Baroty 2013).

Microalgae lipids are very rich in long-chain fatty acids (PUFA) such as Arachidonic acid (ARA), Eicosapentaenoic acid (EPA) Gamma linoleic acid (GLA). These long chain fatty acids in algae have many benefits and functions in dietetic and therapeutic uses, as shown in Table 2. It has positive effects in the treatment of hypertension, premenstrual tension, various atopic disorders, diabetes, coronary heart disease, skin disease, hypertonia, cancer, hyperlipidemia and other cases. 20-carbon polyunsaturated fatty acids such as ARA and EPA are substances that are necessary for the synthesis of the prostaglandin hormone. Docosahexaenoic acid (DHA) is a component that helps the development of brain tissue and vision in babies. Omega-3 fatty acids obtained from microalgae are also used in food and agriculture (Rasheed et al. 2020; El-Baky and El-Baroty 2013).

Vitamins

Humans need micronutrients such as vitamins as well as macronutrients (carbohydrates, proteins and fats) to survive. Vitamins are one of the main micronutrients essential for human health. Because vitamins are required for energy metabolism, immunity, repair of cellular damage, antioxidants, supporting bones and other functions (Kusmayadi et al.

2021). Microalgae are a valuable source of vitamins as they are rich in vitamins A, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C, E (Matos et al. 2017).

Since microalgae used in human nutrition contain more vitamins than traditional foods, they are considered as non-traditional sources of vitamins. It is particularly rich in *Dunaliella tertiolecta*, B_2, B_3, B_{12}, B_9, C and vitamin E. In addition, *Navicula ostrearia* (vitamin E), *Porphyridium cruentum* (C, E vitamins and provitamin A), *Chlorella* (fat-soluble and B group vitamins) are other vitamin-rich microalgae. Some vitamins such as vitamin E and provitamin A that can be obtained from microalgae have a wide market potential, especially because they are used as antioxidants. If microalgae are eaten as a whole, some vitamins necessary for human and animal nutrition are met, while the bioactive properties of other compounds are also utilized besides antioxidant vitamins. Therefore, microalgae can be used as food supplements or food additives for human and animal nutrition (Venugopal 2009; Raposo et al. 2013; El-Baky and El-Baroty 2013).

Other compounds

Sterols are one of the most important bioactive chemical components that can be isolated from microalgae. Although the sterol ratio is generally low in phytoplankton species, it is high in microalgae such as *Thalassiosira* and *Pavlova*. *Pavlova lutheri* contains besides cholesterol, other rare sterols such as brassicasterol, campesterol, stigmasterol and sitosterol. Although high levels of LDL (low-density lipoprotein) and cholesterol pose risk for cardiovascular disease, sterols

have been found to play an important role in lowering LDL and cholesterol levels in their *in vivo* use. However, sterols have bioactive properties such as hypocholesterolemic, anti-inflammatory, and antiatherogenic activity. Also, phytosterols (C_{28} and C_{29} sterols) are important precursors to many compounds, including vitamins. For example, ergosterol is a vitamin D2 precursor (Raposo et al. 2013; Ibañez et al. 2012).

In recent years, extensive studies have been carried out on the production of different compounds with antibiotic, antibacterial and antimycotic activities from microalgae. Researchers obtained cyguatoxin from *Gambierdiscus toxicus* and okadaic acid from *Prorocentrum lima* and demonstrated that these compounds have antifungal activity. In addition, it can be said that the most important feature of okadaic acid in health applications is that it promotes the release of the nerve growth factor. On the other hand, the cytotoxic activity of these compounds is used in anticancer therapy (Nagai et al. 1992).

Gamma-aminobutyric acid (GABA) is the compound that stimulates and regulates the brain development of adult mammals. Derived from *Porfiridyum* microalgae, GABA is used as a dietary supplement in some commercial formulations (Raposo et al. 2013).

The use of microalgae as functional food and food additives

Functional food can be defined as a new food or food item that contains many bioactive ingredients that can increase health and reduce the risk

of disease, rather than a basic diet of humans. The term "functional food" was first used in Japan in the mid-1980s for processed foods containing many nutritious nutrients produced for hypertension patients. In the following years, many foods or food ingredients enriched with chemicals that have health benefits have been developed in some countries to cure many diseases and to maintain health (Venugopal 2009; Lordan et al. 2011; Hasler 2002; Plaza et al. 2008; Borowitzka 2013).

The economic, cultural and scientific development of societies has caused significant changes in dietary habits and lifestyles. For example, with sedentary life and decrease in physical activity, it has led to an increase in obesity, as well as problems such as heart disease, diabetes and hypertension in humans. Thus, people turned to functional foods with health-protective and disease-healing effects (Plaza et al. 2008; Gil-Chávez et al. 2013).

When a bioactive compound is added to a food formulation with a specific purpose, the resulting new product can be considered a functional food. The possible health effect of functional foods is realized by functional ingredients not found (or found in small amounts) in traditional foods. These ingredients are compounds such as vitamins, minerals, fatty acids, pigments, phytosterols and soluble fibers. These ingredients have many beneficial properties such as lowering cholesterol levels, reducing lactose intolerance, cure of Crohn's disease, preventive of cardiovascular disorders, immune enhancing and anticancer, antioxidant, antiviral, antihypertensive, anti-inflammatory

effects (Hasler 2002; Raposo et al. 2013; Michalak and Chojnacka 2014).

All foods are functional to some extent because they offer taste, aroma and nutritional properties (Hasler 2002). For example, foods such as fruits and vegetables represent the simplest form of functional foods as they are rich in a variety of natural bioactive compounds. Containing natural bioactive compounds such as polyphenols and carotenoids, fruits have an antioxidant effect and reduce the risk of developing certain types of cancer. Apart from these compounds, many compounds that have the potential to be used in the food industry are obtained from important sources such as different plants, animals and microalgae (Plaza et al. 2008; Gil-Chávez et al. 2013).

Microalgae are a natural source of many new bioactive compounds that can be used as functional additives thanks to their rich biochemical composition. Thus, isolation and identification of bioactive compounds from microalgae has emerged as a fundamentally trend to develop new functional foods. It is aimed to minimize the risk of chronic diseases with functional foods obtained by using bioactive compounds isolated from microalgae in traditional foods (Kim, 2015; Plaza et al. 2008).

Table 3. The intended use and bioactive properties of some microalgae species used in different studies

Species	Functional Food / Product	Intended Use / Bioactive Property	References
Chlorella stigmatophora, Phaeodactylum tricornutum	The crude polysaccharide extract	Anti-inflammatory and immunostimulatory effects	(Guzmán et al. 2003)
Chlorella vulgaris	Butter cookies	Colorant	(Gouveia et al. 2007)
Chlorella vulgaris, Haematococcus pluvialis	Food emulsions	Colorant / Antioxidant effect	(Gouveia et al. 2006)
Dunaliella salina	Powder *Dunaliella*	Antioxidant effect	(Murthy et al. 2005)
Diacronema vlkianum, Isochrysis galbana	Pasta products	PUFA enrichment	(Fradique et al. 2013)
Haematococcus pluvialis	Cookies	Colorant, Antioxidant effect	(Hossain et al. 2017)
Isochrysis galbana	Biscuits	Colorant, PUFA enrichment	(Gouveia et al. 2008b)
Nannochloropsis oculata	Cookies and pasta	Colorant, PUFA enrichment	(Babuskin et al. 2014)
	Sterol extract	Anticancer and anti-inflammatory effect	(Sanjeewa et al. 2016)
Porphyridium cruentum	Polysaccharide isolate	Anticancer and immuno-modulation effect	(Gardeva et al. 2009; Sun et al. 2012)
	Crude the sulfolipids	Antioxidant, anti-inflammatory and anti-proliferative effect	(Berge et al. 2002)
	Sulfated polysaccharide	Antiviral effect	(Huleihel et al. 2001)
Spirulina maxima	*Spirulina* supplement	Anti-inflammatory and antioxidant effect	(Gutiérrez-Rebolledo et al. 2015)

Spirulina platensis	Pasta	Protein enrichment / Antioxidant effect	(Zouari et al. 2011; Lemes et al. 2012; Marco et al. 2014)
		Colorant, nutritional enrichment	(Özyurt et al. 2015)
	Chocolate biscuits	Protein enrichment	(Morais et al. 2006)
	Yoghurt	Protein, fat and iron enrichment	(Malik et al. 2013)
		Colorant, protein enrichment	(Arslan and Aksay 2021)
	Manioc cake	Protein enrichment	(Navacchi et al. 2012)
	Gluten-free bread	Colorant, protein enrichment	(Figueira et al. 2011)
	Baby food	Nutritional enrichment	(Sharoba 2014)
	Extruded product	Nutritional enrichment	(Joshi et al. 2014; Morsy et al. 2014)
	Peptide isolates	ACE inhibitory	(Lu et al. 2010)
Spirulina spp.	Shake-type powdered food	Nutritional enrichment	(Santos et al. 2016)
	Spirulina supplement	Immuno-modulation, antioxidant effect, and lipid-lowering effect	(Park and Lee 2016)

Used as a functional food source, microalgae are not a new food and have been used for nutrition by people in Asia, South America and Africa for at least 700 years or more (Kavas and Kavas 2009; Chacón-Lee and González-Mariño 2010). For example, people living in Mexico in the 17th century have dried the *Spirulina* microalgae harvested from the Texcoco Lake in the sun and consumed the cake-shaped “tekuitlatl”

food. Africans living around Chad Lake also used the food called "dihe" obtained from *Spirulina* like the Mexican people. In addition to being used as a consumable food product, *Spirulina* is also useful as a functional ingredient. Because *Spirulina* biomass can be included in various food products to improve nutritional quality and its therapeutic effect in chronic diseases (Vonshak 1997; Richmond 2004; Habib et al. 2008; Yılmaz and Duru 2011; Da Silva Vaz et al. 2016; Arslan 2018).

Commercial production of microalgae for human nutrition is carried out in many countries. It is in the form of tablets, powders, capsules and pastilles in the market and is used as nutritional supplements and functional foods. In addition, many functional foods are obtained by including microalgae in foods such as pasta, biscuits, bread, cookies, confectionery, yoghurt, snacks, and drinks (Table 3). Functional foods enriched with microalgae are much more useful and variable sensually. Therefore, functional foods have an attraction for consumers as well as health benefits (Richmond 2004; Gouveia et al. 2008a).

Conclusions

Depending on the increase in the human population, health problems increase and accordingly, people turn to foods that increase health and protect health. Microalgae is also used in nutraceuticals and functional foods produced for health purposes, thanks to the bioactive compounds they contain. These compounds obtained from microalgae have effects such as antiviral, anticancer, antidiabetic, antibiotic, antioxidant, antihypertensive, immune system booster, cardiovascular system

protector, hypocholesterolomic and antiallergic. In this review, the bioactive effect on human health of protein-amino acids, essential fatty acids, vitamins, natural pigments and other components produced from microalgae were discussed as functional food and/or additives for foods.

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Table 1. General composition of different foods and microalgae (% dry matter)

(Richmond 2004; Spolaore et al. 2006; Gouveia et al. 2008a).

Commodity	Protein	Carbohydrate	Lipid
Baker's yeast	39	38	1
Meat	43	1	34
Egg	47	4	41
Milk	26	38	28
Rice	8	77	2
Soya	37	30	20
Anabaena cylindrica	43-56	25-30	2-7
Aphanizomenon flosaquae	62	23	3
Chlamydomonas rheinhardii	43	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechococcus sp.	63	15	11

Table 2. Bioactive properties and application areas of microalgal compounds (Samarakoon and Jeon 2012; Raposo et al. 2013; Koller et al. 2014).

Group of compounds	Components	Microalgae/cyanobacteria	Bioactivity
Proteins Peptides Enzymes	Proteins Peptides Superoxide dismutase (SOD) Carbonic anhydrase	*Amphidinium carterae* *Anabaena* *Chlorella vulgaris* *Dunaliella salina* *Isochrysis galbana* *Pavlova lutheri* *Phaeodactylum tricornutum* *Porphyra yezoensis* *Porphyridium cruentum* *Prorocentrum minimum* *Spirulina platensis* *Synechococcus* *Undaria pinnatifida*	Antihypertensive Antioxidant Anti-inflammatory
Pigments	Carotenoids Chlorophylls Phycobilins	*Botryococcus braunii* *Chlorella vulgaris* *Dunaliella salina* *Haematococcus pluvialis* *Porphyridium cruentum* *Spirulina platensis*	Antioxidant Anticancer Anti-inflammatory Immune-stimulatory
PUFA	Eicosapentaenoic acid (EPA) γ-Linolenic acid (GLA) Arachidonic acid (ARA) Docosahexaenoic acid (DHA)	*Crypthecodinium* *Dunaliella salina* *Isochrysis galbana* *Nannochloropsis* *Nitzschia laevis* *Pavlova lutheri* *Phaeodactylum tricornutum* *Porphyridium cruentum* *Schizochytrium* *Spirulina platensis*	Antimicrobial Anti-inflammatory Anti-aging Anticancer Immune system booster Cholesterol lowering Brain and eye development Preventive cardiovascular diseases Nutraceutical
Vitamins	A, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C, E and K vitamins	*Dunaliella salina* *Isochrysis galbana* *Pavlova lutheri* *Porphyridium cruentum* *Spirulina platensis* *Tetraselmis*	Antioxidant Immune system booster Blood cell formation Blood clotting mechanism
Other compounds	Brassicasterol Stigmasterol γ-Amino-butyric acid (GABA) Okadaic acid	*Chaetoceros* *Gambierdiscus toxicus* *Isochrysis galbana* *Pavlova lutheri* *Porphyridium* *Prorocentrum lima* *Skeletonema*	Antifungal Anti-inflammatory Antioxidant Hypocholesterolemic effect Immune system booster Neurotransmitter Regulation of the nervous system

Table 3. The intended use and bioactive properties of some microalgae species used in different studies

Species	Functional Food / Product	Intended Use / Bioactive Property	References
Chlorella stigmatophora, *Phaeodactylum tricornutum*	The crude polysaccharide extract	Anti-inflammatory and immunostimulatory effects	(Guzmán et al. 2003)
Chlorella vulgaris	Butter cookies	Colorant	(Gouveia et al. 2007)
Chlorella vulgaris, *Haematococcus pluvialis*	Food emulsions	Colorant / Antioxidant effect	(Gouveia et al. 2006)
Dunaliella salina	Powder *Dunaliella*	Antioxidant effect	(Murthy et al. 2005)
Diacronema vlkianum, *Isochrysis galbana*	Pasta products	PUFA enrichment	(Fradique et al. 2013)
Haematococcus pluvialis	Cookies	Colorant, Antioxidant effect	(Hossain et al. 2017)
Isochrysis galbana	Biscuits	Colorant, PUFA enrichment	(Gouveia et al. 2008b)
Nannochloropsis oculata	Cookies and pasta	Colorant, PUFA enrichment	(Babuskin et al. 2014)
	Sterol extract	Anticancer and anti-inflammatory effect	(Sanjeewa et al. 2016)
Porphyridium cruentum	Polysaccharide isolate	Anticancer and immuno-modulation effect	(Gardeva et al. 2009; Sun et al. 2012)
	Crude the sulfolipids	Antioxidant, anti-inflammatory and anti-proliferative effect	(Berge et al. 2002)
	Sulfated polysaccharide	Antiviral effect	(Huleihel et al. 2001)

Spirulina maxima	*Spirulina* supplement	Anti-inflammatory and antioxidant effect	(Gutiérrez-Rebolledo et al. 2015)
Spirulina platensis	Pasta	Protein enrichment / Antioxidant effect	(Zouari et al. 2011; Lemes et al. 2012; Marco et al. 2014)
		Colorant, nutritional enrichment	(Özyurt et al. 2015)
	Chocolate biscuits	Protein enrichment	(Morais et al. 2006)
	Yoghurt	Protein, fat and iron enrichment	(Malik et al. 2013)
		Colorant, protein enrichment	(Arslan and Aksay 2021)
	Manioc cake	Protein enrichment	(Navacchi et al. 2012)
	Gluten-free bread	Colorant, protein enrichment	(Figueira et al. 2011)
	Baby food	Nutritional enrichment	(Sharoba 2014)
	Extruded product	Nutritional enrichment	(Joshi et al. 2014; Morsy et al. 2014)
	Peptide isolates	ACE inhibitory	(Lu et al. 2010)
Spirulina spp.	Shake-type powdered food	Nutritional enrichment	(Santos et al. 2016)
	Spirulina supplement	Immuno-modulation, antioxidant effect, and lipid-lowering effect	(Park and Lee 2016)

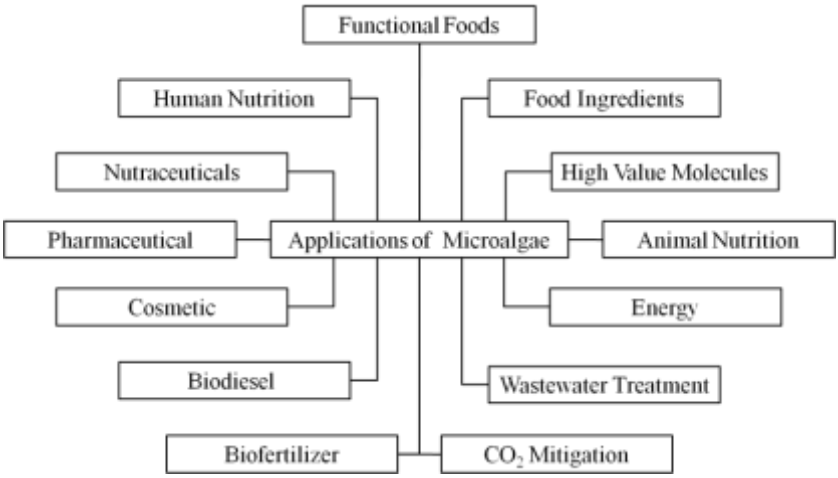

Figure 1. Applications of microalgae

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