ADVANCE CONCEPTS ON NATURAL AND AGRICULTURAL SCIENCES

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Publishing House

(The Licence Number of Publicator: 2014/31220)
TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

It is responsibility of the author to abide by the publishing ethics rules.
Iksad Publications – 2023©
ISBN: 978-625-367-194-5
Cover Design: Ahmet KAZANKAYA
July / 2023
Ankara / Türkiye
Size = 16 x 24 cm

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PREFACE

Outstanding Scientists,

We are delighted to meet the numerous scientists who contributed to the scientific field and the literature in this volume. We would like to give special thanks to the excellent researchers who contributed to this work.

We would like to thank the authors of the insightful studies included in this volume.

Over the years, significant advancements have been made to enhance the quality of life and well-being of humans. Significant developments in recent years have hampered sustainable life and agriculture. In this regard, scientists and chapter authors have made significant contributions to the issues. Humanity must safeguard the ecosystem from greater threats. Small changes in the climate can have a significant impact on the life cycles of living organisms, as well as on the scientific era's overall productivity. Population growth, shifting dietary habits, technological advancements in industry, urbanization, climate change, and chemical disaster are just some of the factors that drive the topics discussed and suggested solutions presented in this book.

This book's primary purpose is not only to discuss current issues in science, but also to provide solutions for problems in natural resources, nanotechnologies, and life sciences, which are primarily driven by population growth, changing dietary patterns, industrial development, urbanization, climate change, and chemical disaster.

This book contains 16 chapters written by different authors. As editors, we would like to thank all our contributors who have made significant contributions to our readers through their knowledge, experience, and suggestions. We hope that this book will raise awareness of new scientific technologies, human benefits, and other living ecosystems, as well as current and emerging scientific research.

Sincerely Yours,

July, 2023

Prof. Dr. Ahmet KAZANKAYA

Assist. Prof. Dr. Mevlüde Alev ATEŞ

CHAPTER 1

MOLECULAR CLOCK ESTIMATIONS IN PLANTS

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INTRODUCTION

Working on the issues the evolution and systematics are related. There should not be any strict line among them. The main contrast is in the purposes of the scientists even if they are geneticist or systematists. Geneticist works on organisms with a perspective to figure out the mechanism and role of evolution. On the other hand systematists is dialed with the evolution and interrelationships of particular organisms(Thorpe 1982)

Since 1960s, scientists has been considering that, the differences within sequences of amino acid were changed practically linear by the time divergence among the species (Margoliash, 1963; Zuckerkandl and Pauling, 1962, 1965; Gaut 1998). This kind of influential observation caused the new term “molecular clock” hypothesis, which maintains the changes at the molecular stages done frequently over time (Zuckerkand and Pauling, 1965). The significant estimation of the molecular clock thought is that the rates of the evolution of molecular times are mostly parallel to divergence of evolutionary lineages (Gaut 1998).

The clock concept is represented by nucleotide changes in DNA sequences, and the clock determines when species diverged. When the evolution period of two or more lineages is the same, a constant rate that determines the number of variations between two samples provides an unambiguous measure of the time until their separation from a common ancestor (Futuyma, 2005). As a result, the phylogeny can be computed by comparing the rate of nucleotide variation between two taxa's sequences.

Generally, the clock hypothesis has significant suggestions to the evolutionary phenomena. Especially using fossil evidence are very useful for estimating divergence times of the species (Gaut 1998). For instance, Sarich and Wilson (1967) discussed that humans diverged from higher animals, primates, nearly 5 million years ago and estimations was fourfold lower than concurrent divergence time estimations based on fossil evidence.

For plants, time estimations by using DNA or protein sequences has appeared to be a significant tool for assuming when the lineages approved in a specific region. The biotic interactions on evolution in floras is the main point

for understanding the evolutionary evidence (Rennner 2005). In higher plants, there is a less attention on molecular clocks because the applications of molecular tools in plant systematics have shed on to a well DNA sequence data for explaining the nucleotide substitution rates. Therefore, in recent studies the molecular clock estimations in plants become an important issue and supported data for taxonomists.

By integrating time restrictions, typically from fossils, with molecular data, new analytical methods can describe how substitution rates change along each branch of a phylogenetic tree. The "relaxed clock" methods could be used on multiple DNA regions that do not have to change at the same rate, and they can also use multiple ancient calibrations at the same time. Since 1995, molecular clocks have been used in at least 100 plant biogeographic studies, and about half of those studies used relaxed clocks. So, the big amount of these show signs that the species moved over long distances. Meta-analyses of studies from the same area can show directional biases caused by things like the direction of the wind or water currents and where and how big the landmasses are in comparison(Renner 2005).

HOW DO THE DATA BUILT FOR ANALYSIS

In the scientific studies, there are some steps for molecular clock-based analysis especially in divergence time events. These are; Firstly; getting genetic distances between 2 species or taxa which is a known time obtaining from fossils. Secondly, the substation rate calculations is important. These are calculating basically by dividing the genetic distance by the known time from fossil records. Lastly, by using the rate to alter genetic distances among taxa of organisms to guess the ages (Renner 2005).

The concept is depicted in Figure 1 in its most basic form. A tree of three species is seen in the illustration. According to the anticipated amount of substitutions per site, the numbers on the branches represent the branch lengths. It should be noted that the tree's branch lengths support the molecular clock idea because the total of the branch lengths from the root to each of the tips is the same (0.4). By imposing this constraint on the branch lengths, one can calculate branch lengths within the framework of the molecular clock hypothesis. The second crucial supposition needed to calculate divergence

times is shown in Figure 1. For the sake of argument, let's say we know the divergence period for at least one of the clades on the tree. Species A and B are assumed to have separated around the 5 mya mark in this hypothetical scenario. We have corrected the atomic clock. The process of calibrating looks like this: In this case, 0.1 substitutions equals 5,000,000 years because A and B descend from the same ancestor who lived 5,000,000 years ago. We may deduce that the ancestor of the three species diverged 20 million years ago using a molecular clock and a calibration (Huelsenbeck and Ronquist, 2005).

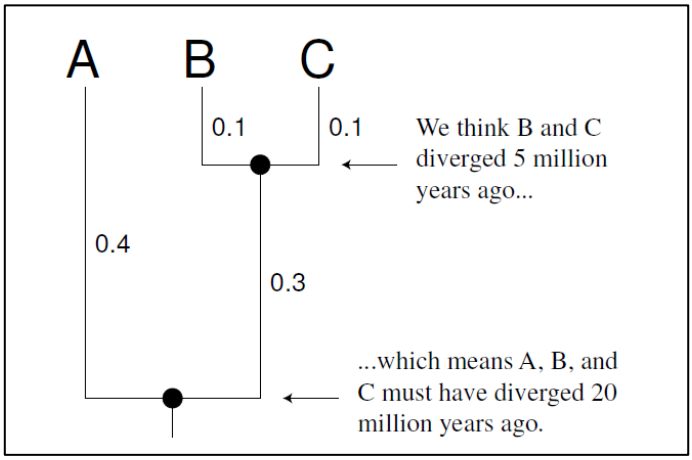

Figure 1: Sample for estimating the divergence times using MC (Huelsenbeck & Ronquist, 2005)

The main point in the studies is to get the DNA sequence data. If your data is clear and all substitution rates can be calculatable, your analysis will be clear and sufficient. Generally, there are many models for substitution models but the main principle is using the genetic distances and substitutions per nucleotide sites or per codon.

The equation for calculating the molecular clock is;

$$Molecular\ clock = \frac{k}{mutation\ rate}$$

Where k is equal to;

$$k = -\left(\frac{3}{4}\right)\ln\left(1 - \frac{4}{3}d\right)$$

d is the number of substitutions per base pair whereas k is the substitutions since divergent time.

The d is calculated as;

$$d = \frac{Variable\ Site}{Total\ Number\ of\ Base\ Pairs\ Sequenced}$$

All of these calculations could be done by hand, but now there are many programs that can do all of these calculations. The programs use nucleotide replacement models to figure out how far apart two sequences are genetically. The amount of base changes between codons is used to calculate how far apart two sequences are. The majority of the time, the number of nucleotide changes increases throughout time. This is because mutation and genetic drift create replacements when changes are neutral (no selection). Substitutions vary across the genome, gene areas, and organisms as a result of selection. They can, however, remain constant within particular genes, sets of codons, or other forms of data partitions, as well as within taxonomic groups (Fleischer, R.C. et al. 1998; Hwang, D.G. & Green, P. 2004). This indicates that variations may build up in local datasets (combinations of taxa and gene areas) at a steady, clocklike rate. Nonetheless, it is patently evident that there is not enough time to go around (Sanderson, M.J. 1998; Cho, Y. et al. 2004).

Studies of plants have shown that the rate of nucleotide substitution between groups is very different (Klak, C. et al. 2004). Estimating the rate of heterogeneity is difficult since the power of relative rate tests or likelihood ratio tests is proportional to the amount of signal in the data. These tests are less effective when there is little variance between sequences or when the chosen sequences are short (Bromham, L. et al. 2000). The "clock assumption," that the amount of substitutions between sister groups does not statistically differ, can be met in small datasets. It is difficult to draw firm conclusions because to the large variances (uncertainty) in estimated branch lengths (and dates) (Baldwin, B.G. and Sanderson, M.J. 1998; Thorne, J.L. et al. 1998; Korber, B. et al. 2000; Sanderson, M.J. 2002, 2003; Renner 2005).

The findings of relative-rate experiments are not always made public when academics seek to estimate divergence times. The second strategy involves searching for molecules that either pass or are more likely to pass a relative-rate test. Either a formal procedure (gather the sequences, run the tests, and revert to the constant-rate Poisson model if you don't like it; Kumar and Hedges, 1998) or an informal one (introns are considered more "neutral" than exons, consequently, introns should be analyzed first, as they have a higher probability of passing a relative-rate test (even if such a test is never run). You should embark on a "molecular shopping spree" if you think the constant-rate Poisson model applies to some molecules. If these substances do indeed exist, all the scientist has to do is track them out, which might require some retail therapy (Cutler, 2000).

SOME FREQUENTLY USED PROGRAMS FOR MC ANALYSIS

Since Zuckerkandl and Pauling first came up with the idea of molecular dating in the 1960s (Zuckerkandl & Pauling 1962, 1965; Kumar 2005; Kumar & Hedges 2016), many different ways have been found to measure timetrees. For instance, modern techniques don't count on the idea that evolution occurs at a constant rate (i.e., that there is a rigid molecular clock). Advanced approaches based on Bayesian statistics have been demonstrated to be particularly useful for forecasting divergence times (dos Reis et al., 2016). These techniques factor in the uncertainty introduced by using calibration data derived from the fossil record, as well as changes in rate between lineages. MCMC modeling Tree (Yang 2007) and BEAST (Bouckaert et al. 2014) programpackage are the two Bayesian molecular timing methods that are used most often in software. But because genetic data has grown so much in the last few decades, it is often not possible to use Bayesian methods because they take a long time to compute (Akerborg et al. 2008; Battistuzzi et al. 2011; Ho 2014; Mello et al. 2017). Timetree inference (Tamura et al., 2012; Ho, 2014; To, et al., 2016) and the resulting scientific knowledge can be sped up with the use of fast-dating approaches (Mello, 2018).

MEGA (Molecular Evolutionary Genetic Analysis)

For over 25 years, scientists have relied on MEGA (Molecular Evolutionary Genetics Analysis), a program that has undergone constant development and improvement (Kumar et al., 1994, 2018). With MEGA, users are able to perform phylogenetic and molecular evolution analyses in the same setting. This makes MEGA very accessible, allowing even inexperienced researchers to do sophisticated evolutionary analyses. In addition, the RelTime technique will receive data on the ages of calibrations from the TimeTree online resource (Hedges et al., 2006; Kumar et al., 2017), which would be utilised to translate relative node ages into absolute timings. This is due to the fact that calendar years are the only units of time for which the dating of species divergences can be reliably applied (Mello 2018)(Figure 1).

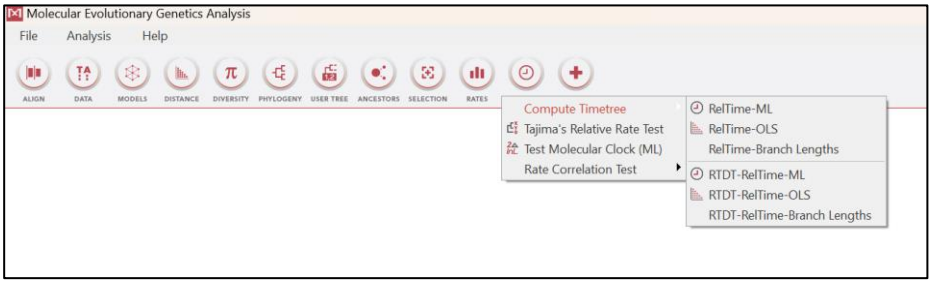

Figure 2: Screen shots of the program interface of the Clock wizard.

BEAST (Bayesian Evolutionary Analysis Sampling Trees)

Multiple-platform BEAST employs Markov chain Monte Carlo to perform Bayesian analysis of DNA sequences. It's all about inferring phylogenies with firm or loose roots based on molecular clock models. But the clock models in BEAST, which are talked about on this page, can also be used to look at sequences that happen at the same time. Several models have been developed and implemented in BEAST to relax the rigid assumption of a molecular clock (Drummond et al 2012). Model selection has been used in Bayesian phylogenetics before for substitution models, nucleotide change rates, and site heterotachy (Huelsenbeck et al. 2004, Gowri-Shankar and Rattray 2007, Suchard et al. 2001). A posterior sample of the likelihood is provided by software programmes like BEAST (Drummond and Rambaut 2007). It can be used to calculate the marginal likelihood by finding the harmonic mean of the

posterior sample. In this method, the target distribution is the prior distribution, and the importance distribution is the posterior distribution, hence the approximation of the marginal likelihood is based on importance sampling (Newton and Raftery 1994). According to Beerli and Palczewski (2010), this approximation frequently yields dreadfully inaccurate estimates of the marginal likelihood since the posterior distribution frequently isn't an appropriate important distribution for the prior distribution. The posterior typically has less variation than the prior, especially when there is a lot of data (Li and Drummond 2012)(Figure 2).

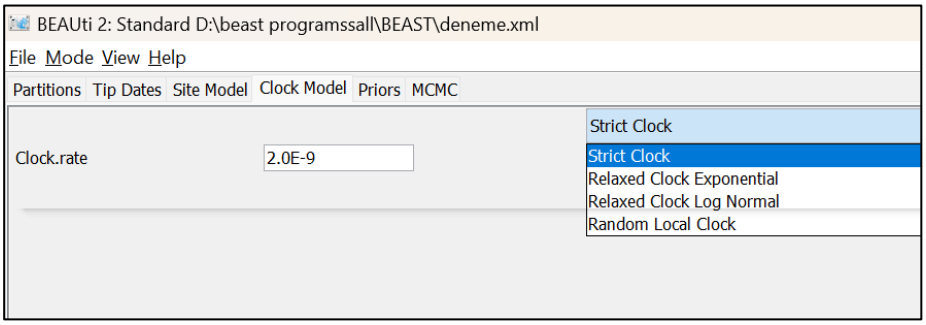

Figure 3: Screen shots of BEUti interface of BEAST program package on Clock Models.

MrBayes (BAYESIAN INFERENCE OF PHYLOGENETIC TREES)

A command-driven programme for Bayesian phylogenetic inference, MrBayes 3 has been fully designed and organised. A strong framework for phylogenetic inference under mixed models that takes data heterogeneity into account is the new program's distinguishing feature. When working with composite data sets, this framework will assist the user in specifying mixed models and utilizing the computing effectiveness of Bayesian MCMC(Markov chain Monte Carlo) analysis (Figure 4)((Huelsenbeck and Ronquist, 2001).

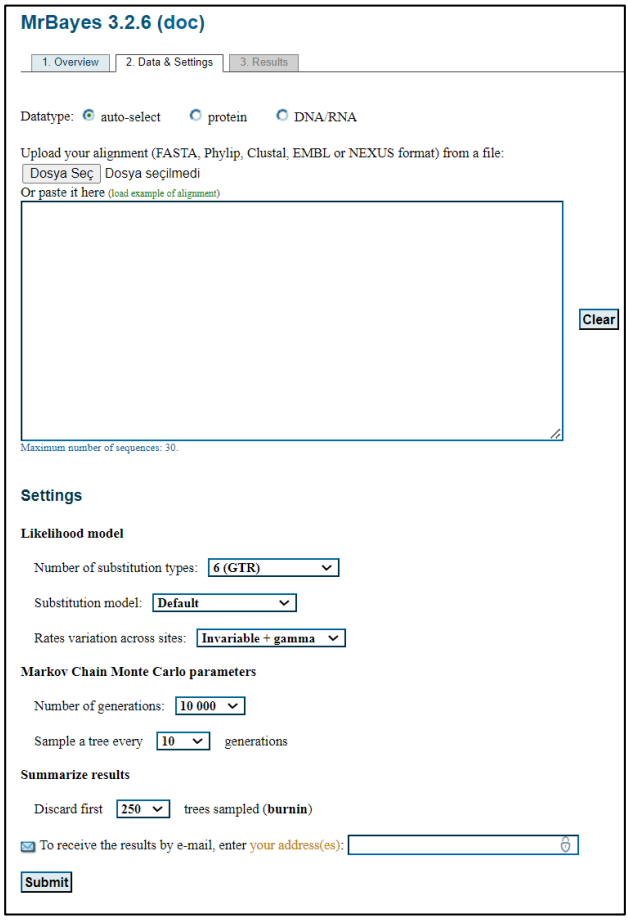

Figure 4: Screen shot of the MrBayes program interface from the web site " http://www.phylogeny.fr/one_task.cgi?task_type=mrbayes"

SOME EXAMPLE STUDIES RELATED WITH MOLECULAR CLOCK ANALYSIS IN PLANTS

In many molecular phylogenetic relationship analysis, MC analysis was additionally done to indicate the divergence times of the taxa. For example Ates et al (2021) were studied ob *Gundelia* genus and in the study they also calculated the divergence times of the taxa. They used a fossil record for calibration point and the speciation times were calculated by using BEAST program with Strict Clock model with a MCMC runs. In the phylogenetic tree the branch times were shown with Fig Tree program. According to estimates of the molecular clock using the ITS region of nrDNA, *G. anatolica* diverged first (21.43 MYA). The remaining species diverged sometime around 17.22 MYA. According to ITS data, *G. vitekii* (Pleistocene epoch, 1.8 MYA) appears to be the species in the genus that has diverged most recently. (Figure 5).

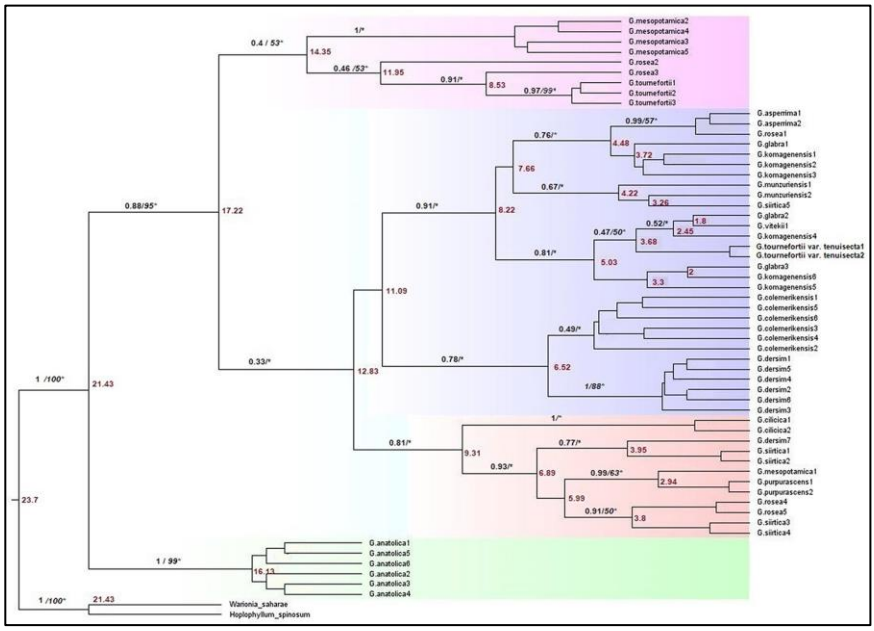

Figure 5: The phylogenetic tree of *Gundelia* sp based on ITS region and molecular times were indicated next to the branches (Ates et al 2021).

Another study was done by Heenan and McGlone (2019). BEAST program with MCMC runs was used for the analysis and New Zealand's vascular flora, comprising 411 extant genera and 477 lineages, has their colonisation history reconstructed using data from the plastid rbcL gene. The Eocene-Oligocene extinction, which occurred around 33.9 Ma, appears to have been pivotal in the development of modern flora since so few lineages, especially ferns and conifers, predate it. Most surviving angiosperm families originated during the mass extinction that occurred during the Eocene-Oligocene transition, as indicated by tree-ring dating. Therefore, the majority of New Zealand's vascular flora likely developed sometime during the late Miocene or later, with some modest input from the Eocene, Oligocene, and early to mid Miocene. (Figure 6).

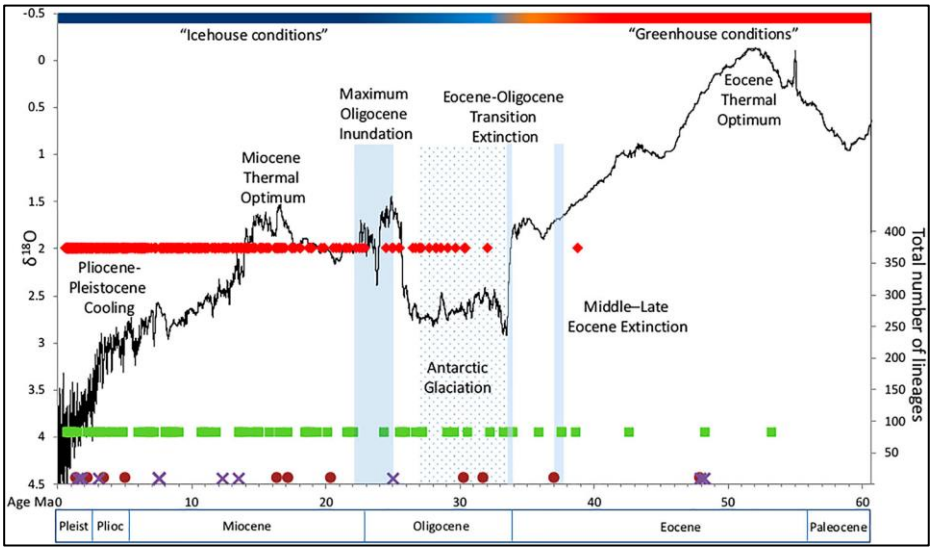

Figure 6: Angiosperm, fern, conifer, and lycopod distribution across the Cenozoic era (crown dates)(Heenan and McGlone, 2019).

Also Acar et al(2022) were studied on Salix genus in Turkiye and they used BEAST program for calculating the time divergences. Based on pooled cpDNA data, two main clades of the genus Salix in Turkey shared the same biogeography and diverged during the Oligocene.Although the origin and split

dating of Turkey's Salix subgenera date to the Eocene (45.1 Mya), the Pliocene is when the majority of Turkey's native Salix species first appeared (Figure 7).

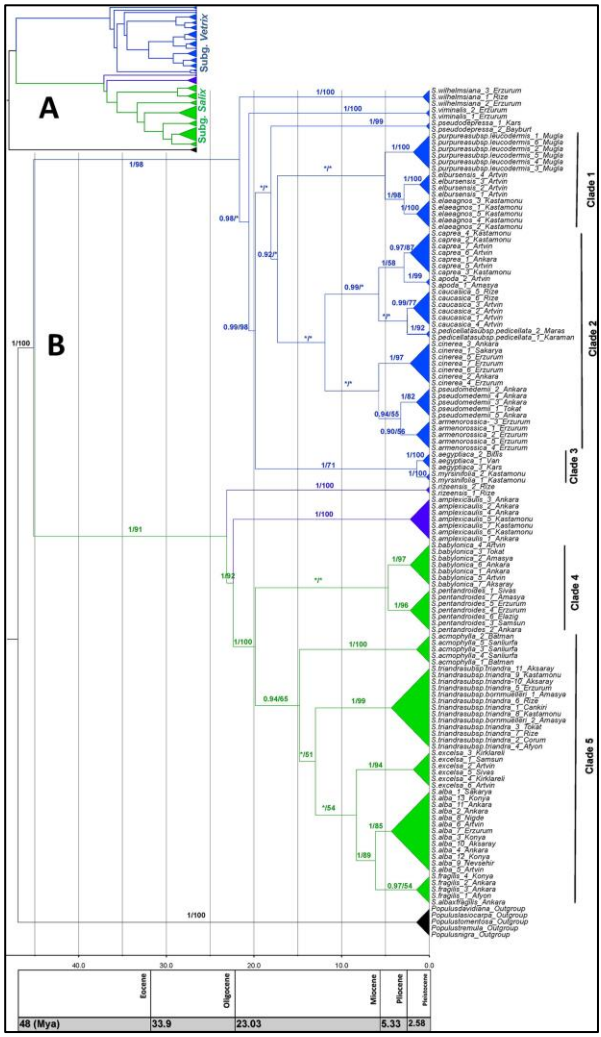

Figure 7: Bayesian molecular dating analysis based on cpDNA sequences (Acar et al, 2022).

CONCLUSION

Molecular clock analysis is an alternative to morphological fossil records, which are used for the estimation of species divergence due to the data type. Nowadays, molecular data are mostly used to support morphological systematic studies for speciation. Taxonomists also try to explore speciation and evolution events by using combinations of both data. Additionally, using molecular data for estimating time divergence between species became an important issue. Therefore, some complicated issues arise in the analysis. Natural hybridization, genetic drifts, founder effects, and bottleneck effects make the evolutionary steps harder; therefore, calculating the speciation times becomes more difficult every day. In that situation, bioinformatic calculations and parameters try to solve these problems. Many different computer programs take over the handling of universal formulas with their different parameters. According to your research, you can easily choose suitable parameters for your species or taxa, and you can minimise the deflections in your calculations.

The molecular clock analysis in plants is the most significant estimation in plant molecular relationship studies these days, and it will become more important in future studies to understand plant biodiversification and indicate how it is reflected in evolutionary times. Because evolution is still continuing...

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CHAPTER 2

INVESTIGATION OF RELATED RELATIONS IN MOLECULAR PERSPECTIVE OF NATURAL ROSEHIP (*Rosa canina* L.) GENOTYPES IN VAN PROVINCE

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INTRODUCTION

Rosehip, which belongs to Rosaceae family, is a perennial plant belonging to Rosaceae family Rosaideae subfamily Rosa genus. It is also known by names such as rose nose, rose apple, wild rose among the people. The plant is a thorny shrub whose canopy height varies between 1-3 m depending on the species, and it is resistant to environmental conditions. Before the fruit is formed, fragrant pink and white flowers occur. With the fleshing of the axes of these flowers, the fruit formation process begins. The fruit, which is mostly cranberry-like, is hairy inside and contains many hard seeds. Since it can grow in all kinds of environmental conditions, it can be seen in valleys, roadsides, orchards fences and cemeteries (Yamankaradeniz 1983; Ozrenk et al., 2012). Rosehip is a valuable raw material preferred in the food and pharmaceutical industry in many European countries such as Germany, Russia, Turkic Republics, Switzerland, Poland and Finland (Yamankaradeniz 1983, Dogan and Kazankaya, 2006). In these countries, rosehip is mainly used in food industries such as baby food, fruit juice, fruit jelly and tea. In the Turkish food industry, however, the commercial use of rosehip is quite new, and rosehip tea and recently rosehip drink and nectar are also produced, along with pulp, nectar and marmalade production from rosehip fruit. In addition to its commercially known products, there are many products that are produced and consumed locally (Dolek, 2013). Rosehip is actually a well-known and consumed fruit in Anatolia and is grown all over Turkey, especially around Tokat and Gümüşhane in the Black Sea region. Rosehip is a kind of fruit that grows wild in Türkiye' s flora, but its importance is better understood day by day. Parallel to this importance, some selection (selection I and/or selection II) studies have been or are being carried out in our regions where rosehip populations are concentrated (Ercisli, 1996; Gunes, 1997; Misirli et al., 1999; Kazankaya et al., 1999; Kizilci, 2005; Dolek, 2008). Registration studies of variety candidate and promising genotypes have reached a certain stage by selection studies carried out in Tokat and Erzincan provinces. Although cultivation studies are still in the beginning stage, rosehip is an important raw material of the food industry in our region. It may be possible to harvested and process more rosehip fruits by the spread of registered varieties or the establishment of their orchards.

Rose (Rosa sp.) species, which are mostly deciduous or rarely evergreen shrub-shaped woody plants, are included in the following systematic classification (Ansin 1996; Dogan et al., 2006).

Section **:** Spermatophyta
Subsection **:** Angiospermae
Taxon **:** Magnoliatae
Subtaxon **:** Rosidae
Ordo **:** Rosalae
Family **:** Rosaceae
Subfamily **:** *Rosaoidae*
Genus **:** *Rosa*

In Türkiye, the molecular studies on rosehips are limited. In this study, it is aimed to reveal the identification at the molecular level in rosehip fruit.

The rapid development of the molecular markers has allowed the rapid advancement of biotechnology. The use of the molecular markers is the first step of this process; therefore it is of great importance. With the recombinant DNA technology, the desired fragments were added to or removed from the DNA; this allowed new products to be obtained. Instead of the classical breeding studies based on Mendelian genetics, the more precise results were obtained as a result of the discovery of molecular markers, the determination of the locations of the desired characteristics and the direct transfer of the found genes, and the inheritance of unwanted genes, which is the disadvantage of classical breeding, was prevented in this way.

MATERIAL AND METHODS

Material

As plant material was used 25 genotypes of *Rosa canina* L. species and one genotype of *Rosa foetida* L. species grown in the same field conditions in the Van region. In the study, the kinship relationships of 21 Rosa canina L. genotypes and Rosa foetida L. species were determined in terms of total phenolic compounds, genotype characteristics and molecular characteristics.

Methods

Within the scope of this study, total phenolic and molecular characterization studies were carried out in some rosehip cultivars.

Molecular Characterization Studies

DNA extraction

DNA isolation from the rosehip leaf samples taken from the Iskele district of Van province was carried out in the Molecular Biology laboratory of the IYTE Department of Genetic Engineering. In order to carry out DNA isolations, the fresh leaves with relatively low levels of polyphenols and other DNA-contaminating compounds were taken. The samples taken for DNA isolation were wrapped in aluminum foil and stored at -85 oC. CTAB DNA isolation protocol was used for DNA isolation. Isolated DNAs were stored at -20 oC.

The used materials

- ✓ 2 ml eppendorf tubes
- ✓ Tweezers
- ✓ Centrifuge device
- ✓ PCR devices (ABI)
- ✓ DNA analysis system (Beckman CEQ8800)
- ✓ Liquithandlingsystem
- ✓ Electrophoresis and power supplies, gel imaging system
- ✓ Extraction buffer: 100 mM Tris-HCl (pH 8.0), 1,5 mM NaCl, 50 mM EDTA (pH 8.0), % 0.5 mercaptoethanol, %1,5 (w/v) CTAB
- ✓ Chloroform-isoamyl alcohol (24% : 1)
- ✓ Phenol-chloroform-isoamyl alcohol (25:24:1)
- ✓ TE buffer (pH 8.0): 10 mM Tris-HCl, 1 mM EDTA
- ✓ 10 mg/mL RNase A (free of DNase)
- ✓ Ethanol
- ✓ 5 M NaCl
- ✓ 70 % ethanol

DNA isolation protocol

1- Small pieces from the ends of the leaf are taken and adds to 2 ml eppendorf tubes.
2- 700 µL of CTAB extraction buffer in each tube are added (There are 693 µL of CTAB, 7 µL of P-mercaptoethanol in 700µL of CTAB extraction buffer).
3- Metal balls are added to Epandorf tubes.
4- It is placed in the TissueLyser II (QIAGEN) device and left for 10 minutes (our aim is to separate the DNA in a shorter time).
5- After the separation process is finished, it is incubated at 60 °C for 60 minutes. During this process, the tubes are continued to be mixed from time to time.
6- At the end of the incubation, 550 µL of chloroform: isoamylalcohol (25:24:1) solution is added to each tube, shaken in a mixer for 15 minutes and centrifuged at 14000 rpm for 10 minutes.
7- 200 µL of cold (-20 °C) isopropanol is added to the new clean Eppendorf tubes and after centrifugation, the supernatant part in the tubes is carefully drawn with a pipette and put into the tubes containing isopropanol.
8- The samples are kept at -20 °C for 1 hour.
9- After one hour, the tubes are removed from -20 °C and centrifuged at 10000 rpm for 10 minutes.
10- At the end of the centrifugation, the supernatant part is carefully poured, the tubes are turned upside down to dry the pellet remaining at the bottom of the tubes.
11- 100 µL of TE buffer is put into the tubes and the pellet is thawed, and 1 µL of RNase is added to each tube immediately after this process.
12- The tubes are mixed gently, kept at room temperature for 15 minutes.
13- 100 µL (-20 °C) cold 70% ethanol is added to each tube.
14- Tubes are kept at -20 °C for 1 hour by gently mixing.
15- After one hour, the tubes are removed from -20 °C and centrifuged at 10000 rpm for 10 minutes.
16- The tubes are kept as open for 3-4 minutes in the refrigerator at 60 °C.
17- 100 µL of dH2O was added into the tubes, kept in the refrigerator at 4°C for 1-2 hours.
18- The samples are stored at -20 °C to be used in PCR

SRAP Analysis

SRAP analyzes were performed according to Li and Quiros (2001). The different combinations of 10 forward and 9 reverse primers were used in the study. The information on the primers used in the study are given in Table 1 and 2. 17 Forward and Reverse primers were used. They were Me11/Em17, Me13/Em17, Me5/Em17, Me6/Em17, Me14/Em17, Me7/Em10, Me14/Em13, Me12/Em14, Me7/Em14, Me3/Em13, Me2/ 17 SRAP primer combinations Em13, Me6/Em4, Me7/Em12, Me8/Em12, Me3/Em11, Me14/Em2, Me6/Em6.

Table 1. The reverse primers used in SRAP analysis

Primers	Reverse Primer	Base Length
Em 2	GACTGCGTACGAATTTGC	18
Em 4	GACTGCGTACGAATTTGA	18
Em 6	GACTGCGTACGAATTTGA	18
Em 10	GACTGCGTACGAATTTAG	18
Em 11	GACTGCGTACGAATTTCG	18
Em12	GACTGCGTACGAATTGTC	18
Em 13	GACTGCGTACGAATTGGT	18
Em 14	GACTGCGTACGAATTCAG	18
Em 17	GACTGCGTACGAATTCCA	18

Table 2. The forward primers used in the srap analysis

Primers	Forward Primer	Base Length
Me 2	TGAGTCCAAACCGGAGC	17
Me 3	TGAGTCCAAACCGGAAT	17
Me 5	TGAGTCCAAACCGGAAG	17
Me 6	TGAGTCCAAACCGGTAG	17
Me 7	TGAGTCCAAACCGGTTG	17
Me 8	TGAGTCCAAACCGGTGT	17
Me 11	TGAGTCCAAACCGGACA	17
Me 12	TGAGTCCAAACCGGGAT	17
Me 13	TGAGTCCAAACCGGTAA	17
Me 14	TGAGTCCAAACCGGGCT	17

SRAP PCR protocol: The SRAP PCR protocol was modified and applied as follows (Table 3).

Table 3. SRAP PCR protocol

Chemicals	Amount used for each Sample (µl)
ddH_2O	9.25
10X	2.0
$MgCl_2$	2.0
Taq DNA Polimeraz (1 unit)	1.0
EM	2.0
ME	2.0
dNTP	0.75
DNA (50 ns)	1.0
Total volume	**20 µl**

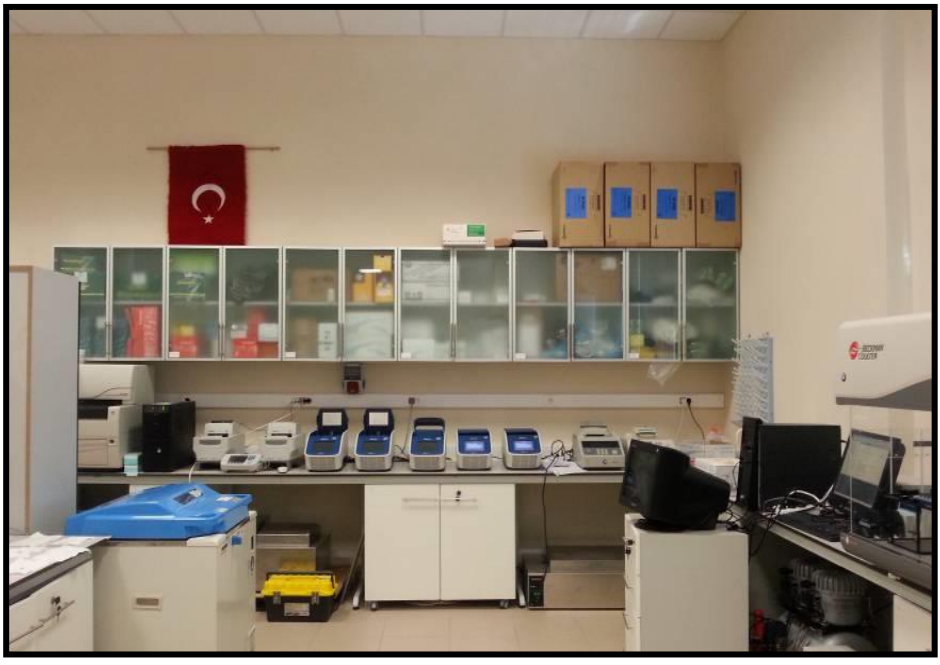


Figure 1. PCR devices

SRAP Agarose Gel Electrophoresis: 5 µl of the obtained PCR products were taken, 2 µl of loading buffer was added, and 1XTAE (Trizma Base, Glacial Acetic Acid, EDTA (Na2.EDTA.H2O) buffer was added in a 2% agarose gel and loaded under 120 volts electric current for 1.5 hours.

Photographs were taken under UV light stained with 0.1% ethidium bromide after electrophoresis (Figure 1). A 100 bp marker was used to determine the size of the obtained DNA bands. In these DNAs, the band sizes are formed as 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp, respectively.

The Determination of DNA Quality and Quantity: The DNAs obtained as a result of DNA isolation were diluted 1:50 in TE solution and the concentration and purity of the DNAs were determined by reading the wavelengths at 260 and 280 nm with the help of an instrument called spectrophotometer (Nanodrop).

The Determination of Polymorphism Rates of Primers: The polymorphism rate of SRAP primers used in the study was found according to the formula below.

Polymorphism Rate (%) = (Number of polymorphic bands / Total number of bands) x100

The Similarity Indexes and The Creating of The Dendograms: To analyze the affinity relationship, SRAP markers were applied to the identified rosehip population and conducted in agarose gel electrophoresis. Polymorphic bands were scored and the scoring file was analyzed in DarWin program to determine the diversity within the population. PIC values were calculated for each marker analyzed by using the Dice method, and a tree was drawn by using the UnweigtedNeigbourjoin method.

RESULTS and DISCUSSION

In order to determine the genetic relationships between rosehip varieties, which is a berry fruit, 22 genotypes determined from naturally grown rosehip varieties in Tusba (Van) district were studied. Research findings were investigated at the molecular level by using total phenolic compounds, color determination studies and 24 SRAP primers.

Molecular Studies

SRAP Analysis Results

24 SRAP primer combinations were tried and 17 of them showed the desired amplification. SRAP primer combinations with no progression (Me1/Em17, Me3/Em5, Me3/Em17, Me5/Em15, Me9/Em17, Me12/Em10, Me14/Em5) were not evaluated. The presence of healthy readable bands obtained as a result of PCR was evaluated as (1) and absence (0). The total number of bands, the number of monomorphic bands, the number of polymorphic bands, the polymorphism ratio and PIC values obtained as a result of this evaluation are presented in Table 4.2.

Table 4. 17 SRAP primer combinations used in Rosehip cultivars

Primers	TBN	BW (bp)	MBN	PBN	PP (%)	PIC
ME2/EM13	3	100-500	1	2	66.66	0.4943
ME3/EM11	2	100-400	1	1	50	0.2355
ME3/EM13	3	100-400	1	2	66.66	0.2004
ME5/EM17	2	100-200	0	2	100	0.4917
ME6/EM4	2	100-200	1	1	50	0.1652
ME6/EM6	2	400-500	0	2	100	0.3161
ME6/EM17	2	100-500	0	2	100	0.4769
ME7/EM10	3	100-900	1	2	66.66	0.4250
ME7/EM12	1	300	0	1	100	0.1107
ME7/EM14	2	100-400	1	1	50	0.1800
ME8/EM12	3	200-500	1	2	66.66	0.4958
ME11/EM17	2	100-300	0	2	100	0.4400
ME12/EM14	4	100-700	1	3	75.0	0.4393
ME13/EM17	2	100-200	0	2	100	0.4338
ME14/EM2	2	100-400	1	1	50	0.0867
ME14/EM13	4	100-500	1	3	75.0	0.2782
ME14/EM17	1	300	0	1	100	0.4081
Total	**40**	**100-900**	**10**	**30**	**Ort: 75.0**	

TBN: Total Bands Number, **BW (bp):** Bandwidith, **MBN:** Monomorphic Bands Number **PBN:** Polymorphic Bands Number, **PP (%):** Polymorphism Percentage, **PIC:** Polymorphic Information Content

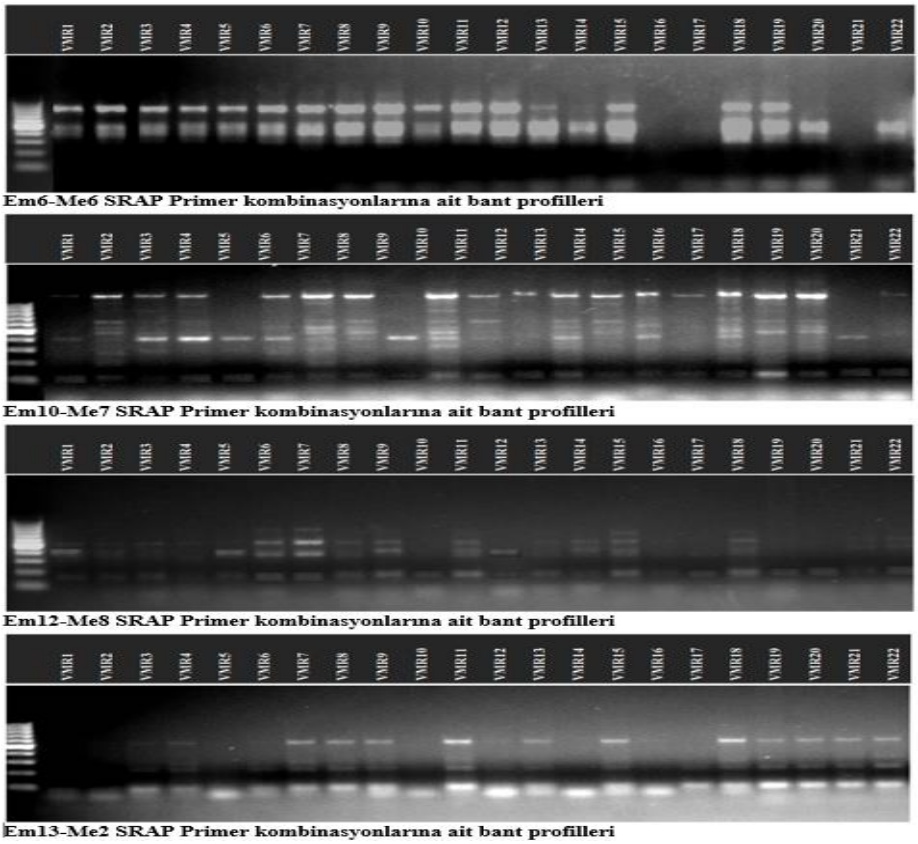


Figure 2. Band profiles of primer combinations

In the study conducted with 17 SRAP primer combinations; A total of 40 bands were obtained, of which 10 were monomorphic and 30 were polymorphic. In line with these results, it was determined that the polymorphism rate was 75.00% and the obtained band gap was between 100-900 bp. Considering the number of the bands obtained, Me14/Em17 and Me7/Em12 primer combinations had the lowest bands number (1 band/primer), but Me12/E1m4, Me14/Em13 primer combinations had the highest (4 bands/primer). It was determined that primer combinations; Me7/Em14, Me6/Em4, Me3/Em11, Me14/Em2 had the lowest polymorphism value with 50% polymorphism in the presence of 1 polymorphic and 1 monomorphic band. The highest polymorphism was observed in primer combinations such as Me11/Em17, Me13/Em17, Me5/Em17, Me6/Em17 and Me14/Em17, Me7/Em12, Me6/Em6 (100%) (Figure 2).

The Dendogram Evaluation Obtained from SRAP Analysis

In the dendogram obtained by SRAP analysis, the rosehip genotypes were divided into three main groups: A, B and C dendogram groups (Figure 3). There were 2 subsets in set A and, these sets were named as A1 and A2. A set consisted of VMR1, VMR2, VMR4, VMR6, VMR7, VMR8, VMR9, VMR11, VMR13, VMR15, VMR18 and VMR21 genotypes. A1 set occurred VMR1, VMR6, VMR7, VMR8, VMR11, VMR15, VMR18 and VMR21 genotypes; A2 set occurred VMR4 and VMR13 genotypes. It was seen that the closest genotypes to each other in the A1 set were VMR11 and VMR18 genotypes that were 0.0948 similarity ratio between. VMR1-VMR15 followed these genotypes with a value of 0.1110 in terms of similarity ratio. VMR6-VMR21 genotypes were found to be the most distant genotypes with a similarity ratio of 0.2755. The VMR4-VMR13 genotypes in the A2 set were similar to each other, with a similarity ratio of 0.1064. In general, it was determined that VMR13 and VMR15 genotypes were close to each other with 0.1018 similarity ratios, and VMR4-VMR21 genotypes were the most distant genotypes with 0.2945 similarity ratios in A set. There were 2 subsets in set B and these sets were named as B1 and B2. The VMR3, VMR5, VMR10, VMR16, VMR17, VMR19 and VMR20 genotypes in the B set constitute the B1 subset. The B2 set consists of VMR12, VMR14 and VMR22 genotypes. B set was different from A set because the similarity ratios were further apart. The VMR19 and VMR20 genotypes in the B1 set were the closest genotypes with a similarity ratio of 0.1589. These genotypes were followed by VMR 5 and VMR 19 genotypes with a similarity ratio of 0.1709. VMR3-VMR17 genotypes were the most distant genotypes in the B1 set with a similarity ratio of 0.5697. It was determined that the VMR12 and VMR14 genotypes in the B2 set were the closest genotypes to each other with a similarity ratio of 0.1892. VMR14 and VMR22 genotypes were the most distant genotypes with a similarity ratio of 0.2604. In general, we can state that in B set, VMR17 and VMR22 are the most distant genotypes with a similarity ratio of 0.7113 in terms of genetic closeness. In group B, it was observed that the VMR2 and VMR3 genotypes got the closest value with a similarity ratio of 0.1741 in terms of genetic similarity. There was 1 genotype in C set, VMR9 genotype was included. A set consisted of 10

genotypes in the population; B set occurred 11 genotypes. It is seen that the smallest set was C set, which consists of 1 individual. Considering A and B sets, it is seen that the individuals in these two sets represent the individuals who are genetically farthest and closest to each other as a result of their mutual comparisons.

The different identification methods have been used in genetic identification studies on rosehips. Lihai et al. (2002) in their study; the rose genetic diversity analysis was analyzed by using the RAPD technique. They found 65 RAPD bands by using 12 RAPD primers on 30 genotypes. One of them was transformed into a SKAR marker. The kinship relationships of 30 genotypes were arranged according to the phylogenetic tree made by the UPGMA method. In the SSR study on Rosa x hybrida (Hong et al., 2013), it was 69 evaluated the suitability of simple sequence repeat markers for 69 Rosa x hybrida diversity identification. 112 SSR primers had been used in the study. In the obtained bands, a series of 43 primer pairs showed 12 types of polymorphism. Twenty-two primer pairs out of 43 primer pairs were found to have high levels of polymorphism and reproducibility. Genotypes were analyzed according to 22 SSR primers for 69 kinds of genetic associations. 114 polymorphic bands were obtained. The mean polymorphism information content (PIC) ranged from 0.211 to 0.813. The optimum PIC value was found to be 0.621. Cluster analysis of 69 rosehips varieties revealed the status of each genotype with these SSR marker sets. SSR markers report that they offer a range of practical applications in the identification of rose varieties and its usability.

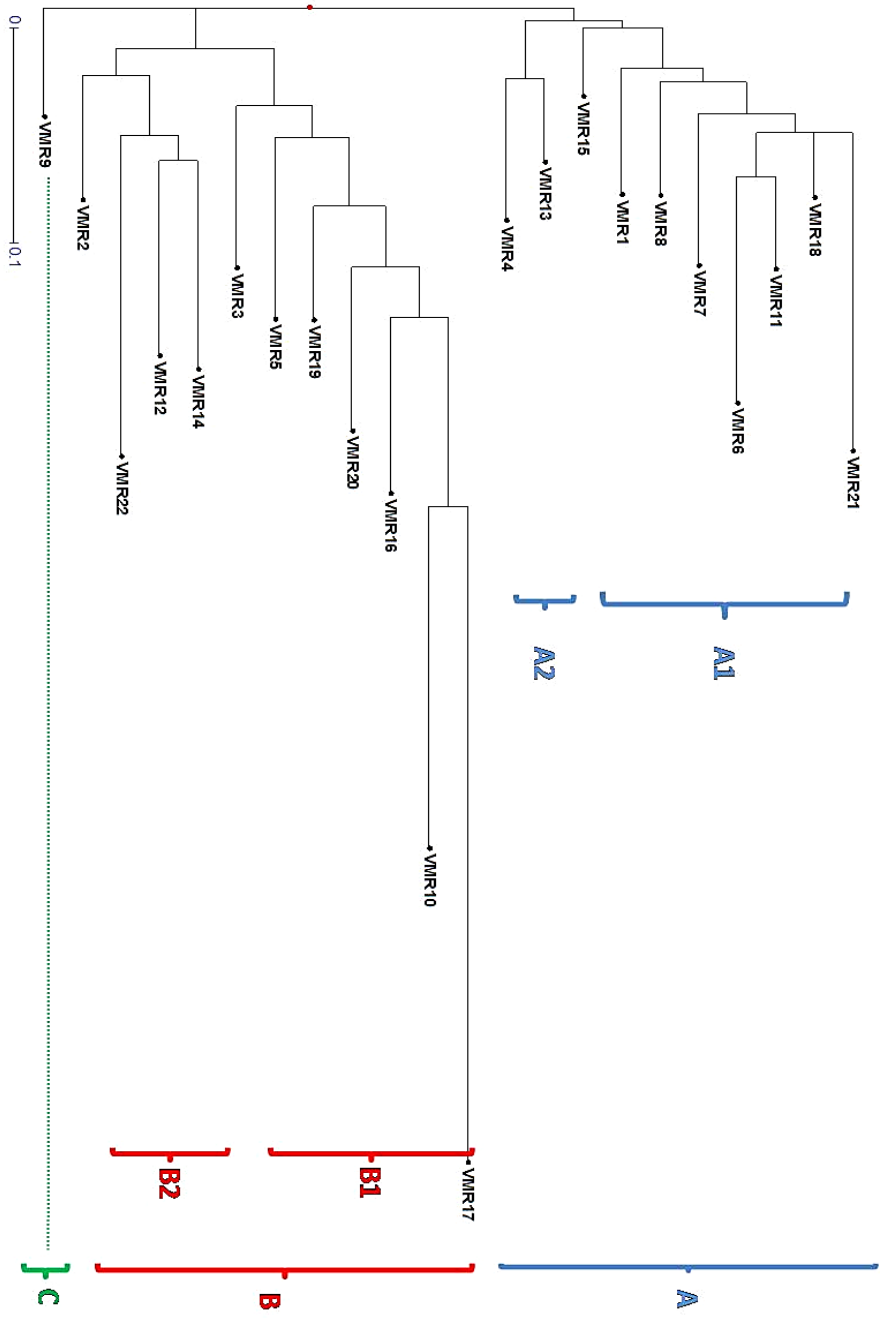
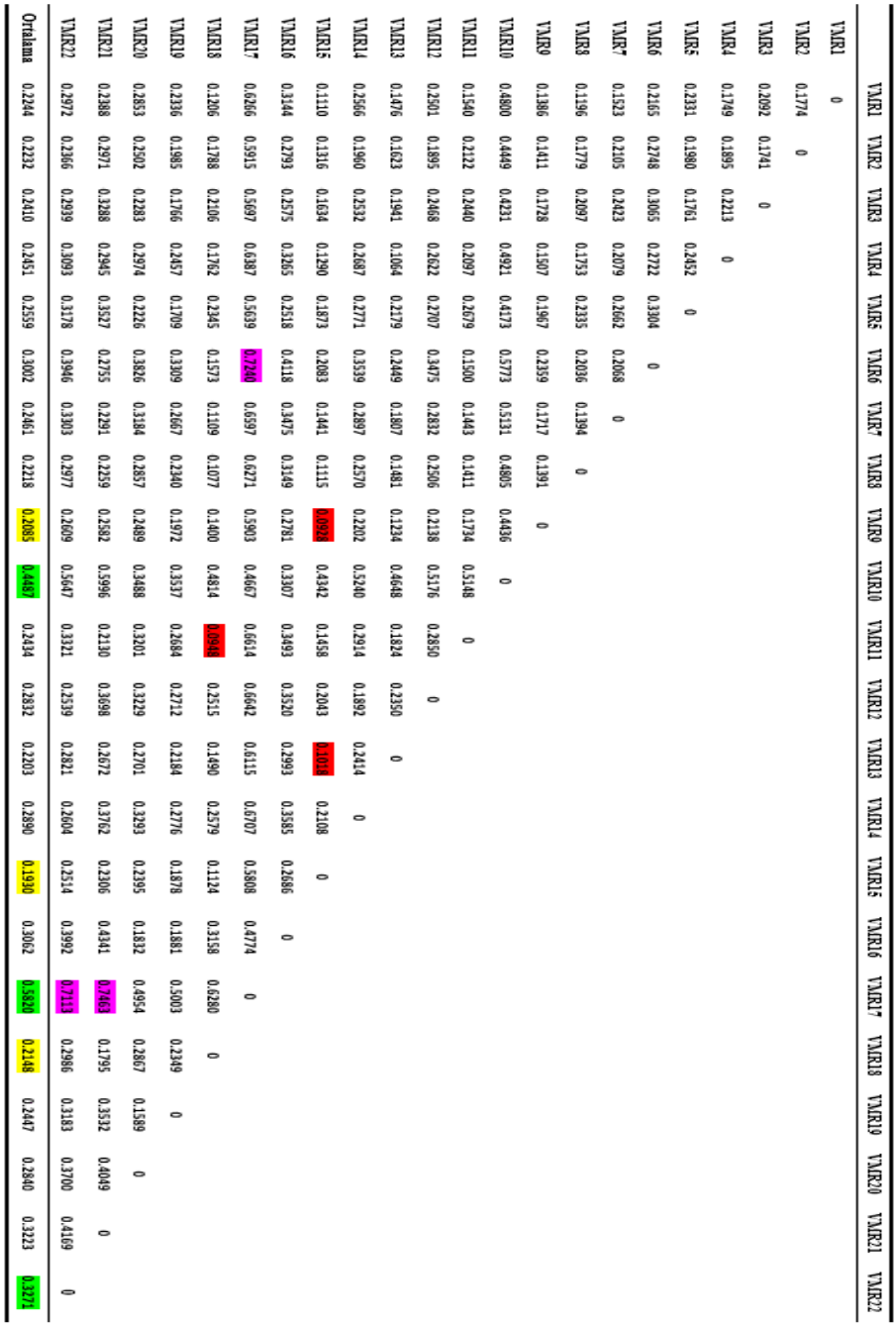


Figure 3. The Dendrogram obtained by SRAP technique in rosehip genotypes



	YAER1	YAER2	YAER3	YAER4	YAER5	YAER6	YAER7	YAER8	YAER9	YAER10	YAER11	YAER12	YAER13	YAER14	YAER15	YAER16	YAER17	YAER18	YAER19	YAER20	YAER21	YAER22
YAER1	0																					
YAER2	0.1774	0																				
YAER3	0.2092	0.1741	0																			
YAER4	0.1749	0.1895	0.2213	0																		
YAER5	0.2331	0.1980	0.1761	0.2452	0																	
YAER6	0.2165	0.2748	0.3065	0.2722	0.3304	0																
YAER7	0.1523	0.2105	0.2423	0.2079	0.2662	0.2068	0															
YAER8	0.1196	0.1779	0.2097	0.1753	0.2335	0.2036	0.1394	0														
YAER9	0.1386	0.1411	0.1728	0.1507	0.1967	0.2359	0.1717	0.1391	0													
YAER10	0.4800	0.4449	0.4231	0.4921	0.4173	0.5773	0.5131	0.4805	0.4436	0												
YAER11	0.1540	0.2122	0.2440	0.2097	0.2679	0.1500	0.1443	0.1411	0.1734	0.5148	0											
YAER12	0.2501	0.1895	0.2468	0.2622	0.2707	0.3475	0.2832	0.2506	0.2138	0.5176	0.2850	0										
YAER13	0.1476	0.1623	0.1941	0.1064	0.2179	0.2449	0.1807	0.1481	0.1234	0.4648	0.1824	0.2350	0									
YAER14	0.2566	0.1960	0.2532	0.2687	0.2771	0.3539	0.2897	0.2570	0.2202	0.5240	0.2914	0.1892	0.2414	0								
YAER15	0.1110	0.1316	0.1634	0.1290	0.1873	0.2083	0.1441	0.1115	0.0924	0.4342	0.1458	0.2043	0.1018	0.2108	0							
YAER16	0.3144	0.2793	0.2575	0.3265	0.2518	0.4118	0.3475	0.3149	0.2781	0.3307	0.3493	0.3520	0.2993	0.3585	0.2686	0						
YAER17	0.6266	0.5915	0.5697	0.6387	0.5639	0.7240	0.6597	0.6271	0.5903	0.4667	0.6614	0.6642	0.6115	0.6707	0.5808	0.4774	0					
YAER18	0.1206	0.1788	0.2106	0.1762	0.2345	0.1573	0.1109	0.1077	0.1400	0.4814	0.0948	0.2515	0.1490	0.2579	0.1124	0.3158	0.6280	0				
YAER19	0.2336	0.1985	0.1766	0.2457	0.1709	0.3309	0.2667	0.2340	0.1972	0.3537	0.2684	0.2712	0.2184	0.2776	0.1878	0.1881	0.5003	0.2349	0			
YAER20	0.2853	0.2502	0.2283	0.2974	0.2226	0.3826	0.3184	0.2857	0.2489	0.3488	0.3201	0.3229	0.2701	0.3293	0.2395	0.1832	0.4954	0.2867	0.1589	0		
YAER21	0.2388	0.2971	0.3288	0.2945	0.3527	0.2755	0.2291	0.2259	0.2582	0.5996	0.2130	0.3698	0.2672	0.3762	0.2306	0.4341	0.7863	0.1795	0.3532	0.4049	0	
YAER22	0.2972	0.2366	0.2939	0.3093	0.3178	0.3946	0.3303	0.2977	0.2609	0.5647	0.3321	0.2539	0.2821	0.2604	0.2514	0.3992	0.7113	0.2986	0.3183	0.3700	0.4169	0
Ortalama	0.2244	0.2232	0.2410	0.2451	0.2559	0.3002	0.2461	0.2218	0.2085	0.4487	0.2434	0.2832	0.2203	0.2890	0.1930	0.3062	0.5826	0.2148	0.2447	0.2840	0.3223	0.3272

Figure 4. The similarity index obtained by SRAP technique in rosehip genotypes

As a result of the matrix obtained by DarWin program (Perrier and Jacquemoud-Collet, 2006) of 22 genotypes; It was determined that the similarity index between rosehip genotypes varied between 0.093 and 0.746. When the studied rosehip genotypes were evaluated in total, it was understood that the Dice coefficient value of 0.7463 and the VMR21-VMR17 genotypes were not similar to each other in terms of genetic relatedness. These genotypes were followed by VMR6-VMR17 with 0.7240 coefficient and VMR17-VMR22 with 0.7113 coefficient. The closest genotypes to each other were VMR9 and VMR15 with 0.0928 coefficient, and the closest genotypes in terms of kinship were VMR11 - VMR18 with 0.0948 coefficient value and VMR13-VMR15 with 0.1018 coefficient (Figure 4). Among all genotypes studied, it was observed that the genotype with the lowest genetic similarity with other genotypes was VMR17 with 0.5820 coefficient, followed by VMR10 and VMR22 genotypes with 0.4487 and 0.3271) coefficient values, respectively. The most genetically similar genotype was the VMR15 genotype with 0.1930 coefficient value. It was followed by VMR9 and VMR18 genotypes with 0.2085 and 0.2148 coefficient value, respectively. In general, the greatest genetic distance was calculated between the genotypes VMR17 and VMR6 in the population (0.7240). At the same time, as a result of phenotypic observations, it was thought that these two individuals were different from each other and even different species. The distance between VMR11 and VMR18 genotypes measured as 0.095, this was the smallest distance in the population, and these genotypes can be said to be identical. A set contains 10 genotypes in the population, B set consist of 11 genotypes. It is seen that the smallest set was is C, which consists of 1 genotype. If we ignore the molecular clock and accept the population content as homogeneous, we can think that the genotypes in this set may be the components of the other two sets as a population structure.

CONCLUSION

The color values and total phenolic content of 22 rosehip genotypes were determined. It has been observed that the obtained data are in agreement with the literature information.

The total phenolic content were higher than the values in studies conducted in different regions. Soil conditions, ecological factors and stress factors were reported to be effective on phenolic substances. It is thought that the excess of ecological factors and stress factors due to the region where the study was conducted causes an increase in total phenolic substances. As a result of our study, it was seen that the rosehip genotypes showed close values between the color values and the total phenolic content, and there was no separation in some genotypes at the molecular level. In the dendograms obtained from the genotypes, it can be mentioned that there are clusters belonging to the species. *Rose dumalis Bechst. Subspp. Boissierii (Crépin) O. Nilson var boisseierii* were made the species descriptions.

It has been defined that VMR3, VMR17 and VMR20 can be hybrids of *Rosa canina* and *Rosa dumalis*. In the SRAP dendogram, it was found important that VMR17, which was thought to be hybrid, was also included in the A2 subset within the A set. Morphological data helped explain this situation. In such characterization studies, molecular and morphological data should achieve to reach more accurate results by complementing each other.The polymorphism rate of SRAP primers (0.77). Although this shows us that SRAP primers are less distinctive than RAPD primers in detecting polymorphism, the higher matrix correlation coefficient obtained from SRAP data (0.94) was due to the fact that these primers amplify regions close to or containing genes.

Six SRAP primers with high polymorphism can be used in future rosehip mapping or identification studies. In the light of the findings we obtained, it was seen that the genotype we named Hass did not show the characteristics of Hass cultivar, so it was thought that it could be a seedling. It is recommended to study Hass cultivar again in future studies.

Our results show that molecular data should be combined with pomological and phenological properties to characterize the information obtained with molecular markers. In addition, by increasing the number of primers used and the using of the different marker systems will be helped to scanning of the more regions in the genome.

In this study, previously unstudied rosehip genotypes were studied and for the first time, the molecular characterization was performed with SRAP

markers and a comparison was made in rosehip. When the total phenolic content and fruit color were evaluated together, VMR3-VMR10, VMR11-VMR18 were in the same group in the dendogram, and it was found to be important in the molecular dengogram to be in the VMR3-VMR10 B1 subset and VMR11-VMR18 A1 subset. In molecular studies, only molecular studies should not be carried out, it is thought that more concrete data can be obtained by integrating pomological and morphological chracteristics in molecular studies in parallel.

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CHAPTER 3

POLYAMINES: A KEY SUBSTANCE INVOLVED in HORTICULTURE, TOLERANCE and RESISTANCE RESPONSES to ABIOTIC STRESS

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1. Introduction

1.1. General Information on Polyamines

Natural compounds with aliphatic nitrogen structure, which are found in almost all living organisms and play important roles in many physiological processes such as cell growth, development and response to environmental stresses, are known as the Polyamines (PA) group. Put, Spd and Spm are the most common PAs in higher plants and can be found in free, soluble conjugated and insoluble bound forms. (Lefevre et al 2001) (Table 1).

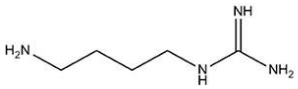
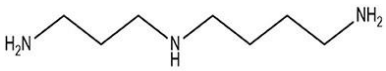
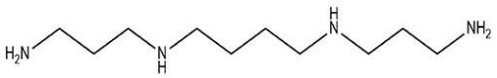
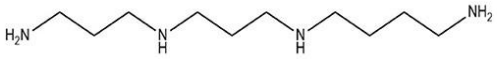
Name	Structure	Molecular formula
Agm		$C_5H_{14}N_4$
Put		$C_4H_{12}N_2$
Spd		$C_7H_{19}N_3$
Spm		$C_{10}H_{26}N_4$
Cad		$C_5H_{14}N_2$
Tspm		$C_{10}H_{26}N_4$

Table 1. Polyamine structure (Chen et al 2019).

Soluble conjugated PAs are covalently bound to small molecules such as phenolic compounds, while insoluble bound PAs are covalently bound to macromolecules such as nucleic acids and proteins (Duan et.al. 2008). Free polyamines are covalently combined with a small molecular substance, such as a phenolic compound and its derivative, in an amide bond. The phenolic compound may be hydroxycinnamic acid, coumaric acid, caffeic acid or ferulic acid. (Luo et al., 2009; Martin-Tanguy, 2010). Other uncommon polyamines have been detected in numerous biological systems, including animals, algae and bacteria.

In higher plants, mainly PAs are found in free form. Polyamines in the cation structure take part in many biological activities with these properties. Responsible for many essential processes including transcription, RNA modification, protein synthesis and modulation of enzyme activities, these organic polycationic structures readily bind to the headgroups of negatively charged phospholipids or other anionic sites in membranes, thereby affecting the stability and permeability of such membranes. They are also involved in the buffering mechanism to maintain cellular pH and ion homeostasis (Pandey et al, 2017). PAs show specific distribution in plant tissue, organ, and developmental stage (Figure 1).

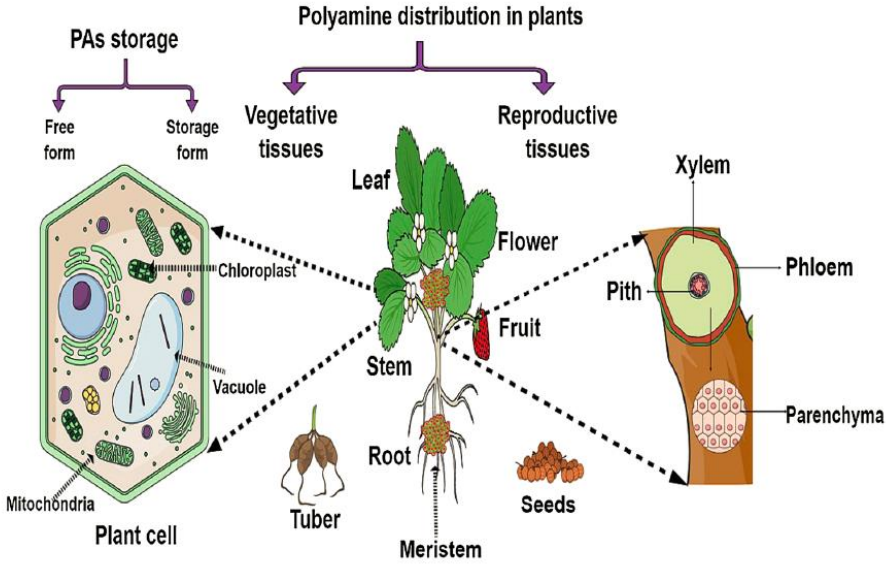


Figure 1. Distribution and storage of PAs in different organs in plants such as vegetative (leaf, stem, root), reproductive (flower, seed, fruit, tuber) organs and other organelle compartments (Tyagi et.al. 2022).

Generally, the level of polyamine is higher at the beginning of development compared to other secondary processes. However, this depends on the plant species and the main polyamine content present in the plant. The decline at the end of the developmental stage serves as a signal for the onset of senescence and death of the plant or part of the plant, while active cell divisions in the early stages of growth require high levels of PA. The growth-promoting

effect of polyamines is thought to be due to their contribution to cellular carbon and nitrogen to plants. (Shawky 2003, Liu et al., 2006). Numerous studies on polyamines indicate that PA catabolism is directly involved in plant growth.

The contributions of polyamines to major biochemical and physiological processes that increase productivity and quality are summarized schematically as follows. (Alcazar et al., 2020) (Figure 2).

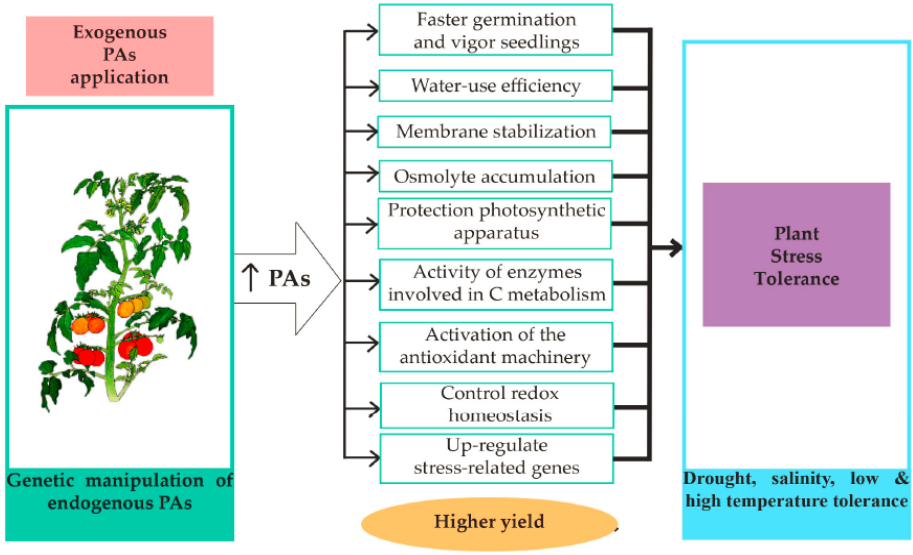


Figure 2. Polyamine accumulation regulates different molecular, biochemical and physiological processes that increase stress tolerance and crop yield (Alcazar et.al 2020).

In the synthesis of polyamines in plants, the decarboxylation of arginine or ornithine catalyzed by Putresin, arginine decarboxylase or ornithine decarboxylase, respectively, constitutes the most important steps. The addition of two aminopropyl groups to putrescine (put) in two reactions catalyzed by spermidine (spd) synthase and spermine (spm) synthase leads to the formation of spermidine and spermine, respectively. The aminopropyl moieties result from the decarboxylation of S-adenosylmethionine by the enzyme S-adenosylmethionine decarboxylase. The level of free polyamines in plant cells depends not only on their synthesis but also on their transport, degradation and conjugation (Groppa and Benavides, 2008) (Figure 3).

Putrescine degradation is catalyzed by diamine oxidase, a copper-containing enzyme that oxidizes diamine in the primary amino group, while spermidine and spermine are oxidized at secondary amino groups by a flavin-containing polyamine oxidase (Flores and Filner, 1985). Polyamines can be conjugated to small molecules such as proteins, antibiotics, and phenolic acids, mostly hydroxycinnamic acid (Martin-Tanguy, 2001). In addition to the effects of polyamines at different stages of plant development, it is known that they also contribute to adaptation to abiotic stress factors. Especially in many plants, abiotic stress factors cause differentiations in intrinsic polyamines. The stress-induced increase in polyamines is a reflection of the upregulation of enzymes associated with their biosynthesis and release from polyamine conjugates (Gupta et al. 2013). Stress-induced increases in polyamine stabilized membrane integrity and functionality, balanced antioxidant enzymes, and induce tolerance by altering hormones.

Productivity in fruit growing is directly related to pollination and an acceptable fruit set afterward. During the initial phase of fruit development, active cell division occurs, which probably needs sufficient polyamines.

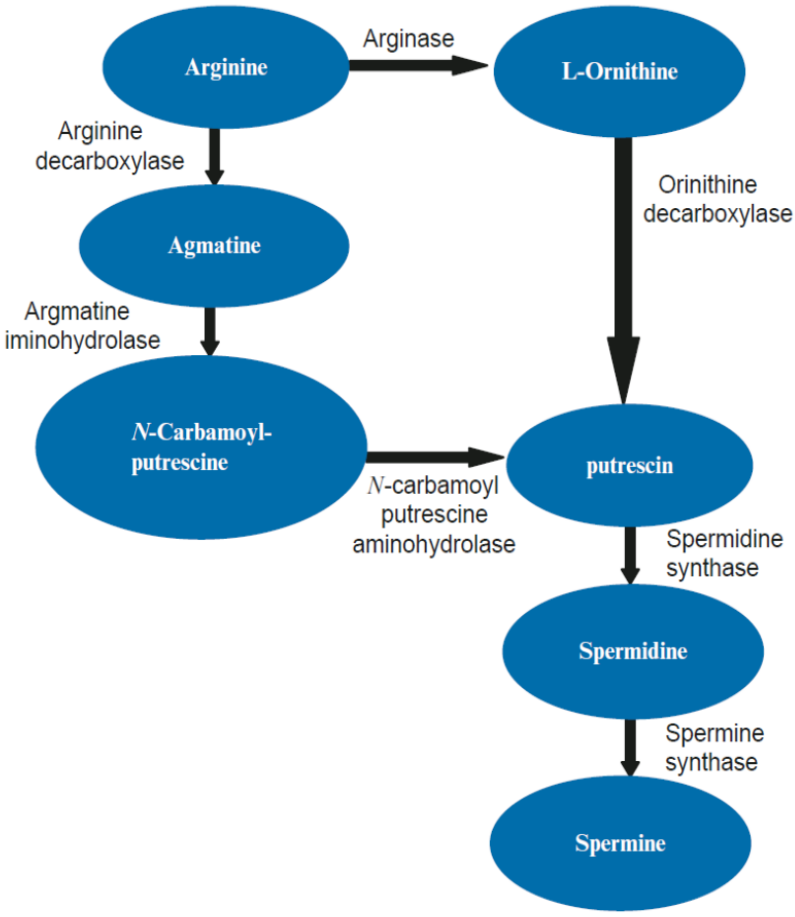


Figure 3. Biosynthetic pathway of polyamines in plants (Ahmad et.al 2012).

In the later stage of fruit development, cell division leads to cell growth where polyamine synthesis is reduced. In the late stage of development, the decrease in polyamines has been considered as a signal for fruit ripening. Exogenous polyamines applied during the flowering period in apples resulted in extended pistil life and improved fruit set (Wang et al., 1996). Putrescine applied during the anthesis period extended the life of the ovule, and increased fruit set and yield, but did not reduce the ethylene levels developed from the flowers during the flowering period (Crisosto et al., 1992). In addition, putrescine increased the effective pollination time in pears (Comice vc.) by

improving the nitrogen and boron contents of leaves and flowers, which are effective in increasing fruit set (Ewart and Kliewer, 1977).

The application of polyamines in other different horticultural crops, including trifoliate citrus seedlings, resulted in increased leaf nutrient content (Wu et al., 2010).

Intense fruit drop, which occurs with the formation of the abscission layer, is an important factor that causes a decrease in yield in many fruit species. Polyamines reduced fruit set by maximizing fruit set and increasing yield in many fruit species, including mango (Malik and Singh, 2003; 2006). The close relationship between polyamines and flower development makes it possible to perform some physiological or biochemical events using polyamine ratios or total polyamines (Rey et al., 1994, Zhu et al 1999). In general, at physiological pH, polyamines are positively charged and thus can interact with negatively charged macromolecules (DNA, RNA, chromatin, proteins, and phospholipids) and protect them from denaturation (Igarashi and Kashiwagi 2015). The stomata, which are responsible for gas exchange between the plant and its environment, are controlled by many factors, including changes that trigger the opening and closing of guard cells, stomatal opening, ion channel activity, and modulation of pumps for the maintenance of turgor (Ward et al., 1995). Especially K^+ ions play an important role in regulating the opening and closing of stomata. In addition, ABA, Ca^{2+} levels and polyamines show activity in these events.

Stomatal regulation is one of the most studied mechanisms of plant responses to stress. Many of the stress factors are known to increase polyamines. Among the ion channels in guard cells, inward K^+ channels play an important role in stomatal regulation and factors/processes that block inward K^+ channels that prevent stomatal opening. It is stated that there is a connection between polyamine levels and regulation of stomatal movements, especially depending on stress conditions (Liu et al. 2000). Aging is a highly regulated and genetically regulated process that can occur at the cell, tissue, organ or whole plant level. The process is largely oxidative and is mainly characterized by cessation of photosynthesis, degradation of organelle structures, loss of chlorophyll and proteins and a dramatic increase in lipid oxidation, with degradation of cell wall components and disruption of cell membranes leading

to cell/tissue loss. The activities of polyamine metabolic enzymes and polyamine ingredients may differ during plant growth stages. In all plants, endogenous polyamines and polyamine synthetase activity were highest in meristem and growing cells, and lowest in senescent tissues. Many changes, such as the gradual decrease in chlorophyll content, the rapid increase in the activities of hydrolases such as ribonuclease and protease, which occur due to the progression of aging, can be inhibited by the administration of exogenous polyamines (Cai, 2009, Chen et al. 2019). It has been reported in studies that external applications of spermidine and sperm can delay aging by increasing the polyamine content of cut flowers and improving flower quality (Yang and He, 2001). Similar results were obtained from the spray application of the GA_3 + Spermin mixture in Ontorium (Anthurium andraeanum), the aging of flowers stored at 20◦C was delayed and the quality of the inflorescences improved (Simes et al, 2018). Reviews summarize that polyamines delay aging by inhibiting ethylene biosynthesis (Anwar et al., 2015). In addition, it has been determined that external applications of spermidine or sperm reduce protein degradation and chlorophyll losses (Serafini-Fracassini et al, 2010; Cai et al, 2015).

During climacteric fruit ripening, an increase in ethylene production occurs with increasing activities of 1-aminocyclopropane-1-carboxylic acid. Carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes (Lelievre et al., 1997), enzymatic changes that cause fruit softening are also associated with fruit ripening. The main enzymes involved in the softening of fruits are polyestrous and endo, exo polygalacturonase. Researchers were particularly interested in slowing ethylene production in climacteric fruits to extend shelf life and preserve quality for longer.

Both polyamine and ethylene have the same precursor, namely S-adenosyl methionine (SAM), so the biosynthesis of polyamines inhibits ethylene biosynthesis by competing for the co-precursor because both have opposite effects on fruit ripening and senescence. Many observations have shown that polyamines can inhibit the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor to ethylene, and thus reduce ethylene production.

Polyamines also inhibited auxin-induced ethylene production and conversion of methionine and ACC to ethylene (Pandey et al, 2017, Chen et al, 2019).

2. Stress Factors and Polyamines in Horticulture:

The ever-increasing human population, the loss of agricultural lands and the gradual depletion of resources pose great threats to world agriculture. Undesirable climatic events such as heavy rains, storms, heat waves, and severe droughts are becoming more and more problems. In light of this information, drought, salinity, and extreme high and low temperatures are among the major abiotic stress factors that negatively affect plant growth and productivity worldwide. Drought is an important abiotic stress factor that adversely affects plant growth and productivity, and its effect can be reduced by methods such as selecting varieties with good drought adaptability and/or improving soil management and irrigation techniques. Land degradation due to soil salinization is a serious problem in many parts of the world, especially in arid and semi-arid regions. Heavy metal toxicity is another abiotic stress factor that negatively affects productivity and quality locally in agricultural lands where the industry is dense and close to these areas. These abiotic stress factors cause more losses in horticultural crops than in other plant species. It has been proven that all types of stress produce reactive oxygen species (ROS), which are toxic to the biological system and cause oxidative stress (Alexieva et al., 2003).

Researchers have observed that polyamines also function as radical scavengers, thereby reducing the damage done by different stresses (Kim and Jin 2006). Some researchers argue that the cell's response to stress is due to the increase in antioxidant defense systems with the application of polyamines (Velikova et al., 2000). Moreover, modulated polyamine levels can act as a signal or a messenger to spatially and temporally express the behavioral response of plants to avoid or overcome stress.

It is known that varying levels of endogenous polyamines (free or conjugated or bound) are involved in the formation of polyamine-RNA complexes, thereby producing structural changes in RNA at physiological concentrations of potassium and magnesium ions. Many studies on the effects

of externally applied polyamines on the tolerance of some stress factors in horticultural crops are summarized in Table 2.

Table 2. Exogenous polyamines application against stress factors for horticultural plants.

Plant Species	Polyamines	Performance	Referances
Cucumber	Spm (Seed Priming-SP)	Drought (Dehydration) tolerance	Shi et al 2010
Cucumber	Spd	Salt tolerance enhancement	Duan et al. 2008
Cucumber	Put	Improved tolerance to salt	Zhang et al. 2009
Cucumber	Spd (Seedling treatment)	Salt tolerance enhancement	Sang et.al. 2016
Cucumber	Put	It relieved the plant from salt stress by maintaining water and nutrition status	Shu et al., 2012
Cucumber	Put	Reduced salt-induced photosynthetic perturbation of leaf through stomatal-aperture modifications	Ma et al., 2020
Damask rose	Spd, Spm (SP)	Alleviate water deficit	Hassan et.al 2018
Lettuce	Spd	Promoted the synthesis of endogenous Put and Spd, which might have great effects on the heat tolerance	Huang et.al. 2021
Lettuce	Put (SP)	Improved drought tolerance	Zhu et.al 2019
Mung bean	Put,Spd, Spm (SP)	Improved seed germination and growth	Sadeghipour, 2019
Mung bean	Spd (Seedling application)	Enhanced tolerance to low temperature	Nahar et.al. 2015
Mung bean	Spm (seedlings Pretreatment)	Enhanced tolerance to heat and drought	Nahar et.al. 2017
Chamomile	Put	Improved seed germination under salinity	Ali et al 2009
Spinach	Spd	Improved seed germination under salinity	Rebeca et.al 2010
Tomato	Spd (SP)	Improved tolerance	Hu et al. 2012
Tomato	Spd (Foliar Spray)	Enhanced tolerance to stress	Zhang et al. 2016a
Tomato	Put (Foliar spray)	Enhanced tolerance to low temperatures	Diao et.al.2017
Tomato	Put	Enhanced tolerance to chilling	Song et.al. 2014

Tomato	Spd	Enhanced tolerance to heat	Cheng et.al. 2012
Tomato	Spd (Foliar Spray)	Enhanced tolerance to heat	Sang et.al. 2017
Cherry tomato	Spd	SA- and Spd-treated plant showed high photosynthesis, antioxidant activity, and proline contents under stress	Fariduddin et al. (2018)
Lemon	Put (Foliar Spray)	Enhanced salt tolerance	Khorshidi and Hamedi 2014
Chrysantemum	Spd (Foliar spray)	Salt tolerance enhancement	Zhang et al. 2016b
Tea	Put (Foliar spray)	Alleviating salt-stress	Xiong et.al. 2018
Cucumber	Spd	Enhaced tolerance to low temperature	He et.al. 2002
Peach	Put	Chilling injury alleviated	Abbasi et.al. 2013
Orange	Spm	Improved plant defense system for dehydration tolerance	Shi et al., 2010
Mango	Put, Spd, Spm	JA MeJA reduce chilling injury on plant by increasing PAs	Tommy Atkins) González-Aguilar et al. (2000)
Apple	Spm	ABA, JA PDJ-/Spm-treated plants are chilling tolerant by ABA accumulation,	Yoshikawa et al. (2007)
Lemon)	Put, Spm	Heat Stress activates PAs production and delayed ABA accumulation	Valero et al. (1998
Grape	Put, Spd, Spm	High number of Total PA and ABA were detected on drought-tolerant plant ABA induce PA accumulation/oxidation	Toumi et al. (2010)

The increase in membrane permeability results from the detrimental effects of chilling injury since it refers to the transition of membrane lipids and proteins from liquid-crystalline to solid-gel state. This increases ion and electrolyte leakage (G´omez Galindo et al, 2004). Cold damage not only spoils the shape of the product, but also affects the quality very badly. The frost damage problem is very common in horticultural crops such as bananas,

mangoes, peaches and pomegranates in low-temperature storage (Mirdehghan et al., 2007). It has been reported that polyamines have a positive effect on cold acclimation, as a result of which cell membrane integrity and fluidity are preserved. The imbalance that occurs during stress causes acidification of the cytoplasm and has serious consequences on the metabolic regulation and homeostasis of living cells (Chen et al. 2019). Increases in phospholipids and other polar compounds have been reported during the cold hardiness of citrus (Waaled 2020). It is reported that these increases are in parallel with the increase in polyamine titers (Figure 4).

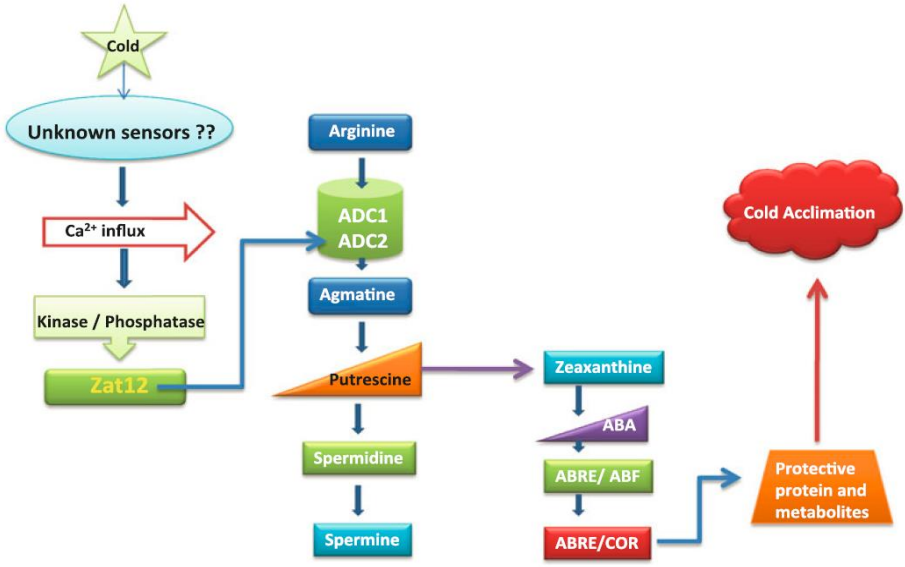


Figure 2 Effect of cold stress on protective proteins and PA synthesis. Cold induces ZAT12, which activates ADC, increasing Put production, resulting in ABA accumulation and activation of protective proteins and metabolites via ABRE/COR.

Another hypothesis on this subject states that the increase in polyamines during stress may have a direct role in maintaining cell membrane thermostability against changes in fluidity and solute leakage (Chen et al. 2019). According to some studies, the accumulation of polyamines preserved the integrity of the cell membrane. It has been noted that citrus trees respond to low-temperature adaptation with a uniform and significant increase in polyamines (Kushad and Yelenosky, 1987) (Figure 5).

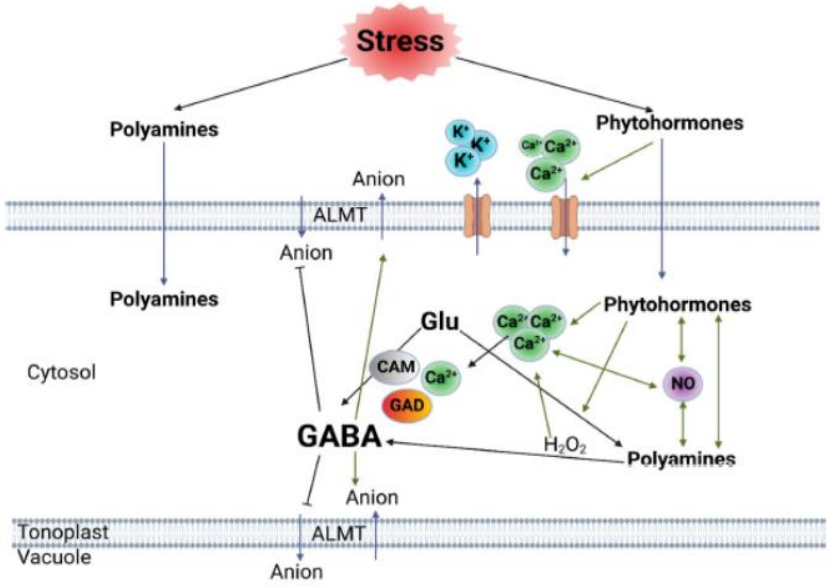


Figure 5. Schematic model of plant stress responses mediated by hormones, polyamines and GABA in different plant cell types, including guard cells and stem cells. (Bown and Shelp 2016).

Exogenous application of polyamines has also been reported to reduce the symptoms of chill damage during storage in a variety of horticultural crops. Postharvest dipping in polyamines was used to reduce chill damage in zucchini (Kramer and Wang, 1989) and cucumber (Zhang et al., 2009). Similar results were obtained from mango fruits treated with exogenous polyamines (Nair and Singh 2004). Pomegranate fruit treated with polyamines and stored at 2 °C developed fewer symptoms of chills injury compared to control (Mirdehghan et al, 2007). Thus, some data emerged that made it possible to evaluate polyamines not only as ROS scavengers, but also as activators of expression of genes encoding antioxidant enzymes such as CAT, SOD and POD (Hiraga et al. 2000; Aronova et al. 2005, Abbasi et al. 2017). It has also been reported that there are changes in cell membrane lipids during chilling injury. This causes an increase in permeability and leakage of ions (Gomez-Galindo et al., 2004). All these changes were significantly reduced when pomegranate fruit treated with polyamines was kept under storage conditions (Mirdehghan et al. 2007). Despite all this information, the mechanism by which polyamines counteract

the effects of stress is still unclear. High temperature is an abiotic stress factor that causes problems up to both yield and quality losses and even plant death and is effective in different intensities all over the world. Synthesis of heat shock proteins is one of the plant's response mechanisms to protect itself from the harmful effects of high temperature stress. It has been reported that polyamines (PAs) can directly affect the synthesis of heat shock proteins at the synthesis level or by affecting cell membrane properties (Königshofer and Lechner 2002). PAO and ADC activities, as well as the concentration of conjugated and free PAs, were found in higher amounts in the callus of non-stress tolerant agricultural crops. In addition, very rare polyamines such as norspermine and norspermidiene increased in amount under high-temperature stress (Roy and Ghosh, 1996, Konigshofer and Lechner 2002).

Drought causes adverse effects on plant growth and productivity. Irrigation alternatives are one of the most important factors for successful plant production in arid regions. Not only is the lack of water, but also the poor quality of irrigation water due to the presence of disproportionate ion concentrations is another major problem of dry areas. The high salt concentration of the water used in irrigation poses a problem in more than one-third of the irrigated lands worldwide (Postel, 1993, Abbasi et al. 2017). Among the crop species, horticultural crops are considered highly sensitive to drought and soil salinity. Significant accumulation of putrescine (Put) and cadaverine (Cad) has been reported in bean and pea plants grown in saline conditions (Shevyakova 1981), a similar situation was noted in Vicia faba (Priebe and Jager 1978). However, a large decrease in putrescine content was observed in the roots of olive plants with increasing salinity (Tattini et al. 1993) (Figure 6).

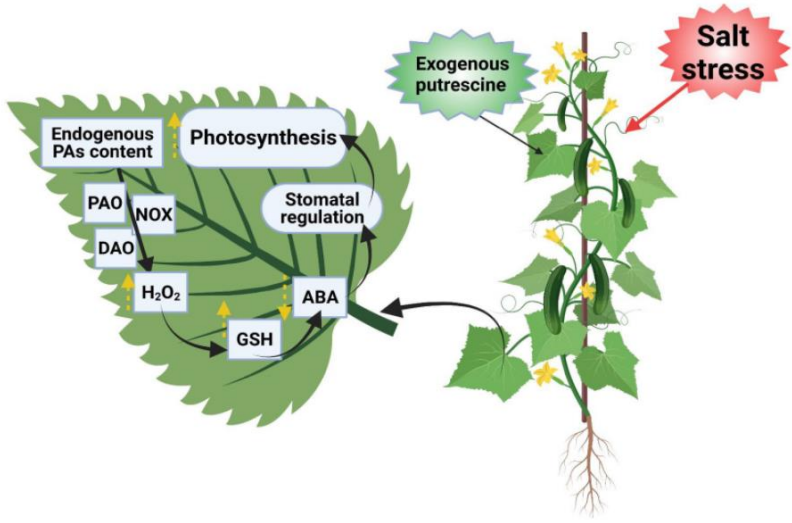


Figure 6. Model diagram of exogenous Put effects on photosynthesis of cucumber leaves under salt stress. (Put-putresin, PAs-polyamines, DAO-diamine oxidase, PAO-polyamine oxidase, NOX-nicotinamide adenine dinucleotide phosphate oxidase, H2O2-hydrogen peroxide, GSH-reduced glutathione, ABA-abscisic acid regulate stomatal opening and closing) (Ma et. al., 2020).

It is known that altered endogenous polyamine levels are effective in the formation of polyamine-RNA complexes, thus causing structural changes in RNA at physiological concentrations of potassium and magnesium ions (Igarashi and Kashiwagi, 2000). In addition, modulated titers of polyamines in combination with epibrassinolides, the active form of brassinosteroids, have been reported to regulate abscisic acid (ABA) and indole-3-acetic acid (IAA) pathways that increase tolerance to metal toxicity (Choudhary et.al., 2012).

Heavy metals are toxic to plants, besides, heavy metals stimulate oxidative stress due to their ionic nature. In combination with brassinosteroids, polyamines modulate the levels of antioxidants such as glutathione, ascorbic acid, proline, glycine-betaine and the like, and antioxidant enzymes such as glutathione reductase, superoxide dismutase, catalase, as well as modulate the ABA and IAA pathways with cascading effects for heavy metal tolerance. et al, 2014).

Under Cd stress, exogenous putrescine administration detoxifies free radicals (ROS) by increasing enzymatic antioxidant enzyme activity (SOD, CAT, APX, MDHAR, DHAR, GR, GST and GPX) and non-enzymatic antioxidants (AsA and GSH) (Nahar et. al, 2016). Seed soaking or foliar application of polyamines significantly increased plant growth and yield under Cd^{2+} and Pb^{2+} stress by improving defense processes (Taie et.al., 2019). The toxic effect of Cd 2+ and Cu^{2+} altered leaf and root membrane fluidity during early seedling growth; however, pretreatment with PAs improved the toxic effects in plants (Benavides et.al., 2018). In addition, PA administration increased the level of free, soluble and insoluble conjugated PAs in Kinnow mandarin, which facilitated tolerance under Cr^{2+} toxicity (Shahid et.al., 2018).

The higher level of PAs can be attributed to the lower catabolic enzymes on metal stress along with the higher activity of the anabolism-related enzymes of PAs (Shahid et.al. 2018). Putrescine application in metal stress can provide osmotic protection by increasing water status and chlorophyll synthesis (Nahar et.al., 2016) (Figure 7).

Protection of fruit from invasion by fungal pathogens is largely dependent on the activation of the biochemical and structural defense system that helps inhibit the spread of pathogens (Schroder et al, 1992, Lawton et al, 1996). It is thought that chitinase and β-1,3-glucanase hydrolysis polymers of fungal cell walls are involved in plant defense mechanisms against fungal infection (Collinge et al., 1993).

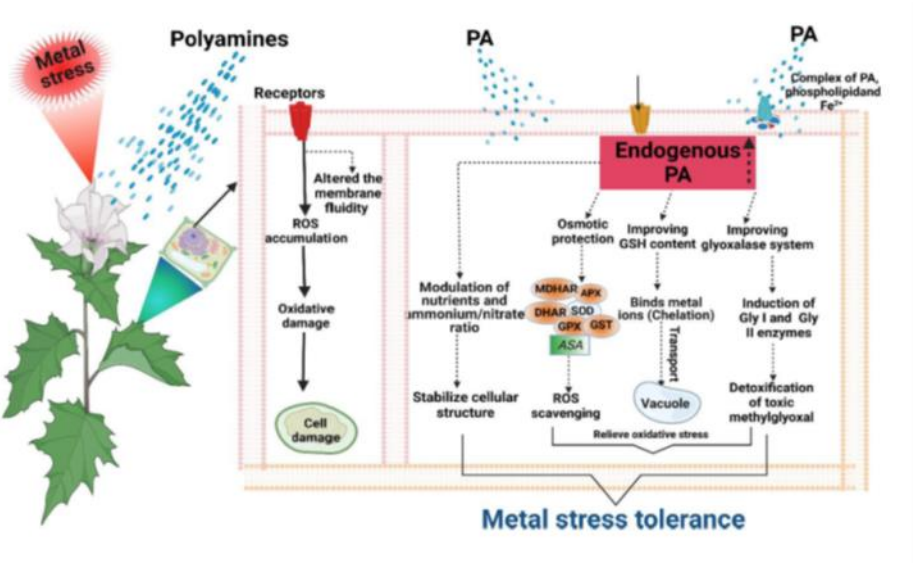


Figure 7. Regulatory roles of exogenously administered PAs in heavy metal stress tolerance. To protect the membrane structure by forming a complex with phospholipids and Fe2+. PAs enhance the glyoxalase system by inducing the enzymes glyoxalase I (Gly I) and glyoxalase II (Gly II), which detoxify toxic methylglyoxal. It increases the level of glutathione (GSH), which includes metal chelation and its transport to the vacuole for detoxification. Enzyme activity (SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GST, glutathione S-transferase; GPX, glutathione peroxidase antioxidant activity) and non- ascorbate, AsA). Therefore, it directly and indirectly reduces oxidative stress (Shao et al. 2022).

Peroxidase (POD) activity generates oxidative power for crosslinking proteins and phenylpropanoid radicals, resulting in strengthening of cell walls against fungal penetration (Huckelhoven et al, 1999; Kristensen et al, 1999). Researchers have also reported that β-1, 3-glucanase, PAL and POD are associated with induced resistance in plants (Mohammadi and Kazemi, 2002; Qin et al, 2003). When polyamines or their precursors are applied to the leaves of the bean plant, an increase in the activity of enzymes such as POD and polyphenol oxidase (PPO) has been noted (Haggag 2005). The increase in the activities of these enzymes has been found to be associated with increased resistance to disease-causing organisms (Kumar and Balasubramanian, 2000).

The increase in ethylene activity during pathogen infection lowers the concentration of polyamines, which explains the possible role of polyamines in the control of viral and fungal infection (Abbasi et al 2017).

Conclusion

The mechanisms of action of polyamines and the precise molecular mechanism underlying their protective effects against stress are still not clearly understood. Polyamines (PA) have a polycationic structure, which allows them to participate in the modulation of cell ion balance and bind to polyanionic molecules such as DNA, RNA, proteins or membrane lipids, preventing macromolecule degradation and protecting cell membranes from stress-induced damage. Therefore, a well-organized protection mechanism consisting of PAs, Ca^{2+}, ABA, H_2O_2 and NO may be coordinating the plant's response to abiotic stress. Thus, PAs can be used to reduce stress and improve crop yield and quality without any adverse effects on crops or the environment. In addition to being known as vital plant regulators, polyamines are now recognized as secondary messengers. However, much research is still required to understand the mechanism of polyamines.

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CHAPTER 4

MOLECULAR APPROACH TO SPECIES IDENTIFICATION IN FISHES: A FOCUS ON *Aphanius mento*

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1. INTRODUCTION

Species determination applications in fishes were generally made by using external morphological features, morphometric and meristic measurements until recent years. However, species identification or affinity relationships of different populations of the same species are carried out at the molecular level in many fishes today. Molecular-based studies allow the systematic identification of fish species to be performed with high reliability using primer pairs on a gene basis. It is a practical and applicable method to determine whether there are intraspecies differences in species with different populations.

In previous studies on the subject, DNA is isolated from the blood or tissues of different fish species and identification processes are encountered with primers synthesized. There are molecular-based studies in the determination of species belonging to some families. However, such studies are very limited in species belonging to the Cyprinodontidae family.

In this study, it is planned to confirm the place of the species distributed in Nemrut Crater Lake in the systematic classification at the molecular level, which is thought to be *Aphanius mento* belonging to the family of Cyprinodontidae in terms of external morphological features. In line with the genomic data to be obtained, it is thought to create a database in order to determine the phylogenetic differences between the populations of the species in other regions.

2. MATERIAL AND METHOD

This study was carried out in Nemrut Crater Lake, located within the borders of Bitlis Province, in the Eastern Anatolia Region of Türkiye (Fig. 1). Nemrut Crater Lake, located on Mount Nemrut, is a formation of volcanic origin located within the borders of Tatvan District. Nemrut Crater Lake has the distinction of being the largest volcanic lake in our country and the second largest in the world (Özdemir and Tuğ, 2009; Kurttaş and Tezcan, 2018). *Aphanius mento* species are distributed in the lake as well as different fish species that have created populations naturally or through fish introductions.

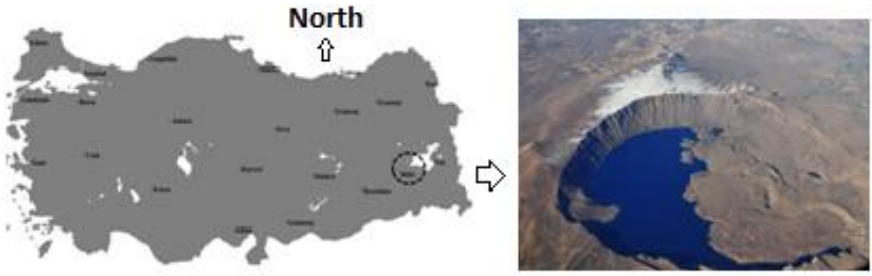

Figure 1. Nemrut Crater Lake and its location (Photo: Original, Map: ODV, 2020).

Aphanius mento, a species belonging to the family Cyprinodontidae, was used in the study. In general, there are freshwater species as well as species of this family that spread in brackish waters and coastal areas of seas. A highly visual blue-turquoise coloration (Fig. 2) is observed in males of the *A. mento* species (FishBase, 2020). This feature gives the species an attractive position in the aquarium industry. The species has populations in Iran, Iraq, Syria, Israel, Jordan, Lebanon and Türkiye (FishBase, 2020; NCBI, 2020). It has been reported that *A. mento* has populations in some inland waters in our country (Ergüden, 2015). The presence of this species, which is generally reported to be detected in the Mediterranean and Southeast Anatolian regions, was first reported in 2001 in Nemrut Crater Lake, located within the borders of Bitlis Province in the Eastern Anatolia Region (Çildir, 2001; Elp et al., 2016). The individuals to be used in the experiment were caught alive in different sexes and heights by using SAMUS 725 MS brand electroshock device and scoops with 1-3 mm mesh sizes.

Figure 2. *Aphanius mento* male and female (Photo: Elp ve Şen).

The caught fish were transported to Van Yüzüncü Yıl University Aquatic Animals Experimental Unit (SUCAN) by taking them into PVC transport tanks of different sizes with 50-120 L volumes. It was taken into aquariums with a volume of 80 L, which was prepared close to the water quality of the lake, and stocked as 1-3 individuals/L (Olivier and Kaiser, 1997). Following a certain adaptation process, DNA isolation process was started in fishes.

2.3. Molecular Identification of Fish Species

2.3.1. Anesthesia and necropsy of fish samples

12 fishes, approximately 3 cm in total length and an average of 3 g ± 0.3 g in weight, were randomly selected from the breeder adapted in 80 L aquariums (Stock ratio: 1 individual/4 L water). Then, these fishes were anesthetized with 0.4 mL/L 2-Phenoxyethanol (Sarıeyyüpoğlu et al., 2017) and necropsy procedures were performed. Before the necropsy procedures, the fishes were taken to the apparatus prepared under aseptic conditions. The skin surfaces were disinfected with 70% ethanol (Perdices et al., 2001). In the necropsy procedure, sterile scalpel tips were used to be changed in each samples. While muscle tissues were taken from the dorsal region, necropsy was performed from the anterior part of the dorsal fin of the fish towards the head of the fish and parallel to the notochord. And then the scales and skin parts were separated from the tissue sample obtained and only the region consisting of muscle tissue was used for DNA isolation. The surface from which 50 mg of tissue sample required for each fish will be taken for the purpose of total DNA isolation, was selected from the dorsal region and the muscle tissues taken from this area were measured on a precision balance (Sartorius-0.001) at 25 mg/fish to be used in DNA isolation (Sarıeyyüpoğlu et al., 2017). Tissue samples of 25 mg were stored at -80 °C until DNA isolation (Yang et al., 2020).

2.3.2. DNA isolation process

25 mg of muscle tissue from fish samples was suspended in 10% b-mercaptoethanol (Sigma) and 180 µL lysis buffer (Qiagen). After the suspension was kept at room temperature for 1 min, tissue lysis was performed with TissuLyser LT (Qiagen) at a rate of 3 ms/sec and for 3 min

(Fig. 3). Tissue dissection was carried out with sterile 0.5 mm diameter steel balls and upside down suspension mechanism. The homogenate obtained after lysis was centrifuged at 10400 rpm for 2 minutes to remove solid particles and cell debris. The supernatant obtained after centrifugation was carefully taken with an automatic pipette (Eppendorf) and placed in the Qiacube (Qiagen) device (Pappalardo et al., 2008; Ferrito et al., 2013; Anonymous, 2016).

Figure 3. Necropsy procedure in *A. mento* samples and performing the disintegration of tissues.

If the tissues taken from the fish were fragmented and the supernatant obtained after centrifugation consisted of liquid homogenate without solid particles, isolation was continued. In the case of solid particles, the samples were centrifuged again and the supernatant was taken again. 180 µL of the obtained homogeneous supernatant was taken and placed on the spin columns in an vertical position. Each fish muscle tissue supernatants were loaded on separate spin columns and placed in rotor adapters (Ferrito et al., 2013; Anonymous, 2016). Isolation of genomic DNA's was performed using the QIAmp DNA Mini Kit (Qiagen) and the QIACUBE automated isolation robot (Fig. 4). In the isolation steps, firstly the supernatant was added and the spin columns were centrifuged in the robot (10400 rpm). And also it was centrifuged again by adding 180 µL of ATL buffer. 20 µL of proteinase-K was added and left for 1 minute, and then vortexed and kept at 56 °C for half an hour using the shaker of the automatic isolation robot (100 rpm). 4 µL of RNase A and 200 µl of buffer AL were added, vortexed for 15 seconds and incubated for 10 minutes at 70 °C after 2 minutes. 200 µL of 99.99% ethanol was added and vortexed and then centrifuged at 8000 rpm for 1 min. 500 µL

of AW1 was added and centrifuged at 8000 rpm for 1 minute, and then 500 µL of AW2 was added and centrifuged at 16000 rpm for 3 minutes. By optimizing the elution stage, the last step, 50 µL of AE buffer was added and after waiting for 1 minute, it was centrifuged at 8000 rpm for 1 minute. The separated total DNA's were either directly studied or stored at -40 °C until studied in Real-Time PCR (Anonymous, 2016; Onalan, 2019).

In order to determine the purity of DNA obtained after isolation, OYD 260 and OYD 280 values were calculated with nanospectrophotometer (Thermo-Nanodrop) and their ratios to each other and DNA purity were determined.

Figure 4. The parts where the QIACUBE automatic isolation robot and buffers are placed.

The isolated DNA values between 1.8 and 2.0 were accepted as pure DNA and without contamination. A ratio lower than 1.8 was considered as protein presence, and a ratio higher than 2.0 was considered as RNA contamination (Nanlohy and Semuel, 2020).

2.3.3. Real-Time PCR analysis

DNA's isolated from the muscle tissues of the fish were used as mentioned before. For species identification, Real-Time PCR was performed using a primer set specifically designed for the mtDNA gene region specific to the *A. mento* species (Esmaeili et al., 2020). While designing the primer specific to *A. mento*, the sequence data generated using the mtDNA gene region of the *A. mento* species sampled from different regions in The National Center for Biotechnology Information (NCBI) were evaluated. Sequences with close base numbers were arranged by nucleotide alignment and a more reliable primer was designed (Fig. 5).

Figure 5. Nucleotide alignment analysis of sequences from *A. mento* available from NCBI.

Sequence data with nucleotide alignment process belong to *A. mento*'s, which are available in the NCBI database and sequenced by sampling from different regions with code numbers on the figure (Moravcikova et al., 2020). The dendogram graph, which expresses the similarities of the nucleotide aligned sequences of *A. mento* sampled from different regions, is given below (Fig. 6). Each different sequence data in the dendogram is named with different code numbers.

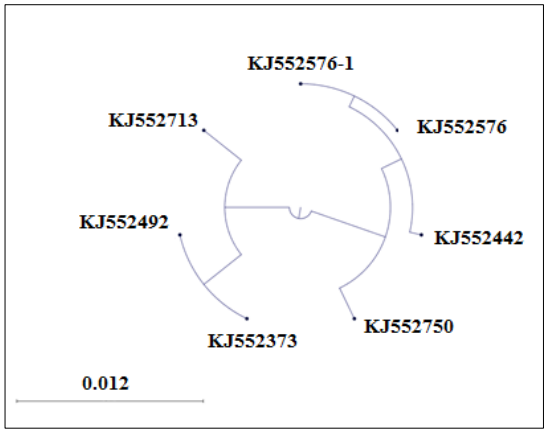

Figure 6. Dendogram plot of sequences of *A. mento.*

In the Real-Time PCR protocol, SYBRGreen qPCR master mix, forward and reverse primer set, 'DNase-RNase free' water and template DNA's were used. Primer design and optimization was performed with SnapGene commercial software. The sequences of the designed primer set are given in Table 1 (Sharma et al., 2020).

Table 1. Primer set designed and its properties

Primer name	Primer sequence	Base Length	GC %	Tm C
Apha_mtDNA_FP	ATCAAGCAAGAGAGCACACCAC	22	50	55.3
Apha_mtDNA_RP	TCAGCGAAAGGTTGAAGAAGGC	22	50	55.5

Control of the designed primers were carried out with the nBlast software on the NCBI website (NCBI, 2020). Only 100% overlapping

sequences were considered in the blast results. Only *Aphanius* sp. to which forward and reverse primers adhere together (Fig. 7).

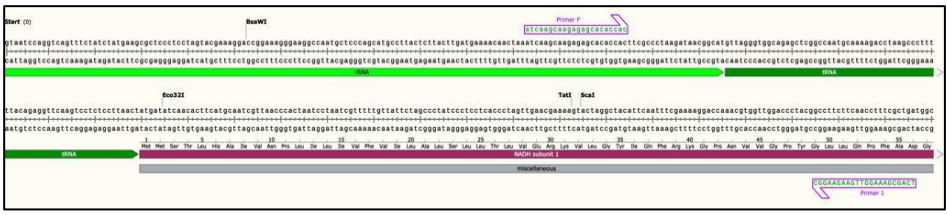

Figure 7. Regions where forward and reverse primers adhere.

For the Real-Time PCR process, qPCR master mix, Forward primer, Reverse primer, DNase and RNase-free water and template DNA were mixed in certain amounts (Table 2).

Table 2. Optimized mixing amounts of Real-Time PCR components

PCR Components	Amount (µL)
SYBRGreen qPCR Master Mix	12.5
Forward primer	1.5
Reverse primer	1.5
DNase-RNase-Endotoxin-free water	7.5
Template DNA	2.0
Total Amount of Mixture	25.0

In the Real-Time PCR stage, following the preliminary denaturation at 95 °C, 45 cycles of 45 seconds at 94 °C, 30 seconds at 60 °C, and 45 seconds at 72 °C were completed. For the final elongation, the PCR step was completed after a 5 minutes treatment at 72 °C. HRM (High resolution melting curve) analysis and standard copy curve analysis were performed to validate the PCR amplicons. The sigmoidal curves obtained after the PCR amplicons plot were considered as positive identification. 0.3 – 0.5 values were used as the threshold value in the Real-Time PCR graph. Positive results were evaluated with above-threshold and sigmoidal curves. Finding all the peaks on the standard single vertical line as a result of the melting curve obtained from the PCR amplicons after the HRM analysis will show the accuracy of the study. DNA's with 3 different copy numbers taken as standard

in the study were used to provide the PCR process and to evaluate the quantitative results if the amplicons were found on quantitative amount and standard curve coordinates after Real-Time PCR (Munoz-Calderon et al., 2020). As a result of the identification analyses performed by molecular methods, positive samples were found to be evaluated as *Aphanius* sp.

3. MOLECULAR IDENTIFICATION RESULTS

3.1. DNA isolation

A260/A280 nm purity ratios of DNA obtained as a result of isolation are given in Table 3.

Table 3. DNA purity rates of samples

No	Sample Name	A260/A280 (nm)
1	A_mento_1	1.88
2	A_mento_2	1.87
3	A_mento_3	1.88
4	A_mento_4	1.89
5	A_mento_5	1.88
6	A_mento_6	1.87
7	A_mento_7	1.90
8	A_mento_8	1.92
9	A_mento_9	1.93
10	A_mento_10	1.89
11	A_mento_11	1.88
12	A_mento_12	1.87

When the table was examined, the purity ratios of A260/A280 nm of 12 samples were found between 1.87-1.93 and it was seen that the isolated DNA's were free from contaminations such as protein and RNA.

3.2. Real-Time PCR analyses

As a result of Real-Time PCR performed with primer sets designed and synthesized specific to *A. mento*, it was observed that all samples gave sigmoidal curves (Fig. 8) and primer and master mix controls gave negative

results according to Ct and Rep-Ct values of PCR samples. Non-Template control sample was also determined to be negative by creating a flat graph. In line with these results, it was seen that both the primer sets and master mixes used in the study did not make non-specific binding, and the non-DNA control sample did not bind, and the results were shaped from the DNA's of the samples.

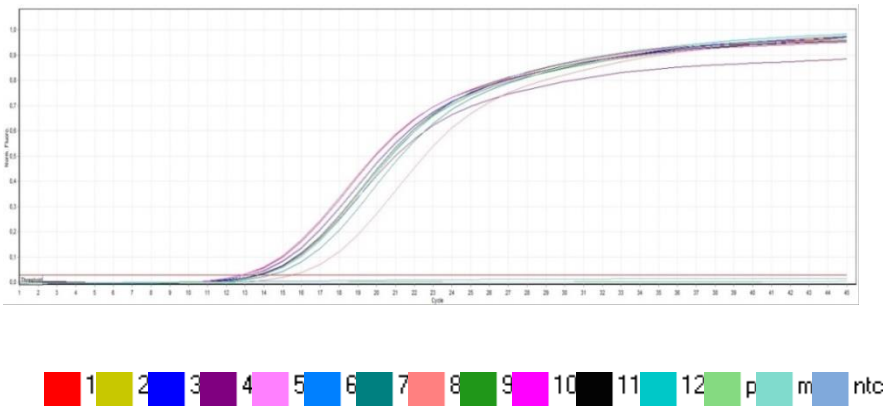

Figure 8. Real-Time PCR analysis image of the fish used in the experiment.

DNA samples used in the study with Real-Time PCR analysis and the Ct values that were positive as a result of the adhesion of the primers are given in Figure 9. In line with these results, the Ct values were "0" due to the lack of binding in the primer, PCR mix control and non template control samples. In the samples containing DNA that isolated from fish, the Ct values were very close to each other. It was observed that the earliest binding was at 13 and the latest at 14.9 Ct. All these results show that the species-specific primers are positive with DNA isolated from the same species of fish, with a difference of almost 1 cycle.

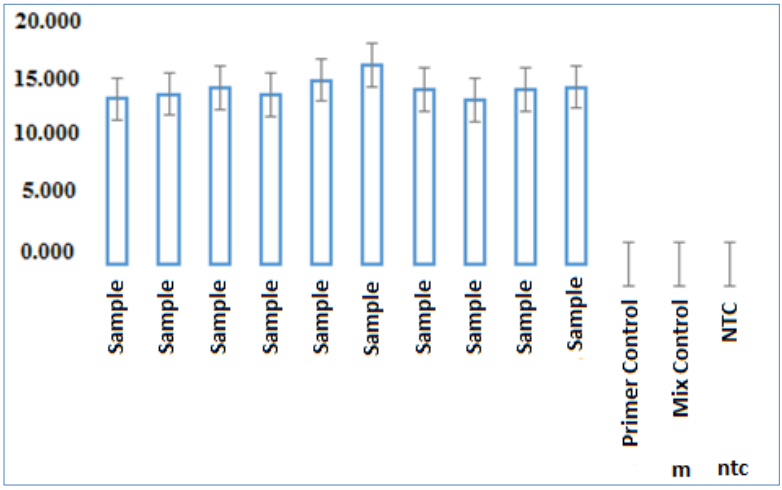

Figure 9. Ct values obtained as a result of Real-Time PCR analysis.

It was seen that all of the samples gave the same graphical curves, as a result of the qPCR analysis performed depending on the PCR amplicon concentration after the Real-Time PCR analysis. And positive sample concentrations were also found to be the same (0.15-0.20). According to these results, it was found that the primers were similar density after 45 cycles (245 copies) and it is understood that the same intensity of concentration is obtained by duplicating the same piece of DNA (Fig. 10).

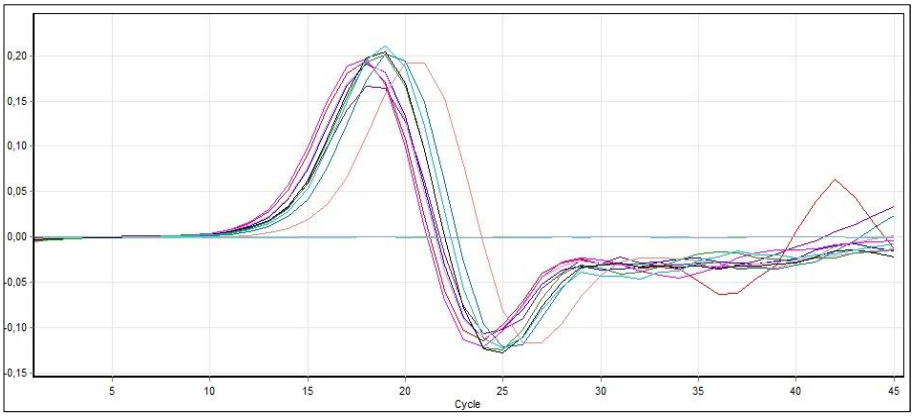

Figure 10. qPCR concentration analysis results.

For the identification of the *A. mento* species, the primer set designed from the mtDNA gene was used by *Aphanius* sp. can be used and it has been seen that species identification has a more specific and verifiable application area with molecular studies.

4. CONCLUSION

DNA-based methods are frequently used in the field of fisheries, especially in molecular studies. In particular, revealing the gene regions of fish species using mitochondrial DNA has become quite common in recent years (Keskin and Can, 2009; Doğaç et al., 2016). The main reason for this is that DNA-based studies are important both in terms of reliability and accuracy of results. In such studies, DNA isolation is carried out with different methods and automatic isolation robots are used as the latest technology in this regard (Pappalardo et al., 2008; Ferrito et al., 2013). In studies conducted for DNA isolation, it has been reported that the manual Trizol method (Kessabi et al., 2010) and the CTAB (Cetyltrimethylammonium bromide) method (Cavraro et al., 2017) have also been used in addition to automatic isolation robots. As in similar studies (Pappalardo et al., 2008; Ferrito et al., 2013; Buj et al., 2015), DNA isolation robot was used in this study in terms of sensitivity and ease of operation. Real-Time PCR devices are used as the latest developed system in PCR studies performed with DNA and other double-chained products. These systems draw attention with their real and instantaneous results and their superiority in data analysis. Nucleic acid stains, beads, primers and probe types are used in Real-Time PCR studies. In this way, real-time monitoring of PCR products can be achieved. PCR amplicons are obtained with Real-Time enabled devices such as HRM and Melting analyses (Onalan, 2019). As in this trial, studies in which DNA isolations were performed with Real-Time PCR (Hrbek et al., 2002; Durna, 2009) were reported by researchers. Similar to the method used in the study, the use of the PCR method for the identification of Aphanius species has been reported by many researchers (Perdices et al., 2001; Vitturi et al., 2005; Pappalardo et al., 2008, Pfleiderer et al., 2014). On the other hand, RNA-based studies are generally using specific gene regions (mRNA) (Kessabi et al., 2010) and RAPD analyses (Alyahya, 2015). Studies on expression are carried out for oxidative stress, differences between

different populations of the same species (Perdices et al., 2001) and regulatory gene expression (Motamedi et al., 2019).

As in similar identification studies (Bardakçı et al., 2004; Doğaç et al., 2016; Teimoria and Motamedi, 2019), the same procedures were applied in the PCR analysis of this study and similar results were obtained. Primer pairs were obtained using the SnapGene and PrimerBlast web bases. Total DNA isolation was performed with the QIACube automatic isolation robot in order to identify the *A. mento* species and also SYBR-based PCR was performed in Real-Time PCR analysis. Results were evaluated as positive and negative according to sigmoidal curves and threshold values. In the PCR results obtained in the study, the fact that all samples gave similar sigmoidal curves showed that they matched exactly with the primers specific to *A. mento* and known as *A. mento* in terms of its external morphological features, the study showed that this species is not a different species or subspecies of the genus Aphanius at the molecular level, but is clearly *A. mento*. These results also constitute important data in terms of revealing the existence of phylogenetic differences between individuals of the same species with populations in different regions.

DNA isolated samples were dissected from dorsal muscle tissue as in similar studies (Perdices et al., 2001; Hrbek et al., 2002; Pappalardo et al., 2008). It has also been reported that studies were carried out by taking tissue from the caudal fin (Motamedi et al., 2019) in studies on mRNA.

Using different gene regions of mtDNA, such as the D-loop region of mtDNA (Cavraro et al., 2017), 12S and 16S ribosomal RNA (Hrbek et al., 2002), *cytochrome b* gene (Perdices et al., 2001) on Aphanius are available. Similarly, the mtDNA gene region was used in this study. The mtDNA gene region used in the study is also important in terms of being used to reveal the intraspecific differences between species belonging to the genus Aphanius. In addition, the gene region from the GenBank dataset was reported from different regions and was selected from mtDNA sequences belonging to *A. mento* only and did not belong to any species other than *A. mento* by nucleotide alignment. This increases the reliability of the results obtained from the study. The dendogram showing the intraspecies differences of the

sequences obtained from the mtDNA gene regions of different Aphanius species obtained from GenBank databases is given in Figure 11.

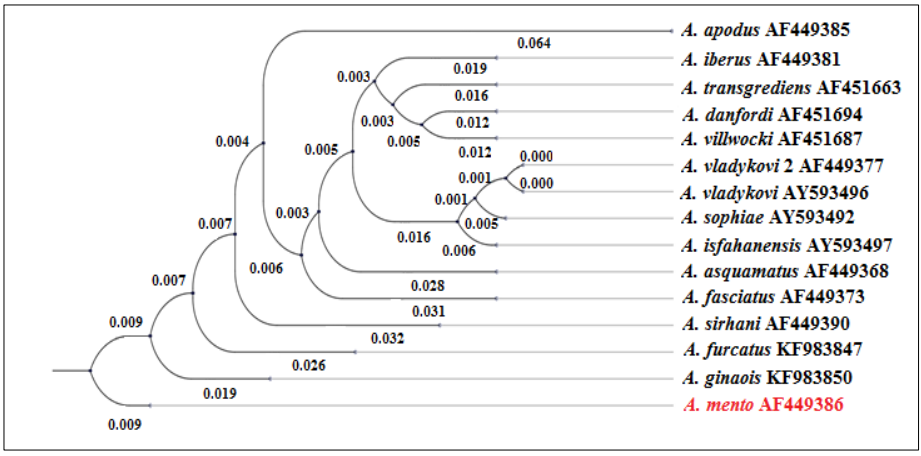

Figure 11. Genomic differences between species of the genus Aphanius.

According to the 12S rRNA gene sequence data in the *Aphanius* sp. mtDNA gene region, the closeness of Aphanius species to each other can be seen. In line with this graph, it is seen that the primers that will examine the differences between the species or the primers used for the identification of Aphanius on a family basis can be realized in future studies. In this study, as a result of molecular identification, it has been seen that systematic identification of Aphanius species can be performed with high reliability by using primer pairs on the basis of gene, and it is a practical and applicable method to determine whether there are intraspecies differences in species with different populations. In addition, the sequence data obtained as a result of this study is important in terms of being the first reported genomic data for the species forming the Nemrut Crater Lake population of *A. mento* and forming the basic database for determining the phylogenetic differences between the Nemrut population and populations in different regions in future studies.

5. ACKNOWLEDGEMENTS

This study was funded by Van YYU Scientific Research Projects Coordinatorship, grant no # **FDK-2019-7979** as part of a PhD thesis. Fish samples were made with the permission (No: numbered 67852565-140.03.03-E.3383136) of the Ministry of Agriculture and Forestry of Republic

of Türkiye, General Directorate of Fisheries and Aquaculture. And also this work was approved by Van Yuzuncu Yıl University, Animal Researches Local Ethic Committee in the season held on 28/07/2020 (decision no2020/07-11).

And we also thank the section editor and referees for their valuable help and suggestions in editing this chapter.

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CHAPTER 5

THE PAST AND PRESENT OF SOILLESS AGRICULTUREIN TÜRKİYE

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1. INTRODUCTION

Greenhouse cultivation is carried out on an area of approximately 5 million decares in the world, 1.8 million decares in Europe, and 810 thousand decares in Turkey. Turkey ranks 4th in the world and 2nd in Europe with its greenhouse area. 93% of the greenhouses in Turkey are covered with plastic and 7% are glass greenhouses. 21 thousand decares of existing greenhouses are modern greenhouses with high technology, and the average operating size of these greenhouses is 27 decares. Almost all of these modern greenhouses are produced with soilless farming technique. Vegetables are grown in 91% of the greenhouses, fruit in 8% and ornamental plants in 1%. Tomatoes are grown in 50.4% of the greenhouses where vegetables are produced, and bananas are grown in 73.9% of the greenhouses where fruit production is carried out (Anonymous, 2022).

Although it has been significantly broken in recent years, can the distrust caused by the perception that greenhouse products are hormonal and especially the intensive use of pesticides for plants grown in low-tech greenhouses can be overcome with greenhouses using high-tech soilless farming techniques?

In terms of commercial production, soilless agriculture, which has a history of about 70 years in the world and 30 years in our country, is seen as sufficient to eliminate this negative perception. As a matter of fact, good agricultural practices, which have a regulation in our country, can be applied more easily in greenhouse production in soilless agricultural enterprises. In order to compete in the globalizing world economy, it is not enough just to have a suitable climate and soil for agricultural production. The technology you use must also be new and advanced. The reason why the Netherlands, whose climatic conditions are more unfavorable than our country, has reached much higher figures in terms of yield per unit area in greenhouse cultivation is due to its use of modern production methods and advanced technology in greenhouse cultivation. Soilless agriculture has become a necessity rather than a choice, as it becomes difficult to produce efficient and quality production as a result of soil fatigue, salinity, soil-borne diseases and increase in the population of pests in regions where greenhouse cultivation is intense in our country.

In recent years, there have been significant changes in consumer demands, especially for freshly consumed products. Consumers, who are more sensitive and respectful to the environment and nature, demand products that are grown in natural or very close to nature with environmentally friendly production techniques instead of classical production systems. With soilless farming techniques, which are accepted among the sustainable agricultural methods all over the world, production that is environmentally friendly and protects consumer health can be carried out all over the world. The current organic agriculture regulation in our country does not allow the issuance of an organic product certificate for production using soilless farming technique. However, in the near future, this practice will change, and organic growing environments and plant nutrition products and production with soilless farming technique will be able to obtain an organic product certificate. The increase in the number of soilless agricultural enterprises using closed systems that save water and fertilizer is also important in showing the extent of environmental awareness. If our country's richness and climatic advantages in terms of geothermal energy, which is used as the most important alternative energy source in greenhouse heating, can be adequately evaluated, it constitutes a great potential power. Increasing the number of enterprises using soilless agricultural techniques, which provide the opportunity to produce with high quality and high efficiency, and to make environmentally friendly production that protects natural resources, will be beneficial not only in economic terms, but also in terms of protection of environment and natural resources and food safety for all humanity.

2. WHAT IS SOILLESS AGRICULTURE?

Soilless agriculture; It is the realization of all kinds of agricultural production in stagnant or flowing nutrient solutions, nutrient solution given in the form of fog, or in solid media fed with nutrient solutions. The purpose of soilless agriculture; To ensure the development of plants with the help of nutrient solution, to meet the nutrient and water requirements of plants without creating stress and to realize this in the most economical way. Soilless agriculture is a farming method that is applied especially in greenhouse cultivation, but has recently been used in open field cultivation (Gül, 2008). In

addition, thanks to the new production techniques and technologies brought by the era, it has started to be used in indoor (single-storey and indoor vertical farming) cultivation methods where artificial lighting technology is used today, apart from soilless agriculture greenhouse cultivation.

3. SOILLESS AGRICULTURE METHODS

Soilless agriculture is classified in many ways according to different researchers and in different countries. However, it is the most widely used classification method to examine soilless agriculture by dividing it into two groups, generally hydroponic and solid media culture.

3.1 WATER CULTURE (HYDROPONICS)

It is based on the principle of giving water enriched with nutrients to the root zone of the plant continuously or intermittently without using any solid media.

- Still Water Culture
  —Aerated
  —Without aerated
- Flowing Water Culture
  —Nutritional film technique (NFT)
  —Deep flowing water culture (Hyponica)
- Nutrient Plum Mist (Aeroponic)
- Aquaponic (It is a soilless farming method made by combining aquaculture and hydroponic system)

3.2 SOLID MEDIA CULTURE (SUBSTRATE CULTURE)

It is named according to the solid medium in which the plants are grown or according to the place in which they are placed.

- According to the solid medium used
  — Inorganic media: sand, pumice, perlite, vermiculite, rock wool, etc.
  —Organic media: peat, coconut fiber (cocopeat), sawdust, bark, etc.

• Depending on where the solid media is placed
  —Bed culture
  —Bag, package and pot culture
  —Vertical bag culture

4. WHY SOILLESS AGRICULTURE?

Increase in Demand for Food Resulting from Population Growth: It is accepted that the increasing need for food, brought about by the rapid increase in the world population, will not be sufficient to feed human beings in the future. According to the 2022 World Food Security and Nutrition Report (SOFI), prepared under the leadership of FAO, 828 million people worldwide are hungry in 2021, while approximately 2.3 billion people are undernourished (FAO, 2022). Therefore, 11.7% of the world's population has problems in accessing food.

While population growth is 0.5% in developed countries, this rate can reach up to 2.5% in developing countries. The rapid population growth of developing countries is expected to increase the world population from 6 billion in 2000 to 16 billion in 2060 (Atilgan et al., 2007). Although the global population growth rate will continue to decrease in the coming years, it is predicted that the world population will increase by 20% to 30% in 2050. In today's world, where the fertile lands that can be cultivated are decreasing day by day, higher yields must be obtained from the existing agricultural areas in order to meet the increasing nutritional needs.

Negativities of Climate Change on Open Field Crop Production: Increase in temperature as a result of global warming, changes in precipitation regime, natural disasters such as drought and flood cause climate change and adversely affect productivity in agricultural production. Therefore, greenhouse or indoor production methods, where plant production can be done under controlled conditions, constitute an important alternative in eliminating the negativities caused by climate change. Soilless farming techniques also find the opportunity to become more widespread, as they allow the air-conditioning to be kept at the most ideal level in terms of plant production.

Narrowing of Production Areas: Despite the rapid increase in the world population, limited agricultural areas; It is gradually decreasing due to erosion, desertification, conversion to residential areas and shifting to tourism with high economic returns. While there was 9 million km^2 of arable land in the world in the 1700s, today there is 27 million km^2 of arable land. In 2050, it is predicted that 3.2 to 8.5 million km^2 of arable land will be needed to meet the food needs of the world population (FAO, 2022). Therefore, in addition to the more effective use of existing lands, the marginal areas that are not suitable for agricultural production should be evaluated in terms of agricultural production.

Soil-Borne Diseases and Pests: Especially as a result of monoculture and intensive agricultural production, the population of diseases and pests in soils becomes more resistant both in quantity and by gaining immunity against chemical control methods. As a result, there is an obligation to use more and more toxic chemicals. However, in the long term, the solution of these problems with chemical control is not sustainable. In addition, it is not possible to control diseases such as tomato moth (*Tuta absoluta*) pest and Rugosa virus (ToBRFV), which have been a major problem in tomato cultivation in recent years, in open field cultivation and low-tech greenhouses. Therefore, soilless agricultural enterprises with high technology, where both greenhouse air conditioning and hygiene conditions can be provided at the maximum level, can also minimize the risk of encountering these problems.

Soil Fatigue: Since the production is carried out on a single type of plant pattern (mono culture), especially in greenhouse cultivation for many years, the problem of soil fatigue, which causes low yield in the long term, is definitely experienced sooner or later. There are two ways to overcome this negativity. The first of these is to change the greenhouse soil or its location, and the second is to use methods such as making changes in the product to be grown (crop rotation). However, the practical applicability of both in greenhouses is very difficult.

Environmental Pollution: The world is faced with the problem of environmental and soil pollution caused by agricultural chemicals that have been used extensively in the last century. Research shows that if alternative methods cannot be developed, agriculture will be carried out by using

chemicals more intensively in the future. In parallel with this, environmental problems arise. The deterioration of ecological balance and biological life, chemical residues in agricultural products have become a threat to human health. Intensive use of chemical fertilizers and pesticides often causes significant hazards for humans and their environment. In order to meet the needs of the growing population, sometimes less attention is paid to issues such as safety and environmental pollution, the effects of which may appear after a long time. As a result, uncontrolled use of chemical fertilizers and chemical pesticides in agricultural areas may come to the fore. High doses of nitrogen fertilizers applied to agricultural fields are converted to nitrate by nitrification by microorganisms in the soil and the nitrate is (-) loaded, causing significant amounts of nitrogen to be found in the ground water by washing the soil (Atilgan et al., 2007). The biggest change in the use of chemical fertilizers in agriculture over time is the increasing dose.

Although the problems caused by excessive fertilizer use in the Antalya region are not on the agenda much in today's conditions, previous studies in the region show that more fertilizer is used in greenhouses (Anaç, 2004).

Decrease in Water Resources: The decrease in the world's water resources due to global warming and the decrease in the quality characteristics of the existing ones necessitate the transition to water-saving production methods. More than 40% of agricultural products in the world are obtained from irrigated lands. These lands constitute 17% of the total lands. With the increase in population and the increase in water use in other areas, the amount of irrigated agriculture areas is decreasing. Irrigated agriculture, which reached the highest amount in 1978, decreased by 17-20% in 2020. Approximately 70% of the currently used water is consumed in the agricultural sector, 19% in industry, and 11% in urban and rural areas (Anonymous, 2022; Tuik, 2022). In order for a country to be considered water rich, it must have a water potential of more than 8,000 m^3 per person per year. Countries with less than 2.000 m^3 water, experiencing water scarcity, and countries with a water potential of less than 1.000 m^3 are considered "Water Poor". According to DSI data, it is predicted that our country, which has a usable water potential of 1519 m^3 per

capita, will decrease to 1120 m^3 of water per capita in 2030 with a population of 100 million (Anonymous, 2022).

Able to Produce in Non-Agricultural Areas: It is to enable agriculture to be carried out with high efficiency in countries lacking arable land. For example, by using the soilless agriculture technique, Israel both gets rid of foreign dependency and adds significant added value to the country's economy by producing its own agricultural product, even though it is located in a geography that is not suitable for agricultural production. In recent years, aid organizations, which have difficulties in helping the hungry and poor people in underdeveloped countries with food problems only with aid, also use soilless agriculture technique as an alternative method. For example; The United Nations Food Organization reports that in 2001, 796 million people in developing countries were on the verge of chronic hunger. The United Nations Food Organization spends several billion dollars each year for these people's expenses such as nutrition and treatment. However, the most suitable method is the project of establishing micro-gardens with soilless agriculture, which costs these people 350 dollars. If implemented, 200 million people will be able to get rid of hunger with an expenditure of approximately 70 billion dollars (Bradley & Marulanda, 2007). Using simplified hydroponic methods, pilot regions from poor Latin America and the Caribbean Islands were selected by FAO in order to ensure safe and healthy nutrition for low-income families in underdeveloped areas of cities. In addition, within the scope of Urban Agriculture; building, container, hangar etc. Crop production can be carried out with soilless farming technique by using completely artificial lighting technology in single-storey or vertical farming concept within sunlight-impermeable structures. In this way, logistics costs can be reduced to cities where agricultural products are consumed intensively from where they are produced, the shelf life of the products is increased by reducing the transportation times, and also because these systems enable production with zero pesticide use, they can also provide a healthier production.

Compliance with Certified Production: The quality of agricultural products and the impact of the production process on the environment have become an important issue all over the world, especially in the European Union

member countries. For this reason, manufacturers have had to consider not only the traditional dimension of products such as price, quality, consumer demands and standards, but also the effects of production stages on the environment and nature. Our companies exporting all over the world are aware of the sensitivity of consumers in these countries to health and the environment, and try to meet the needs of consumers by offering products in accordance with market demand and legal regulations.

In order for greenhouse businesses to export, their production must be made in accordance with GLOBALGAP criteria. The GLOBALGAP Regulation covers the entire production process from planting or planting the plant to the packaging stage. Its requirements do not include difficult conditions as in organic farming production. For example; Although only plant protection products specified in the legislation of the country of production can be used in Organic Agriculture; Under GLOBALGAP conditions, the plant protection product can be used on the relevant agricultural product as written on its label, provided that it is licensed. Soilless agricultural enterprises are in a much more advantageous position in terms of producing in accordance with the GLOBALGAP criteria.

Good Agricultural Practices are defined by FAO as "the processes that must be implemented to make the agricultural production system socially viable, economically profitable and efficient, protecting human health, giving importance to animal health and welfare and the environment". The aim of Good Agricultural Practices is to make production that will not harm human health and the environment. For this, Good Agricultural Practices recommends the application of Integrated Pest Management (IPM) and Integrated Crop Management (ICM) techniques together in production. Soilless agricultural greenhouses are controlled environments where Good Agricultural Practices can be applied more easily.

Increasing Competitiveness: Thanks to the high-tech automation systems used by soilless agricultural enterprises during production, labor and energy savings and higher efficiency compared to grounded greenhouses increase the competitiveness of enterprises with their competitors. In greenhouses with soil; Due to processes such as processing the soil, preparing

for planting, hoeing, making it suitable for irrigation, soil disinfection, fertilization of plants, and weed control, the need for labor is quite high.

Providing High Yield and Earliness: By making use of the technological opportunities of plant nutrition and ambient air conditioning, it is possible to obtain both early and high yields from balanced and well-nourished plants. For example, the average tomato yield of soilless agricultural enterprises in the Mediterranean coastal zone in our country is 30-35 tons/da. This figure can go up to 50-55 tons/da in geothermal heated greenhouses in the inner regions where the continental climate is dominant, where production can continue in the summer months.

Prohibition of the Use of Methyl Bromide: In greenhouse cultivation, where soil disinfection has become a mandatory practice against soil-borne diseases, pests and weed problems, especially in greenhouse cultivation, as a result of the Montreal Agreement signed by our country, the restriction of the application of methyl bromide, which is used as the most common soil fumigant all over the world, even soil As a result of the complete prohibition of its use in disinfection, soilless agriculture is seen as the strongest alternative in all studies investigating alternative applications that can replace this application.

Conservation of Nutrient Element: The amount of chemical fertilizer applied per unit area in greenhouse cultivation is much higher than in open field. As a result of this, both the negative impact of fertilization on the environment and its high proportionality in the economic production inputs require the producers to be more sensitive about the use of chemical fertilizers. In soilless farming methods, plant nutrients are given to the root zone of the plant in the form of dissolved ions in water in frequent periods, and since there is no loss such as being retained by the soil and washed into the lower layers, it provides great savings compared to soil agriculture. In fact, it is known that nutrient savings reach 60% in productions using NFT systems, which is a soilless farming method. Another important reason forcing the transition to soilless agriculture is the suspicion that world fertilizer production will not be able to meet the fertilizer requirement of normal soil agriculture in the future. Caplow (2009), reported that 75.000 tons of water was saved even though the production amount was the same, when he compared open-air cultivation on an

area of 10 hectares and hydroponic cultivation in which the nutrient solution circulated (closed system) on an area of 1 hectare.

High Greenhouse Heating Costs: The aim of an agricultural business is to obtain a high quality and high amount of products from a certain area with the lowest input cost. Greenhouse cultivation is a production method that increases profitability as much as heating expenditures can be reduced. Because heating is included in the total expenditures; It has a share of up to 40-80% depending on the growing season, product variety and location. In soil cultivation, especially in winter, the soil temperature decreases and as a result, root activity decreases. Heating to change the greenhouse climate is generally not very effective on the soil. However, in soilless culture, the heating of the growing medium, and therefore the root zone, occurs spontaneously while heating the greenhouse.

5. THE STATUS OF SOILLESS AGRICULTURE IN THE WORLD

Hydroponic cultivation techniques have been used by many different civilizations in the past: the hanging gardens of Babylon, the floating plants of the Aztecs, and the knowledge gained from ancient Egyptian hieroglyphs can be cited in documents mentioning aquaculture a few centuries ago. The real beginning of hydroponic science, which scientists first laid the groundwork for scientifically in the 1600s, was by Sachs, who created the first standard nutrient solution recipe in 1859-1865, and by Knop, who is called the father of water culture. In 1929, from the University of California, Dr. William F. Gericke was able to grow a 7.5 m long tomato plant using a nutrient solution. He named this new system "hyroponics", which means water work. In fact, hydroponics, which means water work, is used today to cover all soilless culture methods used in organic and inorganic solid environments (Gül, 2008).

Soilless agriculture, which has a very old history, started to be used in plant production in the 1960s in countries such as Italy, Spain, France, England, Germany, Switzerland, Russia and Israel. Soilless agriculture has started to be used as an alternative method to traditional soil agriculture in greenhouse

cultivation, which has more problems with soil structure, fertilization and soil-borne diseases.

Although the use of soilless agriculture in greenhouse vegetable cultivation was achieved in the United States between 1925 and 1935, the use of soilless agriculture in greenhouses was limited until the 1970s. The widespread use of soilless agriculture in commercial greenhouse production coincides with the 1970s, because with the world energy crisis, soil disinfection with steam ceased to be economical. Since the 1980s, soilless agriculture has gained importance as an alternative to chemical soil disinfection, which has negative effects on the environment. Especially in the 2000s, soilless agriculture came to the fore as an alternative to methyl bromide (Tüzel et al., 2004).

It is reported that there was a total of 10.000 ha of soilless greenhouse area in the Netherlands in 2001 and that rockwool was the most preferred growing medium. It is reported that there is a rapid development in Spain, one of the Mediterranean countries. It is reported that there were 3000 ha of soilless greenhouse area in 2000 and 4000 ha in 2001 in Spain (Tüzel et al., 2005), and that the soilless agricultural area in Spain reached around 5000 ha as of 2005 (Urrestarazu and Mazuela, 2005). In addition, the use of soilless agriculture shows rapid development in Morocco, which exports vegetables to Europe (Tüzel et al., 2005). 2.4% of the total 47000 ha strawberry production in Belgium and the Netherlands, that is 1140 ha, is done in a closed system using soilless farming technique (Lieten et al., 2004).

Although soilless agriculture is widely used in the Netherlands, the development of soilless agriculture is slow in greenhouse countries in the Mediterranean basin. The reason for this is explained as the salty waters, the expensiveness of some substrates, the difficulties in the management of the nutrient solution in relation to the substrate properties, the difficulties in the adaptation of closed systems.

China, which had only 0.1 ha of hydroponic cultivation in 1981, reached 50 ha in 1995. Most of these areas produce vegetables and some flowers. From 1996 to 2000, soilless agriculture was 815 ha with a national project. In 2005,

it reached 1250 ha. Organic production was carried out by hydroponic methods such as nutrient film technique (NFT), deep flowing hydroponic (DFH) and Lu-SC system. The eco-organic variety of soilless culture production method, which reached 496 ha with a great increase at the end of 2001, constitutes 60% of the total soilless culture area (Jiang et al., 2004).

Soilless agriculture in the Netherlands is done in 64.2% of the total greenhouse area, this rate rises to 71.6% in vegetable growing. The use of rockwool and cockpit is common as soilless media. Soil/environment disinfection in greenhouses is usually done with steam. More than 70% of the vegetables grown in the greenhouse are produced with environmentally friendly techniques and sold with labels. This ratio rises to 90% in tomatoes and peppers. Producers are members of sales cooperatives and sales are carried out through auctions. 90% of tomatoes grown in greenhouses, 85% of peppers and 80% of cucumbers are exported. It is reported that in the Netherlands in 2000, a closed system was used in 70% of the soilless greenhouse area and the use of closed systems in the entire production area of the vegetable species whose fruit is consumed is provided by law (Van Os, 1999).

Hydroponic production has undergone significant changes in the United States in recent years. The greenhouse tomato, which is considered tasteless and unhealthy, has become preferred and even paid a high price thanks to organic hydroponic cultivation. Organic hydroponic production is known as the 'niche' and is becoming increasingly common in America. Federal Organic Standards, enacted in January 2002, further facilitated the operation of the system. U.S.D.A. Products certified by the organic product label are enriched with pepper, cucumber, lettuce and medicinal plants as well as tomatoes. The industry, which combines hydroponic system and organic production in greenhouse production, will grow even more in the future (USDA, 2022).

According to 2007 data, a total of 950.000 m^3 of different substrates are used in approximately 8500 ha of soilless farming area in Europe. 600.000 m^3 of this is shaped rock wool and other foam products, 250.000 m^3 is perlite and vermiculite-like mineral aggregates, and 100.000 m^3 is coconut (cocopeat) fiber. Hydroculture is becoming increasingly common especially in the cultivation of vegetables, cut flowers and ornamental plants. In particular,

automatic irrigation and fertilization systems developed in recent years continuously measure the humidity in the growing slabs and determine the necessary water and air ratio for ideal cultivation.(Verdonck, 2007) However, due to the fact that rockwool is a synthetic medium, it could not compete with the cockpit because of the high production cost and the waste material after production, and it fell behind the cockpit in terms of usage.

In the Netherlands, the use of closed systems is encouraged in order to ensure sustainability in soilless agriculture. Although this problem has not been discussed in detail in Mediterranean countries, it is possible that open systems will cause environmental pollution with the increase in the greenhouse area where soilless agriculture is made. It is recommended to use the substrates for 4-5 years to reduce the waste substrate (media) problem.

Closed systems (Recirculated) in which the nutrient solution is reused are rapidly becoming widespread in many European countries. The compression made by environmental laws to reduce the use of nutrients, the system's saving of water and nutrients, and the better control of the food source are effective in the widespread use of recirculating systems. Recirculated systems provide up to 40% extra water nutrient savings.

The most important problem that can be experienced in the use of recirculated systems is the possibility that a disease that may arise at any stage of production can spread to the whole system. However, in developed countries where recirculated systems are widely used, this problem can be easily overcome with different solution disinfection methods. The most common of these are given in Table 1 (Van Os, 1999).

Tablo 1. Solution disinfection methods in closed systems in soilless agriculture

Nutrient solution disinfection methods in recirculated systems		
Method	**Dosage**	**Active pathogens**
Temperature Application	95°C for 30s	All pathogens
UV Rays	250 $mJ.cm^{-2}$	All pathogens (most commonly used)
Slow sand filters	100 $l.m^2.h^{-1}$; D_{10}<0.4 mm	Phytophthora, pythium
Hydrogen peroxide+activators		All pathogens
Lava Filter		All pathogens
Ozonization	10g ozone per m^3 for 1 h	All pathogens
Membrane filter		All pathogens

Although soilless agriculture is accepted as the most important alternative especially against soil-borne diseases all over the world, root diseases still emerge as the biggest problem in soilless agriculture. Although solution disinfection methods are very effective, they have disadvantages due to the risk of inability to provide complete disinfection, high installation and operating costs compared to open systems, and being destructive on not only harmful but beneficial microorganisms. Therefore, in recent years, it has been focused on the use of microorganisms against disease agents in soilless culture (Koohakan et al., 2004).For example, *Bacillus subtilis* is successfully used against fungal diseases caused by *Pythium* spp, *Fusarium* spp, *Phytophtora*, *Rhizoctania* and Anthracnose in our country.

6. THE STATUS OF SOILLESS AGRICULTURE IN TURKEY

The commercial use of soilless agriculture in our country started in Antalya in the early 1990s. It is reported that while the greenhouse area for soilless agriculture was 20 ha in 2000 (Sevgican et al., 2000), it was 40 ha in 2002 and 75 ha in 2005 in our country. As of November 2007, it has been

determined by us that there is a greenhouse farm that produces 278 ha of soilless farming technique, as indicated in table 2. This cultivation technique is generally used in modern greenhouse enterprises that produce for export and therefore in accordance with GLOBALGAP criteria. With the prohibition of methyl bromide since 2008, soilless agricultural areas have increased with a higher (Table 2).

Tablo 2. As of November 2007, the enterprises that produce with soilless agriculture technique in our country and their size in decares.

Soilless Agriculture Business	**Decare**	**Soilless Agriculture Business**	**Decare**
Agrobay(Dikili-İzmir)	300	Mehmet Türkmen(Antalya)	20
Özaltın (Serik-Antalya)	120	Alattin Aytekin (Kumluca-Antalya)	18
Haşim Balaban(Serik-Antalya)	120	Makamlar (Mersin)	13.5
Lider(Salihli-Manisa))	105	Biotar (Köyçeğiz-Muğla)	17
Bostan Tarım (Manisa)	100	Sandıklı Tarım(Sandıklı-Afyon)	17
Eskihisar(Mturgutlu-Manisa)	91	Ar-Ser Seracılık(Serik-Antalya)	17
Yükselen (Yanköy Silyon-Antalya)	83	Mehmet Baştürk(Sandıklı-Afyon)	17
Türkeli(Dikili-İzmir)	80	Aydın (Serik-Antalya)	16
Adalya (Gaziler-Antalya)	60	Umg(Denizli)	16
Batman (Karaöz-Antalya)	60	User Tarım(Manisa)	15
Agroser (Serik-Antalya)	60	Osman Kumbul(Serik-Antalya)	14
Cantexttil(Çorlu)	54	Nas Tarım	14
As Tarım Ada Çorap(Denizli)	50	Gümüş Köyü-Germencik-Aydın	14
Uzman Tarım Dönüş (Urfa)	50	Özbey (Aksu-Antalya)	13
Sözmen(Seferihisar)	45	Selahattin Ataç(Adana)	12
Antalya Tarım(Antalya)	42	Muslim Yanmaz(Urfa)	12
Çalışkanlar(Adana	40	Mustafa Çınar(İzmir)	7.5
Agrion(Dikili-İzmir)	40	Cüneyt Doğan (Karaöz-Antalya)	11
Akbulut (Mersin)	40	İsmail Çetin(Antalya)	6.5
Agh (Aksu-Antalya)	32	Flora Sera (Diyarbakır)	11
Vegevital(Dikili-İzmir)	32	Özdoğanlar(Antalya)	11
Halis Seracılık (Adana)	32	Korhan Özada (Hasyurt-Antalya)	10
Yıldız Tarım(Antalya)	31	Meşhur(Kundu-Antalya)	10
Ali Çam (Serik-Antalya)	30	Onur Kumbul(Serik-Antalya)	10

Balabanlar- Bilgin (Serik-Antalya)	30	Egeler (Serik-Antalya)	10
Ts (Gaziler-Antalya)	30	Alduran(Alaşehir-Manisa)	10
Azim Textil(Denizli)Boss	30	Ahmet Altındağ(Manisa)	10
Kipaş(K.Maraş)	30	Yentex(Adana)	10
Akcelep(Dikili-İzmir)	30	Kmk(Maraş)	10
Ayer Tarım(Antalya)	30	Hasan Okutan(Antalya)	10
Ateşoğlu(Antalya)	30	Ofa Tarım (Aydın)	10
Roseland (Mersin)	30	Necmettin Gökkaya(Kumluca-Antalya)	9
Mb.Şrt. Grup-Ege Enerji(Aydın)	29	Afo (Serik-Antalya)	9
Meristem (Aydın)	26	Mehmet Kumbul(Serik-Antalya)	8
Gökşin Kimya(Denizli)	25	Habib Gönül(Antalya)	8
Erten(Antalya)	25	Çimenoğlu(Denizli)	8
Bademli(Seferihisar)	25	Tuğtekin Seracılık(Yalova)	8
Ekinciler (Mersin)	25	Abdullah Kerimoğlu(Serik-Antalya)	7
Salih Şubaşı (Hasyurt-Antalya)	24	Enerji Tarım(Antalya)	7
Tevfik Şekerci (Kumluca-Antalya)	24	Mithat Kadıgil (Boztepe-Antalya)	5
Naturel(Mersin)	22	İpektarım(Antalya)	5
Eylül Tarım (Mersin)	21	Özada(Antalya)	5
Piribeyli (Silyon-Antalya)	20	Ali Çevik (Adana)	5
Tria Tarım(Antalya)	20	Erhan Bafralı(İzmir)	3
Murat Gönçüoğlu(Turgutlu-Manisa)	20	H.Hüseyin Erbudak(İzmir)	2
Hasan Serin(Mersin)	20	Nevzat Güner(İzmir)	2
Ektar(Adana)	20	Şener Piyar(İzmir)	1
Kocabaylar(Denizli)	20		
Total: 2787.5			

While the area produced with soilless farming technique was approximately 278 ha in 2007, it has reached approximately 883 hectares as of March 2015. In the last 8 years, the area produced with soilless farming technique has increased by about 4 times. It has increased from 95 to 188, and the number of businesses has doubled (Table 2, Table 3).

Tablo 3 As of November 20015, the enterprises that produce with soilless agriculture technique in our country and their size in decares.

Soilless Agriculture Business	**Decare**	**Soilless Agriculture Business**	**Decare**
Agrobay (Dikili-İzmir)	650	Altunkaya(Şanlıurfa)	100
Özaltın (Serik-Antalya)	120	Ersezgin(Dikili-İzmir)	40
H.Balaban(Serik-Antalya)	350	Yda Tarım(Dikili-İzmir)	140
Süral(Serik-Antalya)	430	İlkser(Sorgun-Yozgat)	30
Kaltun(Serik-Antalya)	270	Başyazıcıoğlu(Nevşehir)	62
Çekok(Serik-Antalya)	30	Başyazıcıoğlu(Yozgat)	60
Agro-Ser(Serik-Antalya)	75	SemayTarım(Afyon)	59
Ar-Ser(Serik-Antalya)	18	AkasTarım(Afyon)	48
Menderes(Denizli)	280	Vema A.Ş(Karaali-Şanlıurfa)	100
Pekdemir(Denizli)	180	Lidersan(Karaali-Şanlıurfa)	100
Bostan(Manisa-Afyon)	260	Gülizar(Karaali-Şanlıurfa)	110
Lider(Salihli-Manisa)	380	Yıldız(Karaali-Şanlıurfa)	30
İmöz(Serik-Antalya)	30	Haypol(Karaali-Şanlıurfa)	25
MuzafferYıldırım(Serik)	45	Sambur(Karaali-Şanlıurfa)	15
Açanal(Karaali-Şanlıurfa)	10	Eskihisar(Turgutlu-Manisa)	110
Ortanca(Karaali-Şanlıurfa)	10	BirleşikTarım(Serik-Antalya)	100
GazipaşaTarım(Antalya)	40	Azim Tekstil(Denizli)	35
Ada(Sarayköy-Denizli)	50	Ekinciler(Mersin)	55
Smyrna(Sarayköy-Denizli)	200	Özdoğanlar(Karaöz-Antalya)	28
Yükselen(Silyon-Antalya)	102	AFO Tarım(Serik-Antalya)	15
Akdeniz(Serik-Antalya)	30	Güneşler(Gaziler-Antalya)	60
Kıraklılar(Serik-Antalya)	47	Aksoy(Gaziler-Antalya)	43
Aydın-Ser(Serik-Antalya)	16	KurtTarım(Çobanlar-Afyon)	50
Agrocan (Çorlu)	100	KipaşTarım(Kahramanmaraş)	50
Batman(Karaöz-Antalya)	65	BM Agro(Söke-Aydın)	55
Berkmuz(Serik-Antalya)	63	Bademli(Seferihisar-İzmir)	30
Enerji(Kurşunlu-Antalya)	10	Mone Tarım(Şanlıurfa)	30
Aydemirler(Çandır-Antalya)	8	Vegevital(Dikili-İzmir)	100
Piribeyli(Gebiz-Antalya)	48	Agrion(Dikili-İzmir)	40
Tria(Kızıllı-Antalya)	20	Celepler(Dikili-İzmir)	45
TS Tarım(Antalya)	52	Türkeli(Dikili-İzmir)	80

Gökkale Tarım(Aydın)	150	Ulu Tarım(Manavgat)	40
Yiğit Seracılık(Kırşehir)	100	Kahvecioğlu(Antalya)	20
Hatipoğlu(Manavgat)	50	Uzmanlar(Serik-Antalya)	40
ŞabanKüçük(Silifke-Mersin)	7	Selinus Tarım(Manavgat)	65
FerhatTarım(Silifke-Mersin)	13	Yaşa Kunt(Mersin)	34
Endaze(Erdemli-Mersin)	13	Garden ve Koala(Tarsus)	23
Tokuçoğlu(Gebiz-Antalya)	22	Torku(Çumra-Konya)	60
ArifDoğan(Mazıdağ-Mardin)	30	Diyadin Jeotermal(Iğdır)	20
Mopaş(Salihli-Manisa)	70	San Tarım(Sandıklı-Afyon)	45
Eylül Tarım(Denizli)	25	Halim(Kızıltepe-Mardin)	20
NC Tarım(Antalya)	30	Mehmet Erol(Mardin)	30
Arif Kayar(Antalya)	35	SandıklıTar(Sandıklı-Afyon)	27
Meristem(Aydın)	30	MehmetÖzbey(Aksu-Antalya)	13
Yaşaroğlu(Kiriçiler-Antalya)	15	Makamlar(Mersin)	20
NecmettinGökkaya(Antalya)	15	Biotar(Köyceğiz)	25
AlaattinAytekin(Antalya)	20	MehmetBaştürk(Sandıklı)	20
KumlucuTar.(Kumluca)	10	ÇilekSeracılık(Mersin)	30
CemilAlış(Eminceler-Serik)	20	Umg(Denizli)	20
Sözmen(Seferihisar)	50	UserTarım(Manisa)	15
Çalışkanlar(Adana)	50	Özada(Antalya)	8
Akbulut(Mersin)	40	BekirKumbul(Antalya)	10
MehmetTürkmen(Antalya)	20	Adalya(Antalya)	60
OsmanYıldız(Antalya)	18	NasTarım(Antalya)	14
Akcelep (Dikili-İzmir)	50	Gümüşköyü-Aydın	14
Ayer Tarım(Antalya)	30	Gökler(Nusaybin-Mardin)	10
MehmetKumbul(Antalya)	40	SelahattinAtaç(Adana)	12
MuslimYanmaz(Urfa)	15	CüneytDoğan(Karaöz)	11
MustafaÇınar(İzmir)	7.5	İsmailÇetin(Antalya)	6.5
Flora Sera(Diyarbakır)	15	Türkmenoğlu(İzmir)	12
Yeni Önder Seracılık(Adana)	16	Serin Tarım(Mersin)	21
Boss Tarım (Denizli)	35	Gökşin Tarım(Denizli)	30
Dönüş A.Ş (Urfa)	50	İlke Tarım (Antalya)	22
Grup-Ege Enerji (Aydın)	30	Karadayılar(Antalya)	20
Halis Seracılık(Adana)	35	İstanbul Tarım(Antalya)	14

Ali Çam(Antalya)	30	Naturel Tarım (Mersin)	30
Kocabaylar(Denizli)	20	Roseland (Mersin)	30
Sakaryabotanik(Sakarya)	5	TevfikSubaşı(Kumluca)	25
Ansel Tarım(Afyon)	35	VıctoryResortHotel(ANT)	50
Genpor Tarım (Afyon)	30	KöprüçaySeracılık(ANT)	50
HüseyinAltınkaynak(Afyon)	20	Kula Tarım(Alanya)	7.5
SalihSubaşı(Antalya)	25	Naturel Tarım(Tarsus-Mersin)	4
Çimenoğlu (Denizli)	10	Rosella Tarım(Alanya)	17
Fidan Tarım(Diyarbakır)	15	SeçkinZiraat(Mersin)	10
GökhanDemirci(Bursa)	8.1	Vitroplant (Adana)	10
Yedіveren(Sakarya)	5	Ekoturka A.Ş(Adıyaman)	5
Ayık Tarım(Ankara)	5.5	Medeni Tarım(Diyarbakır)	5.5
Sarten Ambalaj(Bursa-İstan.)	5	Karaman 21(Diyarbakır)	7.5
Karataş Petrol(Bursa)	1.5	MNA Seracılık(Diyarbakır)	7.5
Tan Yapı(Eskişehir)	0.6	Aydem İnşaat(Diyarbakır)	7.5
İmamoğlu Çiçek(İstanbul)	1.1	Elita Tarım(Diyarbakır)	7.5
MİA Lojistik(İstanbul)	7.4	Bismil Tarım(Diyarbakır)	7.5
Boydak Holding(Kayseri)	0.2	Deren Fide(Fethiye)	8.1
Tuğtekin çiçek(Yalova)	0.3	ITC Sera(Konya)	1.2
Has San Tarım(Afyon)	15	Sanser seracılık(Afyon)	18
Yaşarlar Seracılık(Afyon)	60	Ali Delil(Mersin)	7
Liva Tarım(Afyon)	50	Şık Tarım(Cizre-Şırnak)	10
Mehmet Aydın(Antalya)	21	Olba Hay.Tar.Tic (Mersin)	16
Olbia Tarım(Antalya)	13	Kuntar Tarım(Mersin)	20
Mehmet Uysal(Adana)	7	Arkadaş Tarım(Mersin)	26
Hasan Cengiz(D.bakır-Silvan)	12	BRY Tarım(Adana)	12
Sultan sera(Aydın)	41	SMS Tarım(Antalya)	24
Serdar Çakır(İzmir)	32.5	Yusuf Ekin(Antalya)	22
Tutku Tarım(Aksaray)	40.4	Korel Tarım(Afyon)	50
Total 8834.4			

The possibility of obtaining high efficiency from the unit area provided by greenhouse cultivation is increasing day by day thanks to the newly developed soilless farming techniques. This situation accelerates the transition to soilless agriculture. Many commercial establishments serving in very

different production branches such as construction, tourism, and textiles, by investing significant amounts of capital, enter the greenhouse sector, which is equipped with modern technology and uses soilless farming technique, as a second investment area besides their main field of expertise.

Modern soilless agricultural greenhouses with high technology, heated with geothermal energy, can provide a return on investment in the 4th year of production; It shows that it is a profitable investment area with its 1.56 benefit-cost ratio, 21% financial profitability and 33.3% internal profitability ratio.

Especially in EU countries and other rich countries with high living standards, consumer demands for freshly consumed products have undergone significant changes. Consumers, who are more sensitive and respectful to the environment and nature, demand products grown in natural or very close to nature with environmentally friendly production techniques instead of classical production. In recent times, the number of those who claim that all greenhouse products are hormone and drug residues and therefore not healthy products is increasing day by day, it is even possible to make organic production using organic product certified substrates, nutrient sources, insecticides, fungicides and biological control methods.

Turkey's 2022 fresh fruit and vegetable export was approximately 5 million tons and reached 2.95 billion dollars (Tuik, 2022).Tomato ranked second among the products exported with 272.6 million dollars. The contribution of our soilless agricultural enterprises, which can produce at European standards in terms of yield and quality, is indisputable in the very high percentage of tomatoes among exported vegetable species. This export success of enterprises using soilless agriculture technique shows us that in order to exist in the foreign market, the number of enterprises that use modern technology that performs high quality and efficient production, that is, enterprises that use soilless agriculture technique, needs to increase.

Greenhouse is a production method that increases profitability as much as heating expenditures can be reduced. In our country, great importance is attached to the use of geothermal resources, which have been considered as an environmentally friendly alternative energy source all over the world in recent

years, in greenhouse heating. Especially recently, Dikili-İzmir, Afyon Karahisar, Sarayköy-Denizli, Manisa, Kırşehir and Nevşehir regions have become the most popular centers of greenhouse companies using soilless farming techniques with their geothermal potentials suitable for greenhouse cultivation. However, it cannot be said that our country, which ranks 7th in the world in terms of geothermal resource richness, and 1st in Europe, can evaluate this advantage in terms of greenhouse cultivation. Approximately 15000 decares of greenhouse area in the world is heated with geothermal energy. In our country, approximately 5.293 decares of greenhouse area is heated with geothermal energy. Its share in our total greenhouse area of 541.000 decares is only 0.98%. A thermal energy of 0.2 MWt is required to keep one decare of greenhouse at 15 ˚C in Turkey conditions. Since the potential geothermal capacity in our country is 31500 MWt, it is possible to heat approximately 150000 decares of greenhouse area using geothermal energy.

Hydroponic systems are preferred especially in the cultivation of salad-lettuce group plants and medicinal and aromatic plants, while soilless agriculture is carried out mostly with the solid media culture technique in soilless agricultural enterprises in our country. However, NFT system is used more widely in advanced countries in soilless agriculture. This system is preferred in the world and in our country, especially in multi-storey production systems made in closed systems using artificial lighting.

7. THE PAST AND PRESENT OF SOILLESS AGRICULTURAL ENTERPRISES IN TURKEY

In order to have more detailed information about the current structure of the enterprises in our country, a survey application was carried out by contacting face-to-face and by phone in 2007 and 2015. In this way, the development process and trends of soilless agricultural enterprises over time have been determined.

Question 1: Name and Location of the Enterprises

As of November 2007, the distribution of the enterprises that produce with soilless farming technique in greenhouse agriculture in our country, according to the provinces, is as in Table 4.

Tablo 4. Distribution of business producing with soilless agriculture technique in our country according to provinces (2007).

City name	**Area (decare)**	**Ratio (%)**
Antalya	1110.5	39.8
İzmir	582.5	20.9
Manisa	351	12.6
Denizli	149	5.4
Mersin	171.5	6.2
Adana	119	4.3
Urfa/K.Maraş/Diyarbakır	83	2.9
Afyon/Aydın/Muğla/Yalova	221	7.9
Total	**2787.5**	**100**

Considering the distribution and decare criteria of soilless agricultural enterprises in our country in 2007, it is seen that 50% are located in the Mediterranean Region and 40% are located in the Aegean Region. in the Aegean Region; İzmir-Bergama, Manisa-Urganlı and Denizli-Sarayköy are areas with suitable geothermal resources for greenhouse cultivation. Especially recently, it is one of the regions where entrepreneurs who want to invest show the most interest. Afyonkarahisar follows these regions again due to its geothermal resource richness.

As of February 2015, the distribution of the enterprises that produce with the soilless agriculture technique in greenhouse agriculture in our country as closed area according to the provinces is as in table 5.

Tablo 5. Distribution of business producing with soilless agriculture technique in our country according to provinces (2015).

City name	Area (decare)	Ratio (%)
Antalya	3920	40.4
İzmir/Aydın	1630	16.8
Afyon	677	6.9
Manisa	685	7.4
Denizli	885	9.1
Adana/Mersin	600	6.1
Urfa/K.Maraş/Diyarbakır/Mardin	792	8.1
Sakarya/Yalova/Tekirdağ	134	1.3
Nevşehir/Yozgat/Kırşehir Aksaray/Konya	355	3.7
Ağrı	20	0.2
Total	**9698**	**100**

Considering the distribution and decare criteria of soilless agricultural enterprises in our country in 2015, it is seen that 48.7% are located in the Mediterranean Region and 33.3% are located in the Aegean Region. There has been a great increase in the last 10 years in the Central Anatolia and Southeastern Anatolia regions. Especially in Manisa-Salihli, Afyon-Sandıklı, Yozgat, Kırşehir, Nevşehir regions, greenhouse cultivation is increasing in these regions due to the use of geothermal energy for greenhouse heating and because it provides a greater profitability than the use of coal for heating, and because the vegetation periods are longer than the Mediterranean and Aegean Regions.

As of 2022, greenhouse cultivation is carried out in 57 provinces in our country by using soilless agriculture technique. In the distribution of enterprises according to the provinces; Antalya is the province with the most soilless agriculture with 22%, followed by Mersin with 12%, İzmir with 11% and Afyonkarahisar with 10%.

Question 2: What is the Size of Your Greenhouse, in which you apply soilless agriculture, in decares?

The classification of the enterprises according to their closed areas is as in Table 6. Due to the high initial investment costs of small entrepreneurs, they could not invest in large areas, so they established their businesses in small areas, and therefore, m² installation costs were higher. The feasibility studies show that the general cost remains the same when the area of the enterprises exceeds 50 da. However, the profitability rate increases due to the increase in productivity. When the relationship between area size and efficiency and profitability is examined, it is observed that the most profitable enterprises are those with 100 hectares and above. When the enterprises using soilless agriculture technique in our country in 2007 are examined, it is seen that the enterprises with a closed area of less than 50 decares constitute 85% of the total enterprises, while those with an area of 100 decares or more constitute only 5% of the total enterprises. Therefore, it is not possible for us to compete with our foreign competitors with the size of the enterprises in 2007.

When the enterprises using soilless agriculture technique in our country in 2015 are examined, it is seen that 62% of the enterprises with a closed area of 50 decares and those with an area of 100 decares or more have a share of 13% (Table 7). Therefore, it is clearly seen that the size of the enterprise has increased compared to 2007. The increase in the size of the enterprises was realized with the business people from different sectors (tourism, construction, textile, industrialists, etc.) entering the sector due to the availability of geothermal energy and the profitability of the investment.

Tablo 6. Distribution of soilless agricultural business by size in 2007.

Size of Business (de)	Business (unit)	Ratio (%)
1–9	19	19.6
10–29	46	47.4
30–59	21	21.6
60–89	5	5.2
90–119	3	3.1
120-above	3	3.1

Tablo 7. Distribution of soilless agricultural business by size in 2015.

Size of Business (de)	Business (unit)	Ratio (%)
1-9	30	15.9
10-29	52	27.6
30-59	62	32.4
60-89	19	9.6
90-119	11	6.6
120- above	14	7.9

As of 2022, geothermal heated greenhouses are carried out in a total area of 10.000 decares in 19 provinces in our country, and especially Aegean, Central Anatolia and Southeastern Anatolia Regions are using their geothermal resource wealth with a rising momentum in the field of greenhouse cultivation. In addition, the establishment of "Specialized Agricultural Greenhouse Organized Industrial Zones", as in İzmir Dikili, contributes to a much higher increase in the areas where soilless agriculture is made. As of 2022, our modern greenhouse with high technology is 21 thousand decares, and our average business size is 27 decares.

Question 3: What Types of Plants Do You Grow?

In 2007, 80% of the holdings grew tomatoes, 10% cut flowers, 9% California-type peppers and 1% beans and cucumbers. pepper, 7% cut flowers, 1% cucumbers and 1% strawberries. California Wonder type pepper is generally grown as a second crop next to tomato in large-scale enterprises.

Question 4: What is your yield per decare in production?

Although the amount of productivity in production among the enterprises varies according to the type and variety cultivated, there are differences in yield according to the region where the cultivation is made. This is related to the vegetation period of the regions. While production is between 9-10 months in the Mediterranean Region, excluding summer months, due to the high

temperature, production is made for an average of 11 months in the Central Anatolia Region, since the greenhouse can be cooled in the summer months. In extreme cases, there are also enterprises that produce for 14 months. Yield levels according to the plant species grown are given in Table 8.

Tablo 8. Yield levels per decare of businesses.

Type of Plant Grown	**Average Yield**
Tomato (Mediterranean-Central Anatolia)	30-55 tons/da
Capia and California type pepper	14-18 tons/da
Strawberry	15-20 tons/da
Cucumber	20-23 tons/da
Rose	70.000-80.000 pcs/year

Question 5: In Which Market Do You Evaluate Your Products (Domestic Market/Export) ?

Especially when large-scale enterprises work mainly on exports, they have started to supply products to the domestic market in recent years due to the high demand for their products from the domestic market. Since 1st class products are generally sent for export, businesses sell their 2nd and lower class products to the domestic market with a separate trademark. Therefore, companies that are seen as exporters can also supply a certain amount of products to the domestic market. While 80% of the existing businesses exported in 2007, this rate reached 85% in 2015 and 90% in 2022.

Although our exports are intense mainly in November, December, January, February and March, the products that go to export are mainly Russia, other Balkan countries and European countries. Exporting enterprises, whose production periods extend into the summer months (June-July), are evaluating their products in the domestic market due to the decrease in prices in foreign markets. However, the disruptions in greenhouse production as a result of the recent energy crisis in Europe created the opportunity to export to Europe in the period after April.

Question 6: Which Solid Media Do You Use in Production?

In 2007, rockwool was used in 40% of the total soilless cultivation areas, perlite was used in 40%, and coconut fiber (cocopeat) was used in 20% of the total soilless cultivation areas, while in 2015, Cocopeat, 10% Perlite, 5% Rockwool. Although the perlite orientation has increased, especially in small-scale enterprises, in recent years, the high increase in energy costs in our country, especially in 2021 and 2022, has increased the production costs during the processing of perlite, which has led to an increase in the price of perlite compared to cocopeat. Since our country has rich perlite reserves, the widespread use of perlite in soilless agriculture is of great importance in terms of both reducing foreign dependency and increasing the use of domestic resources.

Question 7: What are your reasons for choosing the solid media you use?

Producers, who stated in the 2007 survey that rock wool provides earliness and an extra yield of 4-5 tons/da (in tomatoes) compared to perlite, still prefer perlite because it is cheaper. In addition, our observations show that the advantages of rockwool come into prominence when combined with modern technology and success in plant nutrition. In the interviews held in 2007, it was reported that businesses using perlite generally prefer unpackaged open perlite, which is called bulk, because it is more economical, and they do the packaging themselves. The biggest problem faced by the enterprises using perlite in this production period is that the solid medium with low water holding capacity at the beginning causes diseases in the roots of the plants due to insufficient drainage in the following periods. As a reason for this situation, it is stated that in some batches of perlites produced in the last period, the particles do not explode sufficiently and do not form enough pores to hold water on their surfaces, presumably due to the lack of heat during expansion.

Cocopeat medium is preferred because it offers less risk, less mistakes and easy breeding. There is no problem with any delay in irrigation in Cocopeat. Since it is an organic material, it is richer in nutrient content than others. It develops the plant well vegetatively and saves on fertilization due to its high

organic matter content. In addition, due to the low EC value of cocopeat and its organic origin, high lignin, cellulose and hemicellulose contents are other important reasons for preference.

Question 8: How Many Years Do You Use Without Changing the Solid Medium You Grow Your Plants?

Producers who have problems in the root zone of the plant during the production season and those who do not want to take risks due to the possibility of disease transmission change their solid environments at the end of the production season without entering the disinfection process. However, businesses that do not have a disease problem are preparing for the new production season after disinfection processes. The use of Cocopeat is recommended for an average of 2-3 years. Because cocopeat will not be a suitable environment for plant root development as compaction, collapse and dust formation will occur due to its fibrous structure in the coming years. But some exceptions from manufacturers use up to 6-7 years. Since Cocopeat is an organic material, it can be used in different areas (mortar material in ornamental plants, etc.) after its use is over. Rockwool allows one year production. It is an inorganic material and is available in businesses that use it for two years. Perlite is used as an annual, but mostly perennial is used in this material. Businesses use an average of 3-4 years. Because when it is used too much, it loses its material properties and is not a suitable environment for the plant.

Question 9: How Do You Procure Your Nutrient Solution?

Since all of the enterprises are more economical, they prepare their own nutrient solutions as stock solutions. Production managers and consultants undertake these operations. They use recipes that are suitable for the results of water analysis, the growing medium used, the growing season and the plant type.

Question 10: Do you reuse your nutrient solution?

95% of businesses prefer the open system. The lack of a legal sanction in our country in this regard, the fact that closed systems require additional

equipment, increase the investment costs, and the risk of spreading diseases seem to be the most important reasons why businesses do not prefer closed systems. Some of the enterprises using open system use their waste solutions in open field in orchards. In addition, some of the enterprises can reuse the nutrient solution in the closed system in order to reduce costs by taking risks in a few months close to the end of the production season. However, recently, especially large-scale enterprises in our country have started to take serious initiatives towards the transition to the closed system. For nutrient solution disinfection in closed systems, the most commonly preferred system in our country as well as all over the world is the disinfection method using UV rays. However, in hydroponic cultivation (NFT systems, indoor vertical farming applications, etc.), which has increased in number and area in recent years, closed systems are widely used. With the increase in these enterprises, there has been a remarkable increase in the proportion of enterprises using closed systems.

Question 11: Do you plan to increase the size of your business in terms of area in the future?

95% of businesses are considering expanding their businesses in the future. In the soilless production technique, which allows quality and efficient production, there is no market problem and the investment expenses of the enterprises return in a short time like 3-4 years, so the growth tendency of the enterprises as a field in their future strategies is heavy. However, some businesses cannot expand their businesses due to the fact that they do not have enough land to expand and due to the capital insufficiency problems in small family businesses. Businesses selling abroad are considering shifting some of their production areas to geothermal areas because of their customers' demands for 12 months of uninterrupted goods. The lack of sufficient quantity and quality of irrigation water in their regions is also effective in the failure of the enterprises to grow.

CONCLUSION

Soilless agriculture, which started in the province of Antalya, where the first commercial enterprise was established in the 1990s in our country, is carried out today in approximately 15,000 decares of land in 57 different

provinces. While soilless agriculture is increasing with a high acceleration in Turkey, the knowledge, experience and trained technical personnel power obtained in this field are also used in the establishment and operation of new enterprises abroad. In recent years, soilless agriculture has ceased to be a plant production method applied only in classical greenhouse enterprises, plant factories, vertical agriculture, etc. where the light demand of plants is completely provided by artificial lighting. It is also spreading rapidly in light-impermeable indoor cultivation. Therefore, it is necessary to develop systems suitable for this new cultivation method in soilless agriculture in the future.

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CHAPTER 6

THE EFFECT OF IMPROVEMENT AND MANAGEMENT APPLICATIONS ON THE BOTANICAL COMPOSITION AND YIELD OF THE PASTURE

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INTRODUCTION

A significant portion of Turkey's pastures is located in arid and semi-arid zones, and the decreaser species, which constitute the most critical component of climax vegetation, are on the verge of extinction due to grazing pressure. Excessive and continuous grazing has led to the dominance of invasive species in the denuded pastures left behind by the decreaser species, posing a threat to animal product quality and animal health (Babalık & Ercan, 2018; Babalık & Fakir, 2017; İspirli et al., 2016; Koç et al., 2005; Seydoşoğlu et al., 2015; Uzun et al., 2016; Ünal et al., 2012; Yavuz et al., 2012).

In Turkey, pastures cover an area of 14.6 million hectares, with approximately 12.9 million hectares identified, 8.9 million hectares restricted, and 5.7 million hectares allocated. Pasture improvement and management projects have been initiated in some of these areas. Since 1998, a total of 1981 projects have been completed, covering approximately 1.4 million hectares of pasture in improvement practices (Anonymous, 2022).

To restore the productivity and quality of Turkey's natural pastures, which have suffered due to non-compliance with fundamental principles of use such as grazing season and capacity, urgent improvement efforts are required (Altın et al., 2005; Nadir et al., 2012; Yavuz et al., 2020). In addition, grazing pressure on pastures should be reduced during the improvement process. For this reason, increasing the production of high-quality roughage is essential. Since expanding the agricultural production areas today is impossible, this increase is only possible by increasing the yield obtained from the unit area (Kir et al., 2023). To achieve positive results from pasture improvement, it is necessary to apply various improvement methods, sometimes simple and sometimes multiple, in combination (Altın et al., 2005; Aydın & Uzun, 2000; Gençkan, 1985; Gökkuş & Altın, 1986).

Various studies are conducted on weed control, seeding, fertilization, aeration, and improvement methods within the scope of pasture improvement activities. Additionally, infrastructure development such as the construction of water troughs, artificial or natural shade structures, wire fences for perimeter enclosure, rubbing posts, drainage for excess water removal, and promotion of forage crop cultivation are implemented. These practices require significant

resources, and the success of projects depends on identifying the shortcomings of improvement programs and determining farmer approaches to ensure the profitable use of resources.

Since enacting the Pasture Law in 1998, the Ministry of Agriculture and Forestry has extensively utilized resources to carry out pasture improvement and management projects to enhance the country's pastures. Assessing the extent to which the objectives of implemented improvement projects have been achieved is crucial for ensuring the profitable use of resources and identifying challenges or factors that hinder the practical implementation of improvement projects.

This study aims to determine the changes in vegetation and productivity of the pasture resulting from improvement and management practices carried out between 2015 and 2018 in Epçeli Village, Çarşamba district, Samsun Province.

MATERIAL AND METHOD

Materials

Characteristics of the Study Area, the Pasture

The study area, the pasture, is located 2.5 km southeast of the village center of Epçeli in Çarşamba district, Samsun province, on the border of Karakulak village (41°14'26" N, 36°36'56" E). The study was conducted in 2019 following the Pasture Improvement and Management Project carried out between 2015 and 2018 (Figure 1).

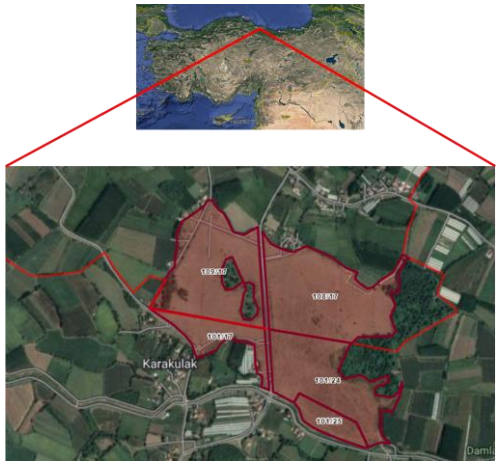

Figure 1. Location and Settlement Information of the Pasture Research Area

The pasture in Epçeli village is situated at an elevation of 10 m above sea level and consists of five adjacent parcels, covering a surface area of 66.5 hectares. It is characterized as a base pasture.

Based on the analysis conducted at the Soil Laboratory of the Karadeniz Agricultural Research Institute, the soil samples taken from Epçeli village pasture before improvement in 2014 exhibited clayey, slightly calcareous, non-saline, and neutral characteristics. The soils in the research area were found to have a low phosphorus content, high potassium content, and a favorable organic matter ratio.

Over several years, the average temperature values in the research area pasture have consistently been higher than the long-term average temperature. Notably, the year before the improvement and the final year of the improvement process showed higher average temperatures (ranging from 15.0-15.6°C) than other years and the long-term average temperature of 14.4°C.

While the total precipitation values during the year prior to improvement (2014) and the improvement process (2017) were lower than the long-term total precipitation (587.3 mm and 758.2 mm, respectively, compared to the average of 849.9 mm), the years 2015, 2016, 2018, and 2019 experienced higher total precipitation (930.8 mm, 955.1 mm, 888.6 mm, and 870.5 mm, respectively) exceeding the long-term average value.

Pasture Management and Improvement Project Applications

The Epçeli village Range Management and Improvement Project (Project No: 2015/55/001), carried out between 2015 and 2018, encompassed various interventions such as subsoiler application for soil aeration, drainage channel cleaning, construction of water troughs and buffalo pools, weed and shrub control through mowing, fertilization, and regulation of the grazing season, complemented by introducing forage crops to alleviate pressure on the pasture.

In 2015, the shrubs within the research area were systematically cut and cleared (see Figure 2c). Chemical herbicides were applied in April-May of 2016 and 2017 to combat young shoots. To improve pasture soil conditions, ventilation was conducted using a subsoiler at intervals of 3x3 meters, following a diamond pattern at a depth of 50-70 cm in December 2015, both in the east-west and north-south directions (see Figure 2b). Furthermore, the drainage channels flanking the road that traversed the pasture parcels underwent cleaning in 2016.

Invasive plants, which the animals did not favor in the pasture area, were annually mowed with chain mowers during the second half of June, except in the first year. Seed formations of invasive plants were carefully prevented. Considering the presence of buffaloes in the village, buffalo pools with a diameter of 7-8 meters and a depth of 150 cm were constructed at three distinct locations within the pasture. Water supply channels were installed near these pools to ensure an increased water supply (Figure 2d).

Figure 2. a) Epçeli Village Meadow Before Improvement, b) Subsoiler Application (Soil Aeration), c) View of Pasture Section After Brush Cutting, d) Buffalo Pool, e) Cages Placed in the Meadow, f) Vegetation Study After Improvement

To enhance forage production for farmers in the village and alleviate animal pressure on the pasture, 7.5 tons of common vetch seeds and 4.5 tons of silage corn seeds were distributed during the project's fourth year.

The pasture underwent annual fertilization between 2016 and 2018, with a dosage of 19 kg da^{-1} of Triple Superphosphate (42%) and 7 kg da^{-1} of Ammonium Nitrate (33%). Phosphorus fertilizer was applied in November during autumn, while nitrogen fertilizer was applied in the first half of April. It was observed that the prescribed grazing period for the pasture, spanning from April 1 to October 30, needed to be adhered to.

Vegetation Data Before Pasture Improvement and Management Project

On July 3, 2014, a vegetation study was carried out in the Epçeli pasture using the "Modified Wheel Point Method." The study aimed to identify the plant species present and determine the percentage of ground cover (Table 1).

Table 1. Species Identified and Ratio of Plant Cover Before Improvement (%)

Order No	Species	(%)
1	*Bellis perennis*	3
2	*Catabrosella parviflora*	3
3	*Centaurea iberica*	4
4	*Crepis armena*	2
5	*Cynodon dactylon*	7
6	*Cynosurus cristatus*	1
7	*Eryngium creticum*	3
8	*Galium verum*	3
9	*Lolium perenne*	2
10	*Lotus angustissimus*	3
11	*Lotus corniculatus*	4
12	*Medicago lupulina*	21
13	*Ononis spinosa*	3
14	*Plantago lanceolata*	3
15	*Prunella vulgaris*	2
16	*Rubus discolor*	6
17	*Taraxacum scaturiginosum*	6
18	*Trifolium dubium*	2
19	*Trifolium pannonicum*	3
20	*Trifolium physodes*	2
21	*Trifolium pratense*	6
22	*Trifolium repens*	6
23	*Trifolium scabrum*	1
24	*Ulmus Minor*	4
	Total	**100**

Before the improvement program, the vegetation study revealed that the Epçeli Village Meadow exhibited no bare areas or rocky terrain, with complete vegetation coverage and a 10% coverage of shrub species (see Figure 2a). The overall condition of the pasture was evaluated as moderate, and its health class

was classified as healthy. A total of 24 plant species were identified in the pasture (Table 1).

Before the implementation of the improvement project, it was observed that the Epçeli village pasture experienced uncontrolled grazing for a duration of 270 days, spanning from March 15 to December 15, with or without the presence of a shepherd. The grazing season was determined as 210 days between April 1 and October 30 during the improvement and management project. Based on the data collected before the project, the pasture's estimated usable dry forage yield was 1350 kg kg ha^{-1}.

Method

The present study's comprehensive data collection effort was undertaken on May 27, 2019, encompassing 1000 readings (Figure 2f). The data collection utilized the "Modified Wheel Point Method" along ten distinct transects established within the pasture area (Figure 3). The vegetation assessment aimed to delineate various land cover types, including bare ground, rocky areas, and plant-covered regions. Moreover, the identified plant species within the pasture were classified based on their ratios in the botanical composition, degree of impact, family categorization, and lifespans.

Figure 3. Reading Lines for Post-Improvement Vegetation Study

The vegetation cover ratio or plant-covered area was calculated by dividing the number of points encountered on plants during the vegetation study

by the total number of measured points. The ratios of each identified plant species in the botanical composition were determined by comparing their counts to the total number of plants, thus providing insights into their representation (Gökkuş et al., 2000). Furthermore, the identified species were categorized as decreaser, increaser, or invasive species, and their ratios in the botanical composition were assessed based on their impact levels.

The plant species comprising the botanical composition were further classified into legumes, grasses, and other families. They were also categorized as perennial or annual species, based on their lifespans, to determine their ratios in the botanical composition.

By considering the ratios of species classified according to their impact levels, the percentages of decreaser species in the botanical composition were summed, while 19 points were allocated for increasing species. For ratios ranging between 20% and 40%, 20 points were assigned, and for percentages exceeding 41%, half of the actual percentage was added. This cumulative scoring system aided in the classification of pasture conditions. The health assessment of the pastures was conducted by transferring data obtained from the wheel-point method to the transect method using the regression equation $y=0.865x-17.498$, $R2=0.9477$ (Koç & Çakal, 2004). The pasture health classification was determined by incorporating the newly obtained plant-covered area ratios and referring to the criteria outlined in Table 2.

Table 2. Pasture Health Class Table

Soil Cover Percentage (%)	Health Class
>40	Healthy
30-40	Risky
<30	Problematic

On March 21, 2019, five 110 x 110 cm cages were strategically placed in different pasture regions to assess forage yield. To ensure accurate measurements, the cages were carefully divided into 10 cm sections to account for edge effects (see Figure 2e). Harvesting occurred

at a consistent height of 5 cm once the vegetation reached grazing maturity. The harvesting events occurred on April 15, May 20, June 30, and August 1, 2019, with each harvest performed within clearly demarcated 1 m² areas. Subsequently, the harvested materials were weighed and subjected to drying in an oven at 60 °C until reaching a constant weight, following the methodology outlined by Sleugh et al. (2000). The dry forage yields obtained from the four harvests within each cage were combined to calculate the cage yields. The average value of cage yields was then converted to yield per hectare, enabling the determination of the dry forage yield of the pasture.

Data Analysis

Given the scarcity of available pre-improvement and post-improvement data regarding the examined topics, a non-parametric Wilcoxon signed-rank test ($p < 0.05$, $p < 0.01$) was conducted on the data (Karagöz, 2010).

RESULTS and DISCUSSION

Vegetated Area

Based on the t-test analysis of the collected data, the observed changes in the vegetated area within the pasture were statistically not significant. The dominant species in terms of soil coverage prior to improvement included *Medicago lupulina, Rubus discolor, Cynodon dactylon, Taraxacum scaturiginosum, Trifolium pratense,* and *Trifolium repens*. After improvement, the species contributing to the vegetated area were identified as *Cynodon dactylon, Ranunculus illyricus, Bellis perennis, Trifolium resupinatum, Lolium perenne, Trifolium repens, Plantago lanceolata, Poa pratensis,* and *Taraxacum scaturiginosum* (Table 3).

Table 3. Plant Cover Ratios of Species (%)

Order No	Before Improvement Species	%	Order No	After Improvement Species	%
	Bare Area	0		Bare Area	0.3
	Rocky Area	0		Rocky Area	0
1	*Bellis perennis*	3	1	*Anagallis arvensis*	1
2	*Catabrosella parviflora*	3	2	*Anthriscus nemorosa*	0.7
3	*Centaurea iberica*	4	3	*Bellis perennis*	10
4	*Crepis armena*	2	4	*Blymus compressus*	0.1
5	*Cynodon dactylon*	7	5	*Bromus hordeaceus*	0.1
6	*Cynosurus cristatus*	1	6	*Carex acuta*	1.1
7	*Eryngium creticum*	3	7	*Carex flacca*	0.2
8	*Galium verum*	3	8	*Carum carvi*	1.5
9	*Lolium perenne*	2	9	*Centaurea ıberica*	0.9
10	*Lotus angustissimus*	3	10	*Convolvulus arvensis*	0.4
11	*Lotus corniculatus*	4	11	*Convolvulus lineatus*	1.2
12	*Medicago lupulina*	21	12	*Cynodon dactylon*	13.3
13	*Ononis spinosa*	3	13	*Cynosurus cristatus*	3.1
14	*Plantago lanceolata*	3	14	*Eleocharis palustris*	0.2
15	*Prunella vulgaris*	2	15	*Eryngium campestre*	0.5
16	*Rubus discolor*	6	16	*Euphorbia helioscopia*	2.2
17	*Taraxacum scaturiginosum*	6	17	*Euphorbia orientalis*	0.2
18	*Trifolium dubium*	2	18	*Festuca pratensis*	0.2
19	*Trifolium pannonicum*	3	19	*Geranium asphodeloides*	0.4
20	*Trifolium physodes*	2	20	*Juncus gerardi*	0.6
21	*Trifolium pratense*	6	21	*Linum hypericifolium*	0.3
22	*Trifolium repens*	6	22	*Lolium perenne*	6.7
23	*Trifolium scabrum*	1	23	*Lotus corniculatus*	2
24	*Ulmus Minor*	4	24	*Medicago lupulina*	0.1
			25	*Medicago minima*	0.1
			26	*Ononis spinosa*	0.4
			27	*Paspalum paspaloides*	0.6
			28	*Plantago lanceolata*	6.4
			29	*Plantago major*	0.8
			30	*Poa annua*	0.6
			31	*Poa pratensis*	5.8
			32	*Potentilla astracanica*	2.1
			33	*Potentilla humifusa*	0.2
			34	*Ranunculus illyricus*	11.1
			35	*Rubus canescens*	0.9
			36	*Rumex acetosella*	0.1
			37	*Rumex crispus*	0.8
			38	*Taraxacum scaturiginosum*	5.1
			39	*Trifolium fragiferum*	0.4
			40	*Trifolium hybridum*	0.2
			41	*Trifolium meneghinianum*	0.2
			42	*Trifolium physodes*	0.1
			43	*Trifolium pilulare*	0.1
			44	*Trifolium pratense*	0.2
			45	*Trifolium repens*	6.7
			46	*Trifolium resupinatum*	9.8
	Total	**100**		**Total**	**100**

After improvement, there was a significant increase in the contribution of *Cynodon dactylon, Trifolium repens, Lolium perenne, and Poa pratensis* to the vegetated area (see Table 3). Specifically, the soil coverage ratio of *Cynodon dactylon* increased from 7% to 13.3% following the improvement practices. This increase can be attributed to using a subsoiler for soil aeration. By employing this practice, the stolons of *Cynodon dactylon* were fragmented, leading to vegetative propagation, and ultimately resulting in an augmented soil coverage ratio.

The plant-covered area in the pasture of Epçeli village has experienced a slight decrease, from 100% to 99.7%. According to the findings of Tosun and Altın (1986), soil breaking is considered beneficial for promoting water infiltration. Altın et al. (2005) emphasized that soil breaking enhances water retention within the soil by mitigating water runoff on the pasture surface. This practice facilitates root and plant development through improved aeration, positively influencing pasture productivity and various characteristics. The observed partial decrease in the plant-covered area could be attributed to several factors, including soil aeration through ripping, vegetation damage, non-compliance with grazing seasons, or soil re-compaction caused by trampling animals during wet conditions. Özaslan (1996) noted that ripping techniques could reduce the vegetation's soil cover ratios.

Species Ratios in Botanical Composition

In the study area, 24 species were identified before improvement, whereas the number increased to 46 species after improvement. The ratios of species in the botanical composition exhibited a range of 1% to 21% before improvement and 0.10% to 13.34% after improvement (see Table 4). It is important to note that only one vegetation study was conducted before improvement, and the plant coverage rate was 100% in the research area. Consequently, the values indicating the ratios of species in the botanical composition coincided with the soil coverage rate values. As a result, the species that exhibited notable soil coverage rates before improvement also constituted a significant ratio of the botanical composition.

Following improvement, identifying a bare area amounting to 0.3% during the vegetation studies brought about changes in the ratios of species within the botanical composition (Table 4).

Cynodon dactylon, Ranunculus illyricus, Bellis perennis, Trifolium resupinatum, Lolium perenne, Trifolium repens, Plantago lanceolata, Poa pratensis, Taraxacum scaturiginosum species were found to be present in the botanical composition between 13.34% and 5.12% (Table 4).

The notable increase in the ratio of *Cynodon dactylon* in the botanical composition can be attributed to the positive effects of aeration with a spiker and the rate of soil cover applied. This treatment has been shown to have a beneficial impact on the vegetative propagation of the plant. According to Aydın and Uzun (2000), practices involving fertilization, overseeding, and aeration increased yield but did not significantly affect the botanical composition. On the other hand, regular grazing positively influenced the botanical composition.

Table 4. Species Ratios in Botanical Composition (%)

Order	Before Improvement		Order	After Improvement	
No	**Species**	**%**	**No**	**Species**	**%**
1	*Bellis perennis*	3	1	*Anagallis arvensis*	1.00
2	*Catabrosella parviflora*	3	2	*Anthriscus nemorosa*	0.70
3	*Centaurea iberica*	4	3	*Bellis perennis*	10.03
4	*Crepis armena*	2	4	*Blymus compressus*	0.10
5	*Cynodon dactylon*	7	5	*Bromus hordeaceus*	0.10
6	*Cynosurus cristatus*	1	6	*Carex acuta*	1.10
7	*Eryngium creticum*	3	7	*Carex flacca*	0.20
8	*Galium verum*	3	8	*Carum carvi*	1.50
9	*Lolium perenne*	2	9	*Centaurea ıberica*	0.90
10	*Lotus angustissimus*	3	10	*Convolvulus arvensis*	0.40
11	*Lotus corniculatus*	4	11	*Convolvulus lineatus*	1.20
12	*Medicago lupulina*	21	12	*Cynodon dactylon*	13.34
13	*Ononis spinosa*	3	13	*Cynosurus cristatus*	3.11
14	*Plantago lanceolata*	3	14	*Eleocharis palustris*	0.20
15	*Prunella vulgaris*	2	15	*Eryngium campestre*	0.50
16	*Rubus discolor*	6	16	*Euphorbia helioscopia*	2.21
17	*Taraxacum scaturiginosum*	6	17	*Euphorbia orientalis*	0.20
18	*Trifolium dubium*	2	18	*Festuca pratensis*	0.20
19	*Trifolium pannonicum*	3	19	*Geranium asphodeloides*	0.40
20	*Trifolium physodes*	2	20	*Juncus gerardi*	0.60
21	*Trifolium pratense*	6	21	*Linum hypericifolium*	0.30
22	*Trifolium repens*	6	22	*Lolium perenne*	6.72
23	*Trifolium scabrum*	1	23	*Lotus corniculatus*	2.01
24	*Ulmus Minor*	4	24	*Medicago lupulina*	0.10

			25	*Medicago minima*	0.10
			26	*Ononis spinosa*	0.40
			27	*Paspalum paspaloides*	0.60
			28	*Plantago lanceolata*	6.42
			29	*Plantago major*	0.80
			30	*Poa annua*	0.60
			31	*Poa pratensis*	5.82
			32	*Potentilla astracanica*	2.11
			33	*Potentilla humifusa*	0.20
			34	*Ranunculus illyricus*	11.13
			35	*Rubus canescens*	0.90
			36	*Rumex acetosella*	0.10
			37	*Rumex crispus*	0.80
			38	*Taraxacum scaturiginosum*	5.12
			39	*Trifolium fragiferum*	0.40
			40	*Trifolium hybridum*	0.20
			41	*Trifolium meneghinianum*	0.20
			42	*Trifolium physodes*	0.10
			43	*Trifolium pilulare*	0.10
			44	*Trifolium pratense*	0.20
			45	*Trifolium repens*	6.72
			46	*Trifolium resupinatum*	9.83
	Total	**100**		**Total**	**100**

The improvement programs such as fertilization and mowing practices have caused changes in the botanical composition of the species. Şahinoğlu and Uzun (2016) found that combinations of fertilization, resting, herbicide application, and spring mowing significantly decreased the ratios of *Eryngium bithynicum* Boiss and *Centaurea sp*. in the botanical composition.

Ratios of Species in Botanical Composition by Impact Levels

Statistical analysis was not conducted on the data pertaining to the ratios of decreaser species before and after the improvement, as the ratios of decreaser species were equal across impact levels. However, the changes in the ratios of increaser and invasive species were found to be statistically significant.

The number of decreaser species with unchanged ratios in the botanical composition increased from 6 to 10 before and after the improvement. The ratio of increasing species rose from 14% to 25.4%, with the number of species increasing from 4 to 6. Conversely, the ratio of invasive species decreased from 63% to 51.6%, while the number of species increased from 14 to 30 (Table 5).

Table 5. Ratios of Species in Botanical Composition According to Impact Levels (%)

Before Improvement			After Improvement		
Decreaser					
Order No	**Species**	**%**	**Order No**	**Species**	**%**
1	*Lolium perenne*	2	1	*Festuca pratensis*	0.2
2	*Lotus corniculatus*	4	2	*Lolium perenne*	6.7
3	*Trifolium pannonicum*	3	3	*Lotus corniculatus*	2.0
4	*Trifolium physodes*	2	4	*Paspalum paspaloides*	0.6
5	*Trifolium pratense*	6	5	*Poa pratensis*	5.8
6	*Trifolium repens*	6	6	*Trifolium fragiferum*	0.4
			7	*Trifolium hybridum*	0.2
			8	*Trifolium physodes*	0.1
			9	*Trifolium pratense*	0.2
			10	*Trifolium repens*	6.7
	Total	**23.0**		**Total**	**23.0**
Increaser					
1	*Catabrosella parviflora*	3	1	*Carex acuta*	1.1
2	*Cynodon dactylon*	7	2	*Cynodon dactylon*	13.3
3	*Cynosurus cristatus*	1	3	*Cynosurus cristatus*	3.1
4	*Plantago lanceolata*	3	4	*Juncus gerardi*	0.6
			5	*Plantago lanceolata*	6.4
			6	*Plantago major*	0.8
	Total	**14.0**		**Total**	**25.4**
Invasive					
1	*Bellis perennis*	3	1	*Anagallis arvensis*	1.0
2	*Centaurea iberica*	4	2	*Anthriscus nemorosa*	0.7
3	*Crepis armena*	2	3	*Bellis perennis*	10.0
4	*Eryngium creticum*	3	4	*Blymus compressus*	0.2
5	*Galium verum*	3	5	*Bromus hordeaceus*	0.1
6	*Lotus angustissimus*	3	6	*Carex flacca*	0.2
7	*Medicago lupulina*	21	7	*Carum carvi*	1.5
8	*Ononis spinosa*	3	8	*Centaurea iberica*	0.9
9	*Prunella vulgaris*	2	9	*Convolvulus arvensis*	0.4
10	*Rubus discolor*	6	10	*Convolvulus lineatus*	1.2
11	*Taraxacum scaturiginosum*	6	11	*Eleocharis palustris*	0.2
12	*Trifolium dubium*	2	12	*Eryngium campestre*	0.5
13	*Trifolium scabrum*	1	13	*Euphorbia helioscopia*	2.2
14	*Ulmus Minor*	4	14	*Euphorbia orientalis*	0.2
			15	*Geranium asphodeloides*	0.4
			16	*Linum hypericifolium*	0.3
			17	*Medicago lupulina*	0.1
			18	*Medicago minima*	0.1
			19	*Ononis spinosa*	0.4
			20	*Poa annua*	0.6
			21	*Potentilla astracanica*	2.1
			22	*Potentilla humifusa*	0.2
			23	*Ranunculus illyricus*	11.1
			24	*Rubus canescens*	0.9
			25	*Rumex acetosella*	0.1
			26	*Rumex crispus*	0.8
			27	*Taraxacum scaturiginosum*	5.1
			28	*Trifolium meneghinianum*	0.2
			29	*Trifolium pilulare*	0.1
			30	*Trifolium resupinatum*	9.8
	Total	**63.0**		**Total**	**51.6**

The pasture of Epçeli village exhibited an increase in increasing species and a decrease in invasive species, indicating a partial improvement in the pasture. One of the main factors contributing to this change is the implementation of clearing practices in the pasture as part of a three-year improvement program. Gökkuş (1999) suggested that mowing annual weeds before flowering and mowing perennial weeds at the budding or flowering stage, repeating the process over several years, is necessary to achieve favorable outcomes.

However, despite the increase in the number of decreaser species, their ratio in the botanical composition did not increase significantly. This could be attributed to excessive and continuous grazing. Herbel and Pieper (1991) noted that overgrazing pastures beyond their carrying capacity negatively alters vegetation structure, reduces soil cover and productivity, and increases soil erosion. Grazing during critical periods, crucial for the development and continuity of plants and vegetation, leads to a decline in pasture productivity (Babalık, 2007; Gökkuş, 1989). According to Kuşvuran et al. (2011), Turkey's low yield and quality of pasture areas can be attributed to the lack of attention to critical periods and excessive grazing.

Despite the decline in the ratio of invasive species within the botanical composition, the increase in species numbers may be attributed to the implementation of improvement programs. The practice of repeated mowing for weed control has proven effective in reducing the prevalence of invasive species. However, it should be noted that certain cultivation practices, such as deep plowing and aeration, may inadvertently activate the weed seed bank in the soil. For instance, Burton and Dowling (2004) reported significant weed eradication through short-term pasture mowing. On the other hand, Aydın and Uzun (2000) found no significant effect of fertilization, top seeding, and aeration practices on the botanical composition of pastures. In a separate study, Gökkuş and Altın (1986) observed changes in the legume ratio of the botanical composition due to fertilization and aeration practices. Koç et al. (2005) determined that early mowing combined with nitrogen application increased the ratio of weeds in the botanical composition. Furthermore, Öten et al. (2016) documented a ratio of decreaser species by 1-38%, increaser species by 2-32%,

and invasive species by 30-91% in the botanical composition of the base pasture in the Antalya region.

Ratios of Species in the Botanical Composition by Families

Based on the research findings, the botanical composition revealed notable changes after improvement. The ratios of species from the legumes family decreased significantly from 51% to 20.4%, while the number of species increased from 10 to 12. Conversely, improvement practices led to an increase in the ratio of species from the grasses, rising from 13% to 30.5%, and an increase in the number of species from 4 to 8. Based on the applied improvement practices, an increase was observed in the ratios of species belonging to other families, rising from 36% to 49.1%, accompanied by an increase in the number of species from 10 to 26 (Table 6).

Table 6. Ratios of Species in Botanical Composition by Families (%)

Before Improvement			After Improvement		
Legumes					
Order No	**Species**	**%**	**Order No**	**Species**	**%**
1	*Lotus angustissimus*	3	1	*Lotus corniculatus*	2.0
2	*Lotus corniculatus*	4	2	*Medicago lupulina*	0.1
3	*Medicago lupulina*	21	3	*Medicago minima*	0.1
4	*Ononis spinosa*	3	4	*Ononis spinosa*	0.4
5	*Trifolium dubium*	2	5	*Trifolium fragiferum*	0.4
6	*Trifolium pannonicum*	3	6	*Trifolium hybridum*	0.2
7	*Trifolium physodes*	2	7	*Trifolium meneghinianum*	0.2
8	*Trifolium pratense*	6	8	*Trifolium physodes*	0.1
9	*Trifolium repens*	6	9	*Trifolium pilulare*	0.1
10	*Trifolium scabrum*	1	10	*Trifolium pratense*	0.2
			11	*Trifolium repens*	6.7
			12	*Trifolium resupinatum*	9.8
	Total	**51**		**Total**	**20.4**
Grasses					
1	*Catabrosella parviflora*	3	1	*Bromus hordeaceus*	0.1
2	*Cynodon dactylon*	7	2	*Cynodon dactylon*	13.3
3	*Cynosurus cristatus*	1	3	*Cynosurus cristatus*	3.1
4	*Lolium perenne*	2	4	*Festuca pratensis*	0.2
			5	*Lolium perenne*	6.7
			6	*Paspalum paspaloides*	0.6
			7	*Poa annua*	0.6
			8	*Poa pratensis*	5.8
	Total	**13**		**Total**	**30.5**
Other families					
1	*Bellis perennis*	3	1	*Anagallis arvensis*	1.0
2	*Centaurea iberica*	4	2	*Anthriscus nemorosa*	0.7
3	*Crepis armena*	2	3	*Bellis perennis*	10.0
4	*Eryngium creticum*	3	4	*Blymus compressus*	0.1

5	*Galium verum*	3	5	*Carex acuta*	1.1
6	*Plantago lanceolata*	3	6	*Carex flacca*	0.2
7	*Prunella vulgaris*	2	7	*Carum carvi*	1.5
8	*Rubus discolor*	6	8	*Centaurea iberica*	0.9
9	*Taraxacum scaturiginosum*	6	9	*Convolvulus arvensis*	0.4
10	*Ulmus Minor*	4	10	*Convolvulus lineatus*	1.2
			11	*Eleocharis palustris*	0.2
			12	*Eryngium campestre*	0.5
			13	*Euphorbia helioscopia*	2.2
			14	*Euphorbia orientalis*	0.2
			15	*Geranium asphodeloides*	0.4
			16	*Juncus gerardi*	0.6
			17	*Linum hypericifolium*	0.3
			18	*Plantago lanceolata*	6.4
			19	*Plantago major*	0.8
			20	*Potentilla astracanica*	2.1
			21	*Potentilla humifusa*	0.2
			22	*Ranunculus illyricus*	11.1
			23	*Rubus canescens*	0.9
			24	*Rumex acetosella*	0.1
			25	*Rumex crispus*	0.8
			26	*Taraxacum scaturiginosum*	5.1
	Total	**36**		**Total**	**49.1**

The decline in the legume ratio can be attributed to the dominance of grasses in the botanical composition, which benefits from deep plowing for aeration and nitrogen fertilization with high organic matter. Deep plowing for aeration resulted in an increased ratio of *Cynodon dactylon*, which spreads through stolon, from 7% to 13.34%. At the same time, fertilization contributed to a higher ratio of *Lolium perenne*, increasing from 2% to 6.7%. *Poa pratensis*, which was absent in the pre-improvement vegetation, accounted for 5.8% of the overall vegetation (Table 6). The rise in species ratios from other families may be attributed to the activation of the seed bank in the soil through deep plowing for aeration.

In a study by Mut and Ayan (2011) conducted under Samsun ecological conditions, aeration alone exhibited a slight reduction in the legume ratio in a pasture plowed and abandoned for approximately 30 years. Petrov and Marrs (2000) have highlighted that fertilization can induce changes in the botanical composition. Similarly, Hatipoğlu et al. (2001) reported an increase in grasses ratios and a decrease in legume ratios within the botanical composition of the pasture as nitrogen doses were increased. According to Reis (2002), nitrogen, phosphorus, and potassium fertilizers significantly influence plant species' participation ratios in the vegetation's botanical composition.

Ratios of Species in Botanical Composition by Lifespan

The ratio of perennial species in the botanical composition decreased from 91% to 85%, while the number of species increased from 20 to 37. Conversely, the ratio of annual species increased from 9% to 15%, accompanied by an increase in the number of species from 4 to 9 (Table 7). Prior to improvement, the dominant perennial species in the vegetation were *Medicago lupulina, Cynodon dactylon, Rubus discolor, Taraxacum scaturiginosum, Trifolium pratense, Trifolium repens,* and *Ulmus Minor*. However, after improvement, *Cynodon dactylon, Ranunculus illyricus, Bellis perennis, Lolium perenne, Trifolium repens, Plantago lanceolata, Poa pratensis,* and *Taraxacum scaturiginosum* became the dominant species. Before improvement, only *Eryngium creticum, Lotus angustissimus, Trifolium dubium*, and *Trifolium scabrum* were identified as annual species in the vegetation, whereas the number of annual species increased after improvement. Notably, *Euphorbia helioscopia*, *Anagallis arvensis*, and *Trifolium resupinatum* were recorded as dominant species (Table 7).

The decrease in the ratio of perennial species in the botanical composition could be attributed to the rapid germination of dormant annual seeds in the soil resulting from aeration with the harrow. Furthermore, despite the soil being wet after aeration, the continued excessive and intense grazing in the pasture may have contributed to the reduction in the ratio of perennial species.

Table 7. Ratios of Botanical Composition According to Lifespans (%)

Before Improvement			After Improvement		
Perennial					
Order No	**Species**	**%**	**Order No**	**Species**	**%**
1	*Bellis perennis*	3	1	*Anthriscus nemorosa*	0.7
2	*Catabrosella parviflora*	3	2	*Bellis perennis*	10.0
3	*Centaurea iberica*	4	3	*Blymus compressus*	0.1
4	*Crepis armena*	2	4	*Carex acuta*	1.1
5	*Cynodon dactylon*	7	5	*Carex flacca*	0.2
6	*Cynosurus cristatus*	1	6	*Carum carvi*	1.5
7	*Galium verum*	3	7	*Convolvulus arvensis*	0.4
8	*Lolium perenne*	2	8	*Convolvulus lineatus*	1.2
9	*Lotus corniculatus*	4	9	*Cynodon dactylon*	13.3
10	*Medicago lupulina*	21	10	*Cynosurus cristatus*	3.1
11	*Ononis spinosa*	3	11	*Eleocharis palustris*	0.2
12	*Plantago lanceolata*	3	12	*Eryngium campestre*	0.5
13	*Prunella vulgaris*	2	13	*Euphorbia orientalis*	0.2

14	*Rubus discolor*	6	14	*Festuca pratensis*	0.2
15	*Taraxacum scaturiginosum*	6	15	*Geranium asphodeloides*	0.4
16	*Trifolium pannonicum*	3	16	*Juncus gerardi*	0.6
17	*Trifolium physodes*	2	17	*Linum hypericifolium*	0.3
18	*Trifolium pratense*	6	18	*Lolium perenne*	6.7
19	*Trifolium repens*	6	19	*Lotus corniculatus*	2.0
20	*Ulmus Minor*	4	20	*Medicago lupulina*	0.1
			21	*Ononis spinosa*	0.4
			22	*Paspalum paspaloides*	0.6
			23	*Plantago lanceolata*	6.4
			24	*Plantago major*	0.8
			25	*Poa pratensis*	5.8
			26	*Potentilla astracanica*	2.1
			27	*Potentilla humifusa*	0.2
			28	*Ranunculus illyricus*	11.1
			29	*Rubus canescens*	0.9
			30	*Rumex acetosella*	0.1
			31	*Rumex crispus*	0.8
			32	*Taraxacum scaturiginosum*	5.1
			33	*Trifolium fragiferum*	0.4
			34	*Trifolium hybridum*	0.2
			35	*Trifolium physodes*	0.1
			36	*Trifolium pratense*	0.2
			37	*Trifolium repens*	6.7
	Total	**91**		**Total**	**85.0**
		Annual			
1	*Eryngium creticum*	3	1	*Anagallis arvensis*	1.0
2	*Lotus angustissimus*	3	2	*Bromus hordeaceus*	0.1
3	*Trifolium dubium*	2	3	*Centaurea iberica*	0.9
4	*Trifolium scabrum*	1	4	*Euphorbia helioscopia*	2.2
			5	*Medicago minima*	0.1
			6	*Poa annua*	0.6
			7	*Trifolium meneghinianum*	0.2
			8	*Trifolium pilulare*	0.1
			9	*Trifolium resupinatum*	9.8
	Total	**9**		**Total**	**15.0**

Altın et al. (2005) found that in continuously and heavily grazed pastures, palatable species cease seed production due to constant animal consumption. These plants require respite from grazing or mowing pressure to mature and release their seeds.

Heady and Child (1994) indicated that soil compaction is inevitable in pastures, particularly during wet conditions, and compacted soils have detrimental effects on water permeability, water storage, soil aeration, plant root development, and soil microorganisms. Gençkan (1985) reported that intensive grazing in rainy regions, particularly clayey soils, leads to soil compaction in early spring. Occasional soil ripping at specific intervals can prevent this issue and achieve desired vegetation yield and quality. However, it

is crucial to have a comprehensive understanding of the root structures of the plant species composing the vegetation and consider the potential negative impacts of vegetation injury and soil ripping during this practice.

Pasture Health and Condition Class

The improvement practices within the research area did not result in any significant changes to the health and condition class of the pasture. Both before and after the improvement, condition of the pasture was evaluated as moderate, and its health class was classified as healthy (Table 8).

Table 8. Effect of Improvement Practices on Pasture Health and Condition Class.

Period	Decreaser (%)	Increaser (%)	Invasive (%)	Pasture Condition	GCR* (%)	Pasture Health
Before Improvement	23	14	63	Moderate	69	Healthy
After Improvement	23	25.4	51.6	Moderate	68.7	Healthy

*Ground Cover Ratio

As part of the improvement program carried out in the pasture, shrub cutting, soil aeration, fertilization, and weed control practices have yielded positive results in vegetation enhancement. Although the number of decreaser species in the pasture has increased, their ratios in the botanical composition have remained consistent, while the increaser species have shown a substantial 78% increase. Moreover, there has been a notable reduction in the presence of invasive species. However, the failure to adhere to prescribed grazing start and finish dates, as well as exceeding the grazing capacity with a higher livestock unit, has had no significant impact on the overall health and condition of the pasture. This finding aligns with Herbel and Pieper (1991) assertion that grazing pastures beyond their carrying capacity adversely affect the vegetation structure.

Dry Herbage Yield

Significant changes were observed in the dry herbage yield due to improvement practices in the Epçeli village pasture Following the implementation of an improvement program, which included cleaning, fertilization, soil aeration, and other methods, the dry herbage yield of the Epçeli village pasture increased from 1350 kg ha^{-1} before improvement to 4233 kg ha^{-1} (Table 9).

Table 9. Results of the t-test analysis on the changes in pasture dry herbage yield

Period	**Dry Herbage Yield (kg ha^{-1})**
Before Improvement	1350
After Improvement	4233
Prob>|t|	0.0051*

* $p < 0.05$, $p < 0.01$

Cages were utilized in the pasture area to conduct mowing events as the vegetation reached grazing maturity. The purpose of these events was to determine the dry herbage yield of the pasture. Specifically, for the Epçeli village pasture, four mowing events took place on April 15, May 20, June 30, and August 1. The respective yield values for these events were recorded as 1091.2 kg ha^{-1}, 921.4 kg ha^{-1}, 1472 kg ha^{-1}, and 748.4 kg ha^{-1}. The cumulative yield for the pasture was calculated as 4233 kg ha^{-1} (Table 10).

Table 10. Post-Improvement Dry Herbage Yield of the Pasture

Cage No	**Dry Herbage Yield (kg ha^{-1})**				**Total Yield (kg ha^{-1})**
	April 15	**May20**	**June 30**	**August 1**	
1	1189	936	1495	772	4392
2	1221	964	1555	858	4598
3	1053	909	1489	758	4209
4	950	880	1364	652	3846
5	1043	918	1457	702	4120
Average Yield (kg ha^{-1})	1091.2	921.4	1472	748.4	4233

During the grazing season 2019, when pasture yield was assessed following improvement, rainfall levels significantly exceeded long-term averages in all months except August and September. In the research area, rainfall was approximately twice the average in April and more than three times the average in June. This substantial increase in rainfall positively impacted pasture yield, leading to an increase in months characterized by excessive rainfall. However, drought conditions in August and low rainfall in September hindered the vegetation from reaching optimal grazing maturity.

The application of fertilizers can be attributed as the primary factor contributing to the increased yield of dry herbage in the pasture. Previous studies have demonstrated that fertilization enhances the yield and quality of natural pastures (Petrov & Marrs, 2001; Yavuz et al., 2008). Gökkuş and Altın (1986) found that fertilization and loosening practices significantly increased the yield of dry herbage and crude protein. Tuna (1990) observed that combinations such as "burning + fertilization + overseeding," "loosening (aeration) + fertilization + overseeding," "herbicide application + fertilization + overseeding," and "fertilization" resulted in dry herbage yield increases exceeding 300% in natural pastures. Aydın and Uzun (2000) Aydın and Uzun (2000) achieved the highest dry herbage yield of 530 kg da^{-1} when applying a combination of fertilization, overseeding, and aeration. Jefferson (2005) reported satisfactory results regarding pasture yield and sustainability through adjustments in spring and autumn grazing management, mid-July mowing, synthetic fertilizer application, and a small amount of farm manure. Şahinoğlu and Uzun (2016) found that a combination of fertilization and resting led to a significant increase in dry herbage yield in the base pasture, surpassing the initial experiment by "2 times in the first year, 7 times in the second year, and 15 times in the third year." This practice was deemed the "most effective and economical combination," according to their findings.

CONCLUSION

The pasture improvement and management project conducted in the Epçeli village base pasture, located in the Çarşamba district, and supervised by the Samsun Provincial Directorate of Agriculture and Forestry, utilized various methods such as shrub control, weed control, subsoiler application for soil

aeration, fertilization, controlled grazing, and construction of grazing regulating facilities. These practices have demonstrated a positive impact on the vegetation and productivity of the pasture.

The pre-existing vegetation coverage in the Epçeli village pasture, which was already high, remained unaffected by the improvement practices. Although the interventions did not alter the ratio of decreaser species, they increased the ratio of increaser species and decreased the ratio of invasive species. Despite the favorable changes in the botanical composition, the decreaser number of species can be attributed to grazing pressure. Furthermore, the improvement and management project led to a decrease in the ratio of legumes while increasing the ratio of grasses and species from other families.

The improvement practices during the project contributed to a decline in the ratio of perennial species and an increase in the ratio of single species within the botanical composition. The reduction in perennial species can be attributed to the soil aeration practice, which damaged their root systems, and excessive and heavy grazing that persisted even after the soil became wet following the application. Conversely, the increase in annual species can be attributed to the rapid germination of reserve seeds of these species in the soil due to soil aeration.

Despite the improvement practices in the research area, there were no changes in the health and condition class of the pasture. Although these practices resulted in some improvements in pasture vegetation, failure to adhere to proper grazing season and capacity guidelines hindered the satisfactory enhancement of pasture health and condition class. Nevertheless, the improvement practices led to a threefold increase in the dry herbage yield of the pasture.

In conclusion, the pasture improvement and management project has yielded successful outcomes regarding forage crop cultivation, vegetation structure, and pasture productivity. However, the long-term sustainability of these results is contingent upon appropriate grazing practices aligned with the grazing season and capacity in the Epçeli village pasture while adhering to management regulations. Uncontrolled and excessive grazing, contrary to the

designated grazing season and capacity, remains a fundamental issue in pastures in Turkey. Therefore, it is crucial to plan and implement training activities related to pasture management in collaboration with the Provincial Directorate of Agriculture and Forestry to ensure proper utilization of the pasture according to the grazing season and capacity.

ACKNOWLEDGEMENTS

This research is a section of Kadir İspirli's master's thesis and was conducted in 2019 within the scope of the project titled **"Determining the Efficacy of Pasture Improvement Studies in the Central and Western Black Sea Region "** supported by the Ministry of Agriculture and Forestry, General Directorate of Plant Production, under the project number **TAGEM/TBAD/B/19/A7/P7/2128**.

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CHAPTER 7

FUNCTIONAL GENE SILENCE TECHNIQUE IN PLANTS: VIRUS-INDUCED GENE SILENCING (VIGS)

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INTRODUCTION

Effective methods for gene silencing have shown a remarkable increase in the field of functional genomics (gene function), as sequencing analyzes of many model organisms reveal a large number of genes whose function is not yet known. Numerous gene silencing mechanisms have emerged over the years, primarily targeting the genes themselves or the mRNAs they encode. Antisense strategies are widely used in these mRNA-targeted techniques. These antisense strategies rely entirely on delivering a DNA or RNA nucleic acid strand into cells that is reverse complementary to the mRNA encoding the targeted protein.

Antisense oligonucleotide studies have led to the discovery of RNA interference (RNAi), a highly effective method for gene silencing in recent years. RNA interference is a post-transcriptional gene silencing mechanism that results in the fragmentation of the complementary mRNA sequence when double-stranded RNA (dsRNA) enters the cell. This mechanism is a natural process and its biological function in living organisms is to play a role in cellular defense by protecting the genome against the invasion of mobile genetic elements such as virus hereditary material and transposons. It also plays an important role in gene regulation by post-transcriptional gene silencing, which is important for the function of the developmental programs of eukaryotic organisms. Because RNAi is a naturally occurring pathway, the efficacy and specificity of this technique differs from other nucleic acid-based silencing/repression techniques such as antisense oligonucleotide and ribozymes. Thus, RNAi has emerged as an important potential alternative to most other classical antisense technologies.

In 1928, resistances were determined in tobacco plants infected with Tobacco ring spot virus. After this study, similar examples in virus infections have been described in many studies. However, these studies could not be detected as gene silencing in those years. However, this and similar studies were assumed to be the typical explanation for gene silencing in later years.

The first signs of the emergence of the gene suppression mechanism, now called RNAi, appeared in studies on genetic modification of plants in the late 1980s. Jorgensen and colleagues tried to obtain more purple petunias by regulating the activity of a gene responsible for the expression of chalcone

syntase (chs), an enzyme that catalyzes pigmentation in petunias, with genetic transformation studies. However, transfer of the exogenic transgene to the petunia plant resulted in whiter petunias with variegated pigmentation rather than darker flower color as expected. Transfer of the extra copy of the chs gene to the petunia plant caused a decrease in its expression, contrary to the expected increase. Studies have shown that this decrease is not related to the decrease in transcription of cytosolic chs mRNA and that transcription continues in the isolated nucleus. Therefore, this situation has been defined as post-transcriptional gene silencing (PTGS), which refers to post-transcriptional RNA degradation. Jorgensen et al used the term co-suppression to describe the loss of both endogenous and transgenic mRNA after this event, which affects not only the transgene itself, but also the expression of the endogenous chs gene, which is transferred to petunia. Subsequent studies have shown that expression of the transgene leads to the formation of double-stranded RNA (dsRNA), thereby initiating PTGS/cosuppression.

Studies in some plant laboratories have shown that the biological function of this mechanism is the response of plants to infection by RNA viruses by cleaving the target viral RNA. In plant systems, dsRNAs from exogenic sources (a large number of transgenes expressed in plants, bacterial or viral sequences) are potential inducers of gene suppression. This phenomenon in plants is defined as post-transcriptional gene silencing (PTGS)/co-suppression or viral-induced gene silencing (VIGS) (Gündoğdu and Çelik 2009).

2. VIRUS-INDUCED GENE SILENCING (VIGS)

2.1. Overview of Advances Prior to the Discovery of Virus-Induced Gene Silencing

Viruses are the most serious plant pathogens. Viruses cause a wide range of responses in their hosts. Plant scientists have been studying these reactions for years in an effort to develop virus-resistant crops and so reduce agricultural losses caused by these illnesses. Early research discovered that virus-infected plants were resistant to infection when exposed to the same virus or strains (McKinney 1929). This is referred to as cross-protection. Early resistance research focused on the incorporation of viral genetic material into plant

genomes. Pathogen-derived resistance (PDR) is the term used to describe this method (Beachy 1997; Wilson 1993). Some pathogen-resistant plants have exhibited phenotypic development. When a virus infected these plants, disease signs arose, but they vanished in newly developing tissues (Lindbo et al. 1993). Post-transcriptional gene silencing (PTGS) was later shown to be the molecular mechanism allowing cross-protection, pathogen-induced resistance, and recovery (Ratcliff et al. 1997).

PTGS, an epigenetic process, causes the endogenous gene to be degraded in a sequence-specific manner. When PTGS was first discovered in plants, it was referred to as co-suppression. PTGS has been discovered in a variety of animals since its discovery in plants. In fungi, this is known as qulling, and in vertebrates, it is known as RNAi (Bruch-Smith et al. 2004).

2.2. Development of Virus-Induced Gene Silencing Technology

Van Kammen (Van Kammen 1997; Bruch Smith et al. 2004) created the term "VIGS" to describe a person's resistance to viral infection. However, the term has come to refer to a technique that uses recombinant viruses to suppress the expression of endogenous genes (Baulcombe 1999; Ruiz et al. 1998). Endogenous genes could be silenced post-transcriptionally (using recombinant viruses with sequences similar to endogenous genes), according to Kumagai et al. (1995). VIGS's promise as a technique for evaluating gene function was instantly recognized because it allows the destruction of a gene's transcript (Baulcombe 1999).

Silencing requires a DNA fragment of at least 23 nucleotides that exactly matches the target gene (Thomas et al. 2001). A sequence of 23 nucleotides, on the other hand, is usually inadequate to begin silencing. Sometimes a longer defined sequence is required (Ekengren et al. 2003; Thomas et al. 2001). Other factors that influence silencing efficacy include the targeted sequence's nucleotide makeup, the thermodynamic properties of the siRNA, and the coupling of the target sequence (Khvorova et al. 2003; Schwarz et al. 2003).

Many vectors are available for use in virus-induced gene silencing. The first is tobacco mosaic virus (TMV), an RNA virus (Kumagai et al. 1995). To effectively silence PDS, recombinant viral mRNA containing a sequence from

the PDS gene was generated in vitro and injected into Nicotiana Benthamiana seedlings. The virus-induced gene silencing (VIGS) approach was successfully applied in the initial study on the highly virus-susceptible wild tobacco species N. benthamiana. Many unique VIGS vectors are derived from the potato virus X (PVX) (Ruiz et al. 1998). Although this vector is more resistant than the TMV-derived vector, it can only infect a narrower range of hosts (plants) than the TMV-derived vector. PVX can infect only three plant families, but TMV can infect nine (Brunt et al. 1996). Furthermore, identifying some minor PTGS traits can be difficult because PVX and TMV-derived vectors generate disease symptoms in inoculated plants (Ratcliff et al. 2001). Furthermore, these viruses are unable to infect their hosts' meristems or growth points. As a result, effective gene silencing is avoided in these tissues (Ratcliff et al. 2001; Hull 2002). Even though the Tomato Golden Mosaic DNA virus did not enter the meristem, *N. benthamiana* acted as the VIGS vector and was successful in suppressing a meristem gene and cell nuclear antigen proliferation (Peele et al. 2001). This TGMV-derived vector has previously been used to silence non-meristamatic genes and foreign transgenes (Kjemtrup et al. 1998).

Tobacco rattle virus (TRV) VIGS vector was created to overcome host (plant) range and meristem exclusion constraints (Liu et al. 2002; Ratcliff et al. 2001).

TRV can spread more swiftly in the plant it enters (including meristem tissue). Infection symptoms are milder than with other vectors. Endogenous genes may be silenced more successfully with increased TRV VIGS vectors (pYL156 and pYL279) (Liu et al. 2002). In contrast to previous early-developed vectors, these vectors incorporate two 35S promoters and a ribozyme at the C-terminus for more efficient viral RNA transcription (Liu et al., 2002) (Figure 1).

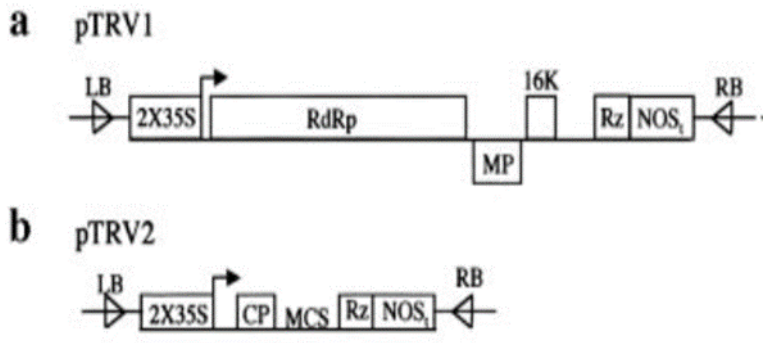

Figure 1. VIGS vector based on TRV. The TRV cDNA clone is located within T-DNA between the CaMV 2x35S promoter and the NOSt (nopaline synthase terminator). Mp (movement protein): movement protein, Cp (coat protein): coat protein, MSC (multiple cloning sites) multi-cloning site, LB (left borders) left border, RB (right borders), 16kDa (cysteine rich protein) cytosine rich protein (Liu et al. 2002).

TRV has a two-part (RNA1 and RNA2) genome and is a positive-strand RNA virus. While proteins encoded by RNA1 permit virus replication and movement within the host plant, proteins encoded by RNA2 permit virion production in viruses and the transmission of viruses across plants via nematodes (MacFarlane 1999). Onto the plant binary transformation vector T-DNA, TRV RNA1 and RNA2 cDNA clones under the CaMV 35S promoter are inserted. The full cDNA clone of the RNA polymerase open reading fragment (ORF) with intron 3 of the Arabidopsis Col-0 nitrate reductase NIA1 gene inserted is found in the TRV RNA 1 construct (pBINTRA6) (Wilkinson and Crawford 1993). The TRV RNA1 cDNA clone in Escherichia coli is unstable without this intron. This intron shields E. coli against the harmful effects of the TRV-encoded proteins. A multiple cloning site (MSC-multiple cloning site) was used to replace the insignificant 29.4k and 32.8k genes in the TRV RNA2 (pTV:00) construct (Ratcliff et al. 2001).

The efficiency of the pYL156 and pYL279 vectors is not restricted to N. Benthamiana, in contrast to the other VIGS vectors previously identified. Tomatoes and other species have been effectively silenced using these vectors (Ekengren et al. 2003; Liu et al. 2002).

A growing variety of plant species are being used in research on viral-induced gene silencing as novel virus vectors are created. In order to enable VIGS in tobacco, a species that is commonly used in plant biology but presents issues for the use of other VIGS vectors, a two-component satellite virus-induced silencing system (SVISS) has been created (Gossele et al. 2002). The helper virus and a satellite of the TMV-U2 generation are both present in this system. Additionally, a VIGS vector has been created to silence monocotyledonous plants like barley (Holzberg et al. 2002). This Barley Stripe Mosaic Virus (BSMV) vector was used to mute the PDS gene, which is the only instance of VIGS in monocots to date (Figure 2).

The development of virus-induced gene silencing as a tool for functional genomics in monocots is crucial. Because these types must be transformed in other loss-of-function procedures. The modified BSMV VIGS vector had more effective silencing despite having more severe phenotypic silencing in monocots. A 40–60 base pair long inverted repeat sequence that generates dsRNA is present in this novel vector (Lacomme et al. 2003). The TMV VIGS vector underwent a similar development by the same team. In order to mute endogenous genes in Arabidopsis, a DNA-derived virus was created (Turnage et al. 2002). The only DNA vector directly employed for temporary VIGS in Arabidopsis has been reported to be this one from the Cabbage Leaf Curl Virus (CbLCV).

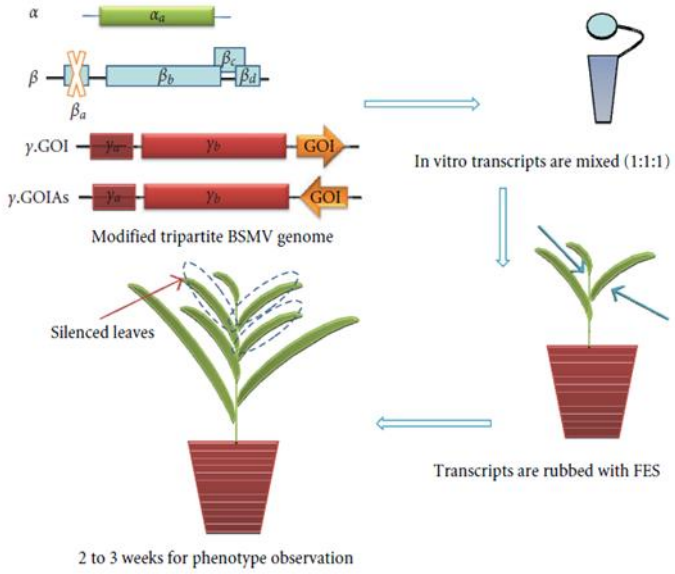

Figure 2. BSMV-mediated VIGS in barley. BSMV has a 3-part genome (α, β, γ). It has been modified for specific VIGS application in barley (Unver and Budak 2009).

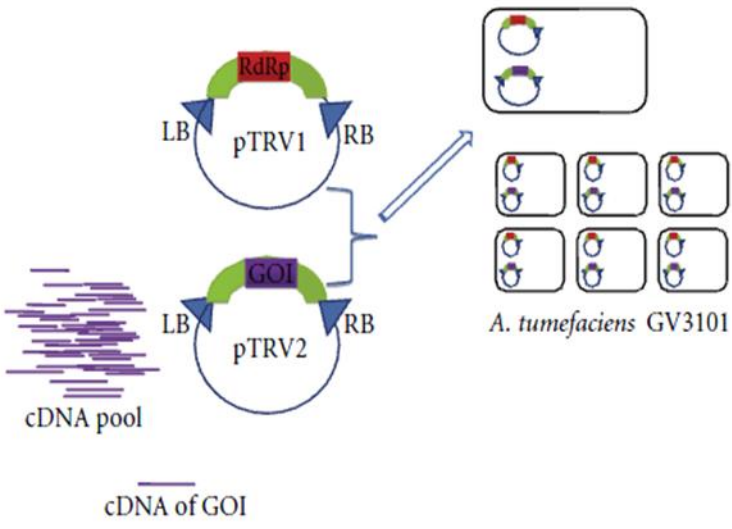

Figure 3. Insertion of the identified gene into the TRV-based ViGS vector and its transfer to *A. tumefaciens* (GV3101) (Unver and Budak 2009)

The plants are then inoculated with Agrobacterium carrying the recombinant VIGS vectors. It appears that target genes are silenced in plants a few weeks after inoculation. In this study, some factors should be considered and optimization should be made in order to maintain the high silencing effect at every stage of the application.

The plants are then inoculated with Agrobacterium carrying the recombinant VIGS vectors. The first methods used in inoculation were toothpick use, microproject and bombardment. Recently, vacuum infiltration, syringing and spray methods are also used (Figure 4.). Recently, the approach of infection to specific organs of plants has been developed. In these studies, infections were applied to tomato fruit tissues and fruit stem.

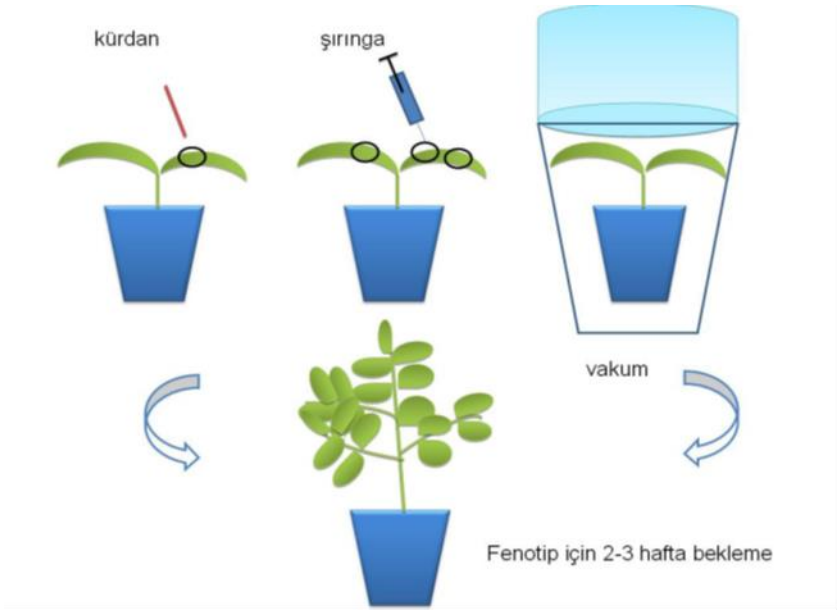

Figure 4. Infection methods (Unver and Budak 2009)

Inoculation of Agrobacterium bacteria carrying the genome of the PVX virus into *N. benthamiana* was successfully performed using toothpicks (Lu et al. 2003). This inoculation method is suitable for many plants for adequate and

effective silencing. However, good results were not obtained from the inoculation of the TRV vector in tomato with this method.

In viruses with a bipartite genome, such as TRV, both viral RNAs are transferred separately to the same Agrobacterium bacterium. These lines are mixed in a vessel prior to inoculation (Figure 5). Then, by making simultaneous inoculations, successful silencing is performed. In the application of the VIGS technique in other plants, the inoculation method should be optimized.

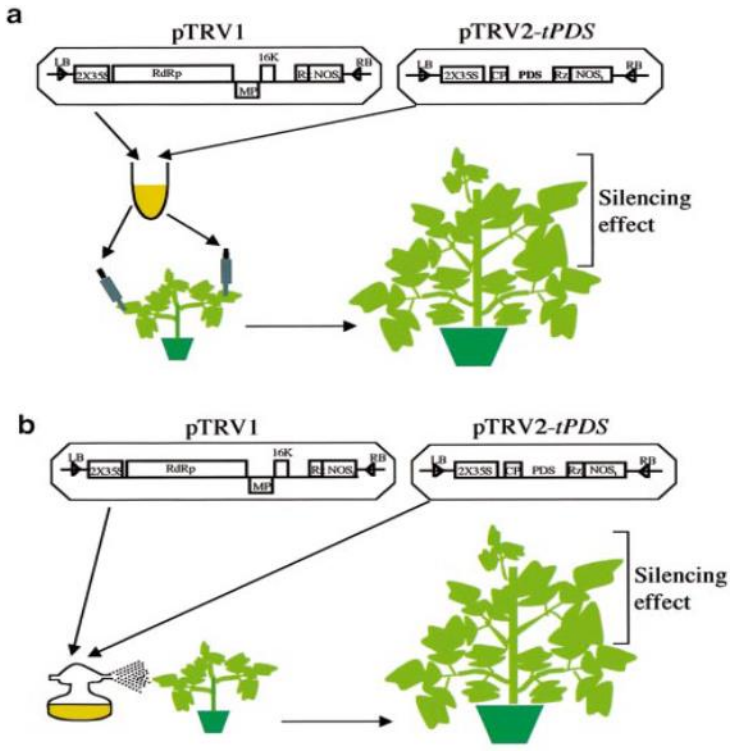

Figure 5. Plant inoculation methods of Agrobacterium containing TRV:PDS clones (Liu et al. 2002)

An interesting method has been developed in which the Agrobacterium suspension containing the TRV-VIGS vector is applied directly to the plant soil. This method is named agrodrenc. With this method, the application of TRV-VIGS vector to young plants whose leaves are not fully opened can be done easily. This method has been used effectively in studies of silencing genes in roots (Figure 6) (Purkayastha and Dasgupta 2009).

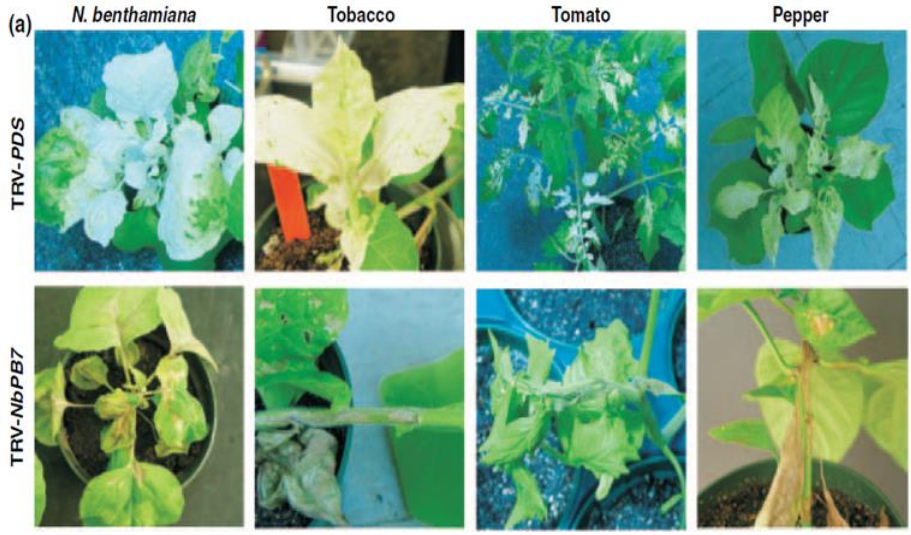

Figure 6. VIGS agrodrench application in many Solanaceae species. PDS and NbPB7 genes in Nicotiana benthamiana, tomato, tobacco, and pepper plants were silenced (Ryu et al. 2004).

Figure 7. Silencing of Actin gene by Agrodench inoculation in roots of *N. benthamiana* plant (Ryu et al. 2004).

In order to maintain a high silencing effect at every stage of virus-induced gene silencing, some factors should be considered and optimization should be made. The size of similar genes affects the effectiveness of VIGS. 300-800 bp is the optimal size for effective silencing of internal genes. If the size exceeds 1.5 kb, the virus cannot spread and it loses most of its insert. The

lowest size for effective silencing was determined as 23 bp. One of the factors to be considered in VIGS applications is the region of the gene targeted for silencing (Değirmenci and Ertunç 2010).

For each VIGS trial, the targeted gene's area that needs to be silenced must be carefully taken into account. It is crucial to establish whether a gene that is a member of a gene family can be suppressed, primarily through BLAST search or DNA gel blot analyses, because any transcript carrying at least 23 nucleotides similar to the targeted gene will potentially be silenced in post-transcriptional gene silencing. The open reading frame (ORF) may be used to silence any portion of a gene that only has one copy. The silencing site must be carefully chosen in order to mute a single gene within the gene family. The 23 nucleotide sequence needed for silencing should not be present in the chosen region, it should be noted. The usage of untranslated regions (UTR) was contemplated as a potential method to distinguish between highly conserved open read segments in genes.

On the other hand, functional diversity is likely to be overcome if conserved sections between genes are chosen to co-suppress multiple members of a gene family (Lue et al. 2003). Given its ability to target a single gene or a large number of genes, virus-induced gene silencing is expected to prove to be a crucial tool for distinguishing overlapping roles within gene families in the future.

Recent research has demonstrated that by adding numerous gene sequences into the virus, it is feasible to simultaneously silence a number of genes. The magnesium chelatase component (water) and proliferating cell nuclear antigen (PCNA) genes were simultaneously silenced by Peele et al. using the TGMV VIGS vector (Peele et al. 2001). Additionally, the PDS and Chlorata 42 genes in Arabidopsis were silenced by the CbLCV VIGS vector (Turnage et al. 2002). Trials of virus-induced gene silencing are becoming more complicated. The reasons of epistatic linkages in pathways (such the formation of lignin) can be attributed to the simultaneous inhibition of the expression of numerous different genes (Abbott et al. 2002). Moreover, a strategy that can be applied for numerous plants that obstruct large-scale imaging is the use of randomly varied gene sequences in the same vector.

The impact of gene silencing may vary depending on the environment. Both the plant's growth and the virus's ability to spread within it are impacted by the environment. One of the most crucial elements for silencing efficacy is temperature. Usually, high temperatures reduce the symptoms in virus-infected plants. According to research by Deirmenci and Ertunç (2010), VIGS is often effective on tomato plants with TRV at temperatures between 15 and 21 degrees.

2.4. Molecular Mechanism of Virus-Induced Gene Silencing

The central process of RNA silencing induced by virus infection, which results in the deletion of related nuclear genes and viral replication, forms the basis of the VIGS mechanism. The RNA silencing mechanism known as VIGS is also referred to as PTGS in plants, quelling in fungi, and RNA interference in animals. Three steps make up VIGS's mechanism. Promoting gene silencing, producing the silencing signal, and spreading gene silencing are these processes.

DsRNAs play a key role in the first stage of gene silencing. The RdRp enzyme converts viral ssRNA that enters the cell as a result of viral infection into dsRNAs. Following recognition by Dicer-like (DCL) enzymes, these dsRNAs are broken down into 18–25 nt small RNA (siRNA). distinct viruses have distinct dsRNA forms. The RdRp enzyme in RNA viruses uses viral ssRNA as a starting point to produce dsRNA. However, a mysterious process indirectly provides dsRNA from DNA viruses. This mechanism is used by geminiviruses, which have a two-part ssDNA structure. With such DNA structures, viruses need the enzymes RdRp6, putative RNA helicase (SDE3), Argonaute1 (Ago1), and SGS3 for this production.

The second stage involves amplifying the gene silencing signal sequence. viruses' ssRNAs. RdRp uses it as a template and creates new dsRNAs using the antisense arms of the acquired siRNAs. The process continues in this fashion as the newly formed dsRNAs reactivate to generate further siRNA. In this process, thousands of siRNAs are generated. When the RISC fuses with the target RNA, the antisense arm of the double-stranded siRNA helps cleave the target RNA at a location in the complementation region. The breakdown of the

target RNA spreads throughout the entire plant by continuing this cycle (Figure 6) (Deirmenci and Ertunç 2010).

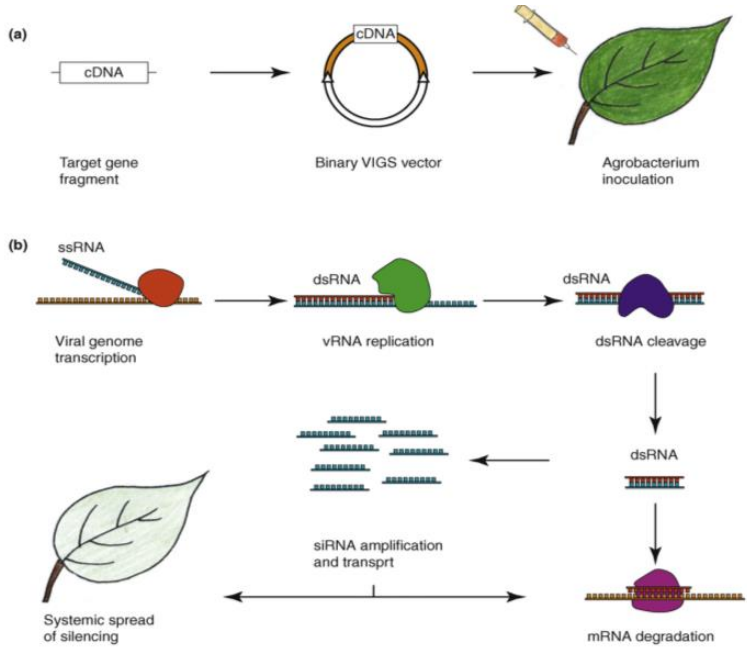

Figure 8. VIGS technique and molecular mechanism in general graphic representation a) Agrobacterium tumefacience is used to transmit the binary VIGS vector containing the viral genome (TRV, etc.) and the target gene (PDS, etc.) to the plant. b) The viral genome is carried by T-DNA, which is inserted into the plant genome and transcribed by the host RNA polymerase (Red). From viral transcripts, RdRp (Green) ssRNA generates dsRNAs. The Dicer (Blue) enzyme recognizes dsRNAs and breaks them down into short silencing RNAs. The RISC complex (Purple) recognizes these silencing short RNAs and converts them to ssRNAs, which they employ as templates to silence the target gene. Single-stranded siRNAs are then amplified and employed as a silencing signal in other plant organs to silence the target gene (Lange and Becker 2009).

2.5. Experimental Controls for Virus-Induced Gene Silencing Trials and Evaluation of the Silencing Effect

Any virus-induced gene silencing study needs appropriate controls to monitor the efficiency of the silencing. According to Kumagai et al. (1995), the PDS gene has an identifiable phenotype that is frequently utilized as a positive

control (Figure 8). Numerous investigations have used the magnesium chelatase gene as a positive control (Kjemtrup et al. 1998; Peele et al. 2001). It is crucial to inoculate the plants with virus blank as a negative control in order to determine whether the phenotypic formation is caused by the virus itself. But it should be remembered that the virus will influence the phenotypic that is seen. Sequences that are anticipated to arise from endogenous genes have also been proposed as useful positive controls for VIGS. These sequences will provide a benign phenotype that can be put into any vector. Researchers will be able to readily locate the silencing region in the plant using these positive controls (markers).

Simultaneous polymerase chain reaction (Real-time -RT-PCR) determines the intensity and specificity of VIGS. This method, which screens for low transcript levels using a tiny sample of tissue, is more sensitive than RNA gel-blot analysis. To compare the corresponding reduced transcript level from silenced genes and virus-infected tissues exclusively, RT-PCR products are amplified (Ekengren et al. 2003; Liu et al. 2004). RT-PCR primers are created from sequences other than the target transcript that were cloned into the VIGS vector in order to prevent amplifying the viral sequence. The target transcript or transcripts are precisely amplified in the PCR process thanks to subsequent cloning and sequencing of the PCR products.

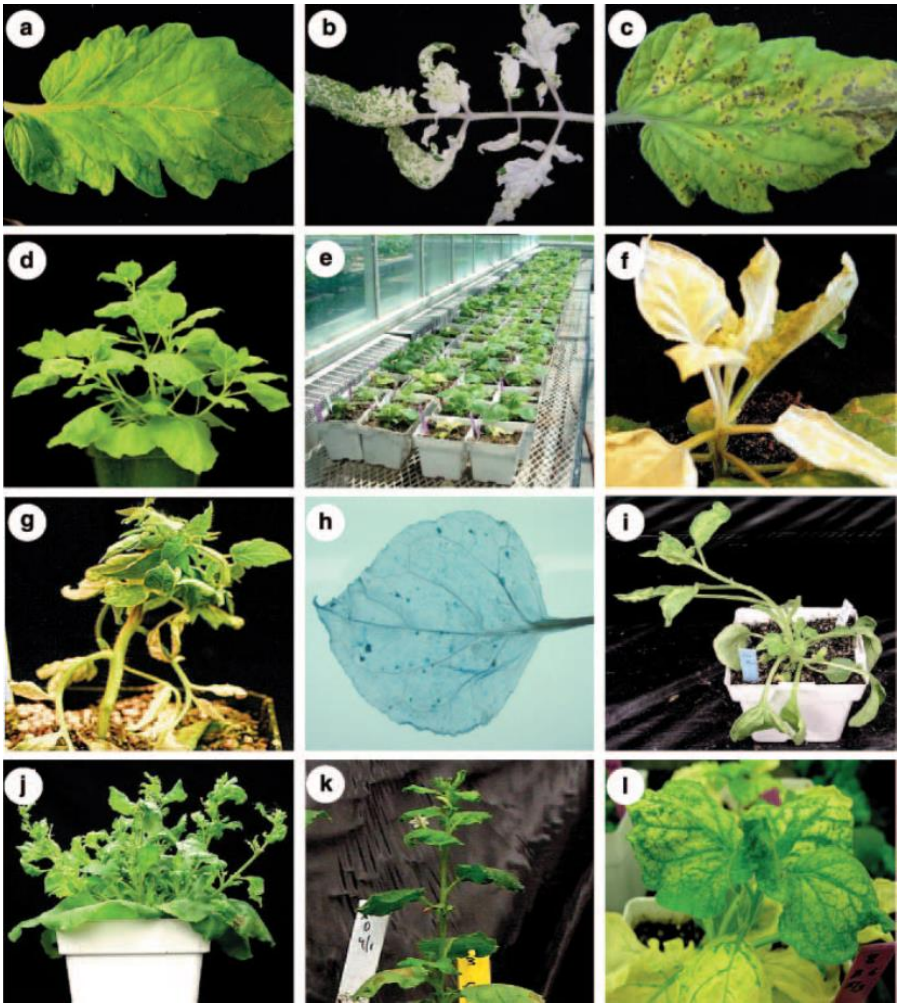

Figure 9. Some of the phenotypes produced by VIGS trials. A) Tomato leaf infected with TRV virus alone b) Silencing of PDS gene using TRV construct c) Silencing of TRV:Prf gene in tomato leaf d) PVX virus infected Nicotiana benthamiana plant e) VIGS screening of random cDNA library f) Silencing the PDS gene in *N. benthamiana* using the TRV construct in the second g) Silencing the CTR1 gene in tomato plant h) Silencing the tomato PP2AC gene in *N. benthamiana* resulted in cell death in particular regions i-l) Using random cDNAs, abnormal fentype formation was seen in N. Benthamiana. i) Silencing of an unknown gene resulted in leaf form and internode abnormalities. j) The plant has a short stature, numerous branching, small and mixed leaves as a result of silencing the gene producing the nuclear shuttle protein.

2.6. 'Fast-Forward Genetics' using Virus Induced Gene Silencing

Vast VIGS studies are now possible thanks to developments in vectors and inoculation methods. The closest method to Forward Genetics is virus-induced gene silencing, in which a random cDNA column is inserted into the virus vector and the appropriate phenotype is shown. This method, known as "Fast-Forward Genetics," has the major benefit of making it simple to isolate and identify the gene sequences responsible for the relevant traits. With TMV and BSMV-based VIGS vectors, comprehensive functional genomic imaging of *N. benthamiana* and barley is carried out. According to a recent study, almost 5000 cDNAs are silenced in *N. benthamiana* in order to analyze the function of the HSP90 genes in defense signals and to ascertain their contribution to disease resistance. Normalizing cDNA libraries with single sequences is crucial for the success of Forward Genetics (Bruch-Smith et al. 2004).

2.7. Advantages of Virus-Induced Gene Silencing and Comparison with Other Functional Genomic Approaches

Reducing a gene's expression or introducing a mutation in the gene that codes for a protein is the easiest and most reliable way to ascertain a gene's or protein's function. There is a need to employ more methodologies when analyzing the activities of the genes in the genome now that the complete genome sequences of many plants are accessible. Loss-of-function analysis can be conducted using a variety of conventional techniques, including TILLING, T-DNA insertion, transposon amplification, and chemical and physical mutagenesis. The application of virus-induced gene silencing, a recently created technology, can get around the drawbacks of these techniques. As previously mentioned, quick phenotypic production has frequently used virus-induced gene silencing, such as Agrobacterium or in vitro transcription-based ad hoc tests (Burch-Smith et al. 2004).

One of the most popular methods for plant functional genomic analysis (loss of function) along with chemical/physical mutagenesis, TILLING, T-DNA insertion, and transposon actication—is virus-induced gene silencing (VIGS). Key genes involved in the metabolic and regulatory processes of the

plant that cannot be silenced in mutation analysis studies can be studied using the virus-induced gene silencing technique. Traditional VIGS is superior than other approaches in several ways. Gene silencing is a simple and quick process, which is the fundamental benefit of conventional VIGS. The partial sequence of the gene that is to be silenced is enough to silence the gene without the need for stable plant transformation. It takes a lot of time and effort to transfer genes in a stable manner. Forward and reverse genetic studies can both be conducted using conventional VIGS. Additionally, using this method, genes in plants with polyploidy traits, like wheat (*Triticum aestivum*), can be silenced. In addition to all of these benefits, conventional VIGS is employed in the characterisation of genes associated to disease resistance, insect and nematode resistance, food production, and tolerance to abiotic stress in many plant species (Kumar and Mysore 2011). A distinct molecular framework governs how VIGS functions. It can be applied to plant species where the establishment of transgenic plants is challenging (Burch-Smith et al. 2004).

A single species' gene's numerous roles can also be swiftly tested using VIGS. Multiple backcrosses are necessary to test mutant alleles in various genotypes, even though many mutant populations are formed in a single genotype. This is a significant problem. Due to the fact that certain mutations might result in various phenotypes depending on the genotype. The VIGS's adaptability allows for more thorough gene function analyses and the possible avoidance of genetically specific effects-related issues.

Technically applied in functional genomics, VIGS' most significant benefit is the ability to phenotypically define a gene's loss of function within a single generation. Because VIGS specifically targets the gene of interest. To find a mutation in a particular gene, large populations are not necessary. Additionally, using the VIGS vector, it is possible to swiftly sequence and identify the gene in charge of the desired phenotype. As VIGS does not result in loss of phenotypic function, which causes mortality in the early developing period of plants, it can be performed on young plants or mature seeds. This indicates that VIGS research can be used to investigate various genes that are involved in the developmental phase. Functional gene diversity is overcome by VIGS. The target sequence from highly conserved portions of the gene family can be used to mute a subset or all of the family's genes. Only by choosing

sequences unique to those members may specific members of a gene family that have undergone conservation be targeted. VIGS makes it simple to compare gene functions between different species. The ability to compare gene function between various species is another benefit of VIGS. In both *N. benthamiana* and tomato, the TRV-VIGS vector created by Liu has been utilized to describe gene function (Ekengren et al. 2003; Liu et al. 2002). The roles of the MAPK kinase and COI1 genes in tomato resistance to bacterial pathogens and viruses have recently been demonstrated using this vector.

2.8. Limits of Virus-Induced Gene Silencing

Although VIGS has benefits, it has certain inherent limitations as a method for loss-of-function research. The fact that VIGS rarely entirely suppresses the expression of a target gene is one of the most significant of these limitations. The phenotype won't be visible in the silenced plant since the reduced transcription level will still be enough to create enough functional protein. In conclusion, VIGS is unable to identify the participation of a gene in a certain functional context if the phenotype is lacking. VIGS might miss the phenotype that functional abundance between gene families has hidden. The fact that a gene does not turn off at the same pace in all regions of an infected plant is another drawback of VIGS. There may or may not be silence. When silencing does not immediately result in a visible phenotype, confusion can occur in the interpretation of the findings. The creation of an intrinsic positive control for the VIGS vector of the region to be silenced with an evident phenotype may be one approach to solving this issue.

There are a number of drawbacks to VIGS' reliance on a pathogen-host interaction. Developmental alterations, particularly in total weight and leaf shape, can only be brought on by the virus being injected into the plant. With TMV and PVX viruses in VIGS applications, this might be especially true. The resulting mild phenotype from the silenced gene may go unnoticed since it is covered up by the virus symptom. PVX and TMV are examples of VIGS viruses that can persist outside of the meristem tissue. The evaluation of gene functions in the growth of shoots, leaves, flowers, and fruits may not be sufficient as a result. The TRV virus is used to solve these issues. Last but not least, it is frequently observed that non-target genes unintentionally result in

silence in VIGS and general PTGS techniques when working with genome-deficient plants. When working with plants that lack genomic sequences, challenges are challenging to overcome. Although there are some valid points in this argument, the huge potential of VIGS to deliver quick approximations of gene function insights should not be overlooked. As mentioned previously, VIGS has been used for functional research in some notable experiments. The risk of unintended co-suppression will diminish for many existing plants as genome sequence data and sizable EST collections grow. However, for the viability of VIGS, it is critical to expose the complete nucleotide sequence of the plants. Numerous research have been successful in silencing the target gene family member without affecting other members. This has demonstrated the viability of highly targeted gene silencing with VIGS (Ekengren et al. 2003; He et al. 2004; Liu et al. 2004).

2.9. Other Functional Genomic Approaches

Chemical mutagenesis, taranspoon, and Agrobacterium T-DNA (insert insert that will produce fragmentation in the coding region) are the most often utilized methods in investigations of loss of function in plants. These technologies are successfully and widely employed. Additionally, for the model organism Arabidopsis, these techniques are still recommended. These methodologies (methods), it is believed, have a number of significant drawbacks when applied to other plant species. In order to screen for mutations in the target gene using these techniques, a large population must first be generated. 2001 (Bouche & Bouchez). Second, the generation of T-DNA or transposon mutations requires huge populations, and these lengthy processes. Third, a measurable phenotype cannot be produced in mutation studies because of the sizeable gene families and gene duplications in the plant genome (Bouche and Bouchez 2001). Since many mutations still have unanswered questions, the gene whose function was researched is unknown. Therefore, even if mutations are found in transposon and T-DNA lines, they might not be isolated. According to Henikoff and Comai's 2003 interpretation of the silent phenotype, this scenario is undesirable. It takes several backcrosses to find the appropriate mutation. This procedure might be laborious and time-consuming. VGS stays clear of these issues. As a result, it is considered to be an addition to other conventional treatments.

2.10. Other Silencing Methods

Other PTGS-based technologies are employed in addition to transitory VIGS to wash away the expression of the target gene. These procedures employ antisense RNA. These methods necessitate the creation of stable transgenic lines, however these lines are frequently unreliable. Despite the time-consuming process involved in creating viable transgenic RNAi lines, this method has a number of advantages over antisense approaches (Waterhouse et al. 2001). Transferring hpRNA constructs to plants results in stable RNAi lines (Smith et al. 2000). These constructs produce findings that are effective, trustworthy, and reproducible (Smith et al. 2000; Wesley et al. 2001). Additionally, all plant species can utilise these constructions uniformly. The gene sequence within the hpRNA construct can be cloned onto a variety of vectors with excellent efficiency (Helliwell and Waterhouse 2003; Wesley et al. 2001). The transformation issue still exists, and this method makes large-scale imaging difficult.

3. AREAS OF USE OF VIGS TECHNOLOGY

3.1. Using VIGS to Understand Plant Defense System Against Pathogens

The VIGS approach was utilized to comprehend plant pathogen defense. ViGS is used most frequently in research on plant defense. A gene known to be involved in defense can have its role determined using VIGS, as can novel genes. For instance, Shirasu et al. (1999) initially described the involvement of the Rar1 gene in disease resistance through the isolation of mutant barley. Later, the Rar1 gene's equivalent function in tobacco was demonstrated by the VIGS method (Liu et al. 2002). Similar to this, VIGS was used in *N. benthamiana* to identify the involvement of the STG1 gene in resistance to bacteria and viruses (Liu et al. 2002c; Peart et al. 2002b). Additionally, N-mediated resistance to the TMV virus was produced via the silencing of the EDS1 and NPR1/NIM1 genes. Additionally, VIGS *N. benthamiana* has been used to show that a number of kinases are involved in TMV protection. NPK1, WIPK, SIPK, NtMEK1, and NtMEK2 are the kinases in question. The function of the protein phosphate type 2A catalytic subunit in plant defense has recently been studied using VIGS. It

was identified by the increased expression of the particular tomato PP2a and PP2Ac1 genes during pathogen attacks and pathogen responses. The tomato PP2Ac gene's sequence was used to silence this gene in N. Benthamiana, and the results included enhanced production of defense genes, self-localized cell death in the leaves and stems, and R-linked signaling.

According to Ekengren et al. (2003), VIGS was used to find the genes necessary for tomato resistance to the bacterial disease Pseudomonas syringae. The functions of the transcription factors TGA1 and TGA2.2 as well as the MAPKKs MEK1 and MEK2, MAPKs NTF6, WIPK, and defense-associated NPR1 gene were also identified by this work. Some of these genes had known defense pathway activities in other plants. In addition to the candidate gene technique, VIGS has been utilized to determine the function of hitherto unidentified genes in illnesses. P58IPK was proven to be required for viral pathogenesis in plants by demonstrating a decrease in virus density in *N. benthamiana* plants when P58IPK was silenced (Bilgin et al. 2003). It's interesting that P58IPK silencing results in cell death in the virus-infected host cell. HSP90 has been demonstrated to be necessary for N-mediated resistance to TMV using a similar method. With the use of VIGS, other genes involved in TMV resistance, such as the protective COP9 signalosome, have also been examined.

3.2. Using Virus-Induced Gene Silencing to Understand Metabolic Pathway and Plant Growth

VIGS has also been used in studies in other areas of plant biology. Many metabolic pathways such as plant sterol synthesis, formation of jasmonate-stimulating inhibitor products against pathogen attacks and photosystem II in biosynthesis have been analyzed by VIGS (Darnet and Rahier, Saedler and Baldwin, 2004). According to Button et al. Using the PVX-based VIGS vector, *N. benthamiana* also silenced CesA, a cellulase synthase gene. The existence of a large number of genes carrying a homologous sequence to the gene known as CesA has emerged in the Arabidopsis genome. VIGS was used to determine the function of the CESA-1 gene in *N. benthamiana* instead of traditional silencing approaches. Although it shows 80% similarity to CesA-2, a specific phenotype has been successfully observed with silencing of CesA-1. It is

difficult to mutate genes in developmental stages because of the many severe deforming or lethal phenotypes that result from mutation. For example, genes such as the meristem gene NFL and the flower organ gene DEFICIENS in *N. benthamiana* and the LEAFY and AP3 genes in Arabidopsis were silenced using VIGS, respectively.

4. RECENT DEVELOPMENTS IN VIRUS-INDUCED GENE SILENCING

4.1. Long Term Virus-Induced Gene Silencing

Long-term virus-induced gene silencing throughout the plant life cycle has the potential to replace mutant or stable RNAi lines. Long-term VIGS is used to assess the overall efficacy of biotic and abiotic trials. Plants exposed to stress from seedling to final growth stage, for example, can be used to assess the function of a gene imparting stress tolerance. VIGS can live for many years in suitable conditions. Long-term virus-induced gene silencing allows researchers to investigate the roles of genes implicated in senescence and a variety of other metabolic pathways in annual and perennial plants. In plants where transformation is difficult, long-term virus-induced gene silencing is employed. A substantial mutant collection is unnecessary. Long-term VIGS has been reported to be used in vegetatively propagated plants. In vitro approaches have been used to demonstrate VIGS in potato tuber utilizing the Potato virus-X (PVX)-based VIGS construct. Researchers can use these techniques to create dormant plants with the same genotype. Infecting fresh shoots of ginger with Barley stripe mosaic virus (BSMV) further revealed the virus's ability to silence genes. Virus-induced gene silencing has been used in plant propagation via tissue culture, callus development, and other in vitro stages.

4.2. Transferable Nonintegrated Post-Transcriptional Gene Silencing

VIGS, unlike fixed RNAi and mutant plants, does not change the genetic composition of the target plant. However, VIGS has all of the advantages of fixed RNAi. One of the key disadvantages or weaknesses of VIGS was that it could not be inherited. However, new research indicate that VIGS can be transferred to first generation facilities. VIGS based on BSMV have been found

to be stable over 6 generations in barley and wheat. In the first generation, gene silencing was reported at a rate of 11%. For the third and subsequent generations, this rate has reached 100%. In addition to wheat and barley, BSMV-based VIGS effectively silenced genes in other monocot plants including as oats, rice, and Brachypodium distachyon. ASLV-based VIGS have been demonstrated to be transmitted at a rate of 33% in the first generation and at a rate greater than 55% in future generations in soybean. Following generations of hushed people demonstrated the same strong silencing effect. The ASLV-based VIGS vector has the ability to silence genes in 15 plant species from the families Brasicaceae, Leguminosae, Cucurvibitaceae, and Solanaceae. TRV-based VIGS is widely employed in plant species and is one of the most prominent VIGS systems in dicot plants. TRV-based VIGS transmission has been proven in *N. benthamiana* and tomato. In addition, various different VGS vectors or viruses may be passed on to first generations.

VIGS-based non-integrated post-transcriptional gene silencing describes the phenomena of gene silencing in first-generation plants. This approach provides a number of advantages over typical short-term VIGS. One of these benefits is that it causes minimal or mild viral symptoms in the first generation. However, the chemical mechanism behind this mild viral symptom has yet to be discovered. The ability to quiet genes expressed during dormancy, seed germination, and seedling production is the second advantage. The third advantage is the systematic silencing of the seedling's root, stem, and cotyledons. Viruses can survive multiple generations of plants. It has been claimed, for example, that BSMV can survive in seeds stored for 20 years. Seeds from VIGS-treated plants can be preserved for an extended period of time, and silent plants can be obtained afterwards. The epigenetic foundation of VIGS transfer to the first generation is still unknown. Furthermore, large-scale VIGS vector and host experiments are required for virus stability, silence of the same genes, and seed delivery efficiency. However, new research has revealed that there are seeds in plants that have transferred the VIGS vector. As a result, first generation VIGS seed transmission may be a viable alternative to stable gene silencing approaches. Unlike seed transmission, it has been demonstrated that VIGS can be passed to subsequent generations of plants by vegetative propagation.

4.3. Inherited Transcription Gene Silencing Through VIGS Vectors

Virus (VIGS) vectors, unlike post-transcriptional gene silencing, can cause transcriptional gene silencing (TGS) by DNA methylation. siRNAs produced from dsRNA can methylate gene promoters. Recent studies in plants support epigenetic changes in the promoters of endogenous genes targeted by dsRNAs. In petunia and tomato, a cucumber mosaic virus (CMV)-based vector effectively promotes hereditary endogenous gene silencing via dsRNA. Because the viral genome that enters the plant does not bind to the plant genome, the target cells are referred to as non-transgenic cells. Continuous gene silencing in plants is done using this method. In this case, neither the presence of a transgene nor the presence of a virus is required for the persistence of gene silencing through generations. TGS (transcription gene silencing) using viruses is simple and effective. It can also be used to a wide range of plant species, tissues, and organs. The virus-based non-transgenic stable TGS method is gaining traction (Figure 7). The virus-based non-transgenic stable TGS technique is appropriate for seed and vegetative plant multiplication. Although mutant plants are useful for studying gene function, invalid mutations in many genes can result in severe developmental problems and exhaustion. Virus-induced TGS may then be useful for investigating the roles of these genes. In addition to the limits of transmitted PTGS and TGS, it has some limitations of its own. First, effective gene silencing varies among generations. As a result, transcript and/or protein activity must be quantified over all generations. Second, environmental conditions during seed storage and germination may influence virus propagation and gene silencing. Another drawback is that not all viral vectors are transmitted by seed. As a result, in the first generation, these vectors are challenging to use for post-transcriptional gene silencing. Only two vectors have been reported to date for use in transcriptional gene silencing. The approach (PTGS and/or TGS) employed for gene silencing is determined on the type of virus vector being examined.

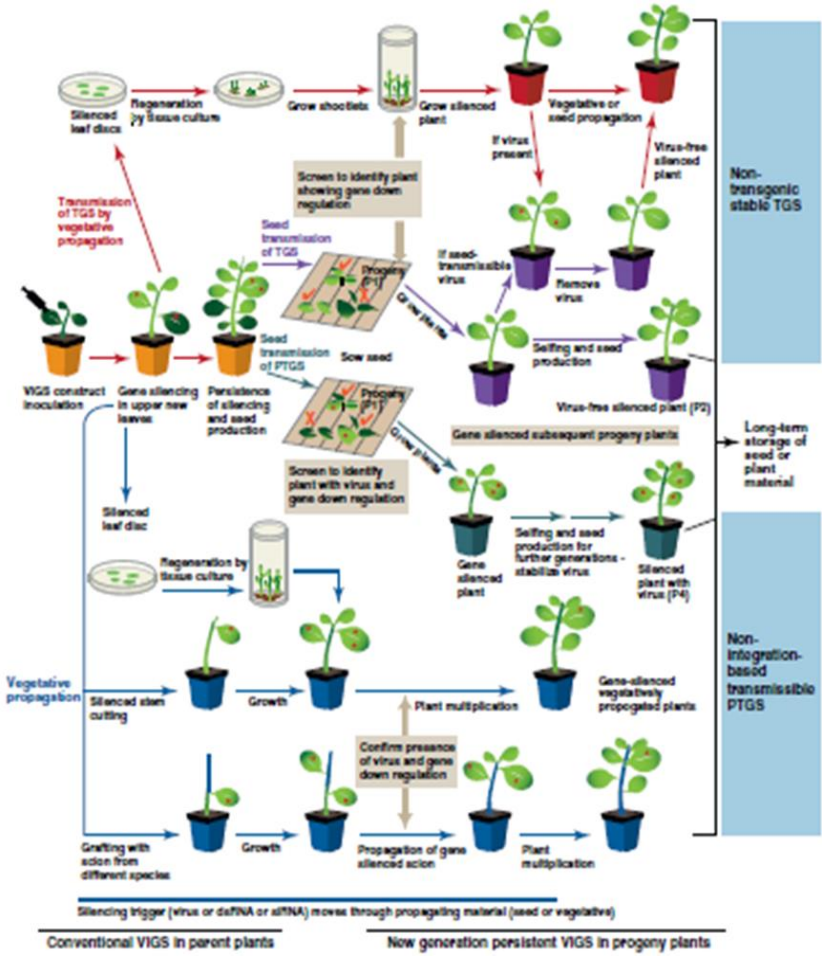

Figure 10 . Using the VIGS vector, strategies for the establishment of persistent gene silencing in plants. The target plant is injected with the appropriate vector containing a portion of the desired gene. The insert must share at least 21 nt of homology with the target endogenous gene's mRNA. As VIGS vectors, either inherited or non-inherited viruses are utilized. If the VIGS vector employed for post-transcriptional gene silencing is transmissible, it can cause transduced post-transcriptional gene silencing in seed-producing plants. Many viruses are known to be capable of permanently silencing genes in first generation plants or sister plants. Cuttings can be used to cause VIGS in many species. This non-integrated base qualifies as transferrable PTGS since the virus genome does not connect with the plant genome. TGS can use both inherited and non-inherited viral vectors. Seeds from gene-silenced plants can be collected, and germinated and gene-silenced seedlings can be obtained. These plants can be propagated and utilized to conduct gene silencing experiments. If the virus arises in the

plant, it can be eliminated by meristem culture or heat shock. Leaf fragments taken from plants silenced by tissue culture can also be used for regeneration.

4.4. Recent Applications of Virus-Induced Gene Silencing Technology in Plant Biology

Recent developments in virus-induced gene silencing procedures allow researchers to combine VIGS with other functional approaches. Many genes with different nucleotide sequences can be silenced at the same time by co-inoculation of their respective VIGS constructs. Similarly, a single VIGS vector can mute numerous genes with high nucleotide homology. To evaluate the function of a genetic pathway, the VIGS approach is also employed to silence the other allele gene in a mutant plant. As a result, the problem of double loss of function in genes is solved. VIGS has been shown to be an effective and useful tool for comparing unlinked genes that are overexpressed or silenced in stable transgenic plants with the VIGS target gene. VIGS can also be utilized in conjunction with molecular breeding approaches. A significant number of genes in certain plant processes are evaluated using the combination of cDNA-AFLP and VIGS. Furthermore, VIGS plants can be employed in the breeding program and during map-cloning to check gene expression. Similarly, VIGS plants can be used in downstream applications. Protoplast and cell cultures created from certain organs of the silenced plant, for example, allow for a more in-depth investigation of specific pathways.

The VIGS approach has been used successfully to silence genes in strawberry and tomato fruits, even after the fruit has been harvested from the plant. This approach is used to mute genes involved in basic metabolic functions without deforming the plant, which is particularly useful during the early stages of vegetative growth. It is employed in the functional investigation of processes related to cell wall loss, ethylene generation, and particular research of genes related to maturation. VIGS is also utilized in the plant's early growth phase to prevent cell disruption and other vegetative abnormalities in the fruit.

VIGS vectors contain many cDNA libraries. These are easily applicable for gene silencing. Following gene silencing, a wide range of genes in plant pathways are investigated. An advanced genetics method, for example, has

been used to study genes involved with disease resistance and new metabolic pathways in N. Benthamiana. VIGS vectors and inoculation methods for silencing genes in numerous plant organs have been established in recent studies.

4.5. Future Vision of Virus-Induced Gene Silencing

In plants, VIGS outperforms other previously developed gene silencing techniques. Both post-transcriptional and transcriptional gene silencing (internal mRNA silence) have recently been shown to be long-lasting and transmissible. However, studies on hereditary and long-term VGS in a variety of plant species have expanded to other genes. VIGS's application potential in the development of cultivated plants has just recently been recognized.

Given the current benefits of VIGS technology, numerous VIGS applications in modern biology appear promising. VIGS vectors can be utilized to generate crop plants through genetic engineering as well as traditional or molecular breeding (Figure 8). Using viral vectors, for example, it is possible to reduce and vary the flowering time of the main genotype or indeterminate variants in wild relatives or natural lines. The late blooming genotype can be crossed with the early flowering genotype when flowering is silenced. This guarantees that the pollination barrier is lowered or removed in the late flowering plant. Reduced flowering time can hasten plant reproduction. The first generation plants are then protected from viruses using heat or ice shock. Meristem culture can be used to create a virus-free plant from meristem tissues that viruses cannot enter. Virus-free plants can be recognized among first generation plants. Because virus transmission to the seed is not always perfect. However, non-transmitted seeds should be used as much as possible to prevent viral transmission to future generations. Breeders use advanced genetic results mediated by VIGS to identify genes critical for plant activities (for example, salt tolerance). Furthermore, these findings are utilised in the quick identification of candidate genes for critical traits in crop plants during the map-based cloning procedure. RNA from a resistant genotype (for example, rust-resistant genotype) can be used to create a cDNA library. By combining the advanced genetics approach mediated by VIGS with these libraries, it is

possible to identify the genes responsible for the resistance trait within the genotype quickly.

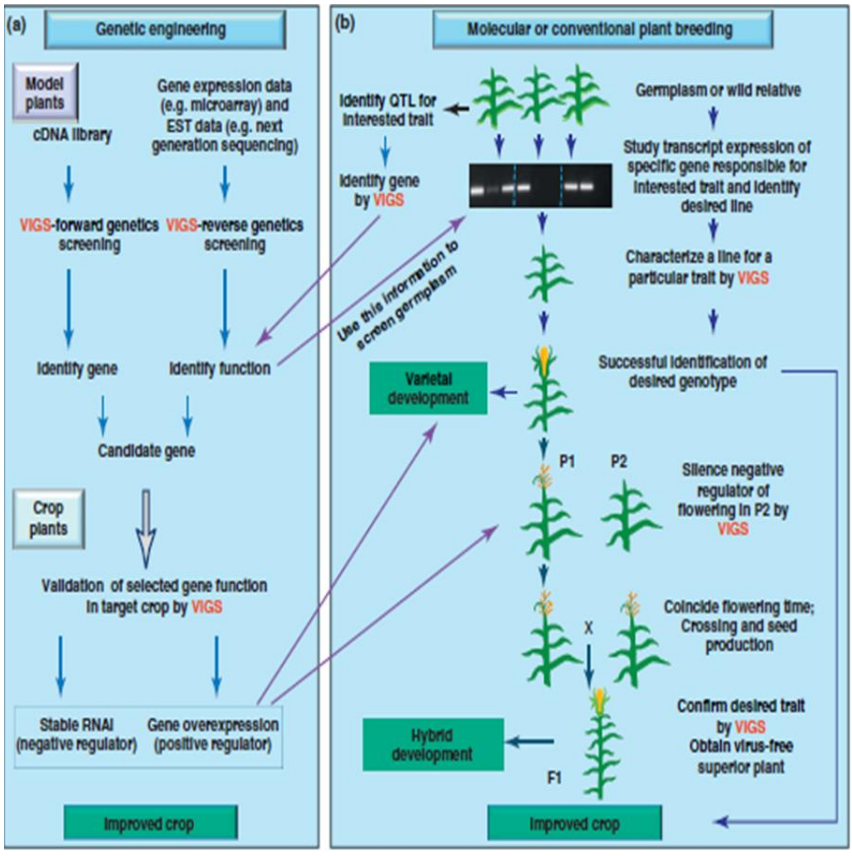

Figure 11. The use of VIGS in a plant breeding program. a) VIGS can be utilized to develop a specific plant in both forward and reverse genetic techniques. VIGS can be utilized in both molecular and traditional plant breeding.

This information can be utilized to develop plants using the transgenic technique. Furthermore, this information can be employed for selection in the molecular breeding program using markers. To find host genes, researchers have long employed a mix of cDNA-AFLP and VIGS techniques. Similarly, with the combination of VIGS, microarray, and next-generation sequencing technology, functional analyses of genes can be carried out with high efficiency. Breeders can now work directly on target crops such as tomato, barley, wheat, soybean, rice, and maize using current VIGS methods.

Approaches such as mutant, RNAi, and overexpression are helping to hasten the development of biotic and abiotic stress-tolerant cultivars.

Non-integrated delivery-based post-transcriptional gene silencing and transcription silencing (internal mRNA silencing) approaches have the potential to be utilised in future plant improvement projects. Non-transgene-bearing modified plants' products can be successfully altered at the gene expression level. VIGS can be used to improve crop plant genetic transformation resistance to Agrobacterium-mediated plant transformation. For example, VIGS can quiet genes that operate as negative regulators of Agrobacterium-mediated plant transformation, and the silenced plant explant can be employed for stable transformation. The viruses are then extracted from the transgenic plant in the manner described above. Viral vectors and stable TGS techniques are utilized to silence mitochondrial and chloroplast DNA by targeting homologous dsRNAs to their DNA.

One of the most recent new VIGS techniques is the usage of microRNAs (MIR-VIGS). MIR-VIGS is mediated in plants by a virus vector carrying the engineered microRNA sequence. MIR-VIGS and VIGS vectors are predicted to be useful for gene silencing in developing plants. VIGS viruses are easily transmitted to other (non-virus-infected) plants growing on the same land as the affected plants. As a result, an adequate biosafety regulation encompassing seeds and other plant materials harboring the VIGS vector is required.

CONCLUSION

Gene silencing, or RNA silencing, is a resistance mechanism found in plants more than in other eukaryotic species. PTGS, in particular, is thought to be an effective viral resistance mechanism. Because plants lack immune-boosting chemicals, the establishment of an effective gene silencing mechanism is regarded to be more critical in these organisms than in animals.

With the discovery of the viral-induced gene silencing mechanism (VIGS), the general mechanisms of gene silencing were well known. The VIGS data serve as a starting point for investigating gene functions. These investigations yielded some insights into the gene's particular features. These techniques have been widely exploited in plant disease resistance and large-

scale gene silencing projects since the introduction of gene silencing, infection methods, and vectors pushed by viruses (Deirmenci and Ertunç 2010).

Virus-induced gene silencing (VIGS) as a reverse genetics technique has many advantages for functional genomic and even proteomic studies. The VIGS technique will be utilized more widely and frequently as the full genomes of several important crop species are sequenced. Although it has considerable promise for widespread application, it has numerous limitations that must be addressed. To begin, the host range of viral vectors must be extended. The VIGS technique relies heavily on sequence information. As a result, the entire genome sequence and EST databases will substantially aid in the application of VIGS. The VIGS-based technique can detect important and astonishing traits on a large scale (Unver and Budak 2009).

In recent years, research have been conducted by merging genetic and molecular biological techniques between distinct processes in the known models of the VIGS mechanism. Furthermore, the discovery of the reporter gene system has boosted the robustness and impact of VIGS. The VIGS mechanism will be applied in larger investigations in the future (Deirmenci and Ertunç 2010).

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CHAPTER 8

RESEARCH THE ALLELOPATHIC AND ANTIFUNGAL POTENTIAL OF *INULA VISCOSA*: A STUDY ON MEDITERRANEAN PLANT EXTRACTS

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INTRODUCTION

The increasing world population and rising welfare levels have led to a higher level of consumption. Changes in dietary habits have caused agricultural production to fall short of meeting the global food demand. Limited agricultural lands and the growing population pose a significant challenge in feeding the world (FAO, 2009). In the latter half of the 20th century, various solutions were sought to address the increasing food needs and improve productivity per unit area. This period, known as the Green Revolution, witnessed increased mechanization in agriculture, widespread use of chemical fertilizers, and the introduction of chemical pest control (Kılıçgil, 2014). However, the unplanned use of agricultural systems and excessive reliance on chemical inputs led to the loss of sustainability in agriculture. The intensive use of synthetic pesticides resulted in issues like reduced plant vigor, environmental damage, and toxic residues (Isman, 2000). Consequently, extensive research has been initiated to find alternative methods that minimize harm to human health and the environment. As a result, alternative approaches to increase crop yield have been explored (Altieri, 1995). One such method involves utilizing secondary compounds naturally occurring in plants. These compounds, known as allelochemicals, play a role in allelopathic interactions between plants. Allelopathy refers to the phenomenon where one plant affects another through the production and release of secondary metabolites, exerting positive or negative effects (Erez and Battal, 2022). The concept of allelopathy, associated with naturally occurring secondary compounds among plants, has gained interest from pharmacologists, herbologists, ecologists, and physiologists. Understanding the concept and mechanism of allelopathy requires a comprehensive understanding of the effects and causes of allelochemical substances in plants. These allelopathic secondary compounds also impact processes such as germination, shoot and root growth (Li et al., 2020), cell division, ion absorption, water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme functions, and gene expression (Singh and Thapar, 2003). The responses exhibited by plants to allelochemicals, produced and released by plants into the environment, vary based on factors such as allelochemical structure, dosage, genetic potential of the target plant, and its metabolic activities (Bao et al., 2019; Inderjit and Mukerji, 2006).

Allelochemicals such as cinnamic acid, vanillic acid, coumarin, and sorgolonone can influence the efficiency of photosystems by affecting chlorophyll function (Erez and Battal, 2022). Furthermore, these allelochemicals can impact not only other plants but also plant pathogenic fungi, insects, and other organisms in nature. Phenols, tannins, peroxidase, polyphenol oxidase, and compounds like Bt proteins (insecticides produced by *Bacillus thuringiensis* bacteria) can suppress insect populations (Gajger and Dar, 2021). Additionally, phenolic compounds produced by plants have been found to be effective against plant pathogens such as *Pseudomonas putida*, *Pseudomonas pyocyanea*, *Corynebacterium xerosis* bacteria, *Fusarium oxysporum* and *Penicillium italicum* (Kocaçalışkan et al., 2006). Various plant species belonging to the Asteraceae family have demonstrated allelopathic effects on other plants, leading to reduced seed germination and crop yield (Muehlchen et al., 1990). *Inula viscosa* L. Aiton (syn. *Dittrichia viscosa* L. Greuter), which belongs to the Asteraceae family, is an evergreen plant that grows in the Mediterranean region. This plant exhibits several properties, including anti-inflammatory effects (Hernández et al., 2007), antidiabetic activity (Yaniv et al., 1987), antipyretic and curative properties, as well as antiseptic and antiphlogistic activities (Lauro & Rolih, 1990). It has also shown antiviral properties (Abad et al., 2000), antifungal effects (Cafarchia et al., 2002), and antibacterial activity (Squalli et al., 2007).

The objective of this study is to determine the allelopathic and antifungal potential of the *Inula viscosa* plant, which is naturally distributed in the Mediterranean region..

MATERIAL AND METHOD

1. Plant Materials: *Inula viscosa*, a plant naturally distributed in the Mediterranean, Aegean, Marmara, and West Black Sea regions of Turkey (Figure 1), was used as the main material for the experiment. *Inula viscosa* plants (Figure 2) were collected from the Alanya district of Antalya province during the flowering stage of the 2022-2023 vegetation period. The collected plant materials were dried in the shade and stored in the laboratory.

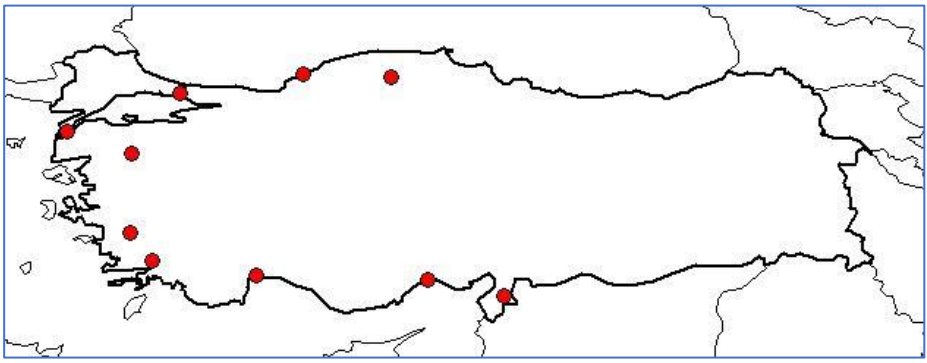

Figure 1. Areas where *Inula viscosa* is distributed (Anonymous, 2023a)

Figure 2. *Inula viscosa* plant (Anonymous 2023b)

2. Fungal Cultures: The plant pathogenic fungus *Cercospora beticola* isolates used in the study were obtained from stock cultures in the Phytopathology laboratories of the Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University. The fungal cultures were grown in 90 mm petri dishes containing 20 ml of Potato Dextrose Agar (PDA) at 23±2 °C for 7 days before being used in the study.

3. Preparation of Plant Methanol Extracts: 100 g of ground plant material was weighed and placed in Erlenmeyer flasks. To each flask, 600 ml of methanol and ethanol were added. The mixture was then subjected to agitation at 120 rpm in an orbital shaker at room temperature for 24 hours. The extract was subsequently filtered through filter paper, and the methanol was evaporated at 32°C until a solid material was obtained. Different concentrations (0.5, 1, 2, and 4 mg/mL) were prepared by diluting the remaining extract with pure water-acetone (Kadioglu and Yanar, 2004).

4. In vitro Antifungal Activity Study: The antifungal effect of the plant extracts was determined using a modified agar plate method (Nwosu and Okafor, 1995). The prepared PDAs were autoclaved and cooled to 40°C. The plant extract was mixed with sterile PDA at different doses (0 (control), 0.5, 1, 2, and 4 mg/mL). The PDAs were then transferred to 60 mm diameter petri dishes (~10 mL/petri). Mycelium discs (5 mm in size) from 7-day-old fungal cultures were transferred to the petri dishes. The fungal cultures were incubated for 10 days at 23±2 °C after inoculation. Fungal growth was recorded daily for a period of 7 days. The colony diameter was measured by measuring the diameter of the fungus colony in separate directions perpendicular to each other. The percent mycelium growth was calculated by comparing the inhibition of growth with the growth in the control. Thiram 80% (Hektaş group), a standard fungicide, was used as a positive control at the recommended dose by the commercial company, while 50% acetone was used as the negative control. The experiment was replicated three times with two repetitions each. The percent mycelium growth was calculated using the following formula (Jabeen and Javaid, 2008):

Percent inhibition (%) = [(Growth in control - Growth in extract) / Growth in control] × 100

5. Effect of Plant Extracts on Seed Germination and Seedling Growth of Test Plants: A study was conducted to investigate the effects of plant extracts on seed germination and seedling growth of test plants. The experiment was carried out using 9 cm diameter petri dishes. In each dish, 25 seeds of the test plants were evenly distributed on two layers of blotting paper. The methanol extract was prepared and then moistened by adding 6 ml to the petri dishes using a mixture of distilled water and acetone at different concentrations (0 [control], 0.5, 1, 2, and 4 mg/mL). The petri dishes were incubated for 1-3 weeks at an average temperature of 24 °C. After this period, the germination rate, rootlet length, and shoot length of the test plant seeds were determined (Önen, 2003).

Data Analysis: The significance of differences between treatments was determined by conducting an analysis of variance (ANOVA), and means were

compared using the Duncan test. The statistical analyses were performed using the SPSS 15.0 computer program.

RESULTS AND DISCUSSION

The results of the experiments conducted in petri dishes to determine the effectiveness of the methanol extract of *Inula viscosa* plant on *Cercospora* isolates, which are important plant pathogenic fungi in sugar beet, as well as on some weeds and cultivated plants, are summarized in the tables and figures below.

In vitro Antifungal Activity of *Inula viscosa*: The study investigated the biofungicidal effects of the methanol extract of *Inula viscosa* against *Cercospora beticola*, an important plant pathogen in sugar beet. Table 1 and Figure 3 present the mycelium growth inhibition rates of the isolates in response to the plant extracts. Statistical analysis revealed significant differences at a significance level of $P<0.05$ among the different doses. The 4 mg/mL dose of the plant extract inhibited the mycelial growth of Isolate-1 and Isolate-2 by 58.03% and 59.35%, respectively, compared to the negative control. Inula viscosa extract exhibited similar effects on the mycelial growth of *Cercospora beticola* isolates (Table 1, Figure 3).

Table 1. Mycelium growth inhibition of methanol extract of *Inula viscosa* against *Cercospora beticola* isolates (%)

Doses (mg/mL)	***Cercospora beticola***	
	Isolate-1	Isolate -2
N-	$0,00\pm0,00^{e*}$	$0,00\pm0,00^{d}$
0,5	$38.34\pm2,95^{d}$	$34.93\pm7,39^{c}$
1	$46.71\pm2,80^{cd}$	$47.76\pm3,08^{bc}$
2	$51.43\pm2,63^{bc}$	$54.85\pm3,38^{b}$
4	$58.03\pm1,48^{b}$	$59.35\pm2,05^{b}$
P+	$100,00\pm0,00^{a}$	$100,00\pm0,00^{a}$

N-: Negative control; P+: Positive control; To compare the means *Means with different letters in the same column differ from DUNCAN at $p<0.05$ significance

level.

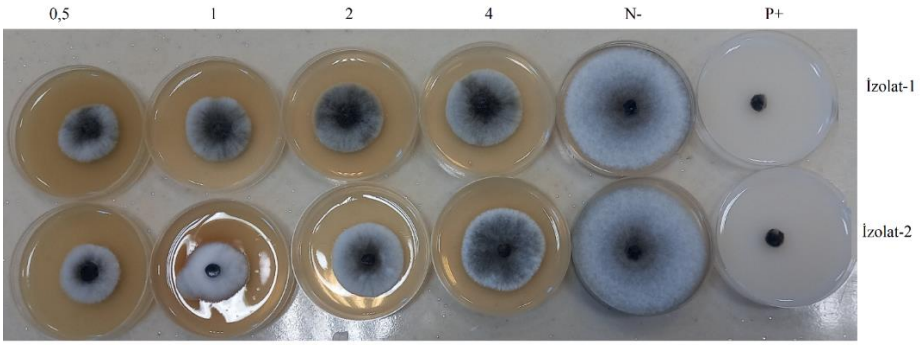

Figure 3. *Effect of methanol extract of Inula viscosa on mycelium growth against Cercospora beticola isolates.*

In previous studies, researchers have reported that extracts of *Inula viscosa* exhibit antifungal and antibacterial activity (Bayar and Genç, 2021; Erdal et al., 2022). The effectiveness of *Inula viscosa* methanol extract was investigated against soil-borne plant pathogens, including *Rhizoctonia solani* (RS), *Sclerotinia sclerotiorum* (SS), *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), and *Fusarium oxysporum* f. sp. *melonis* (FOM). The study reported that the highest dose of 4000 ppm inhibited the growth of FOC, FOM, SS, and RS mycelium by 68.23%, 72.04%, 83.51%, and 100%, respectively (Bayar and Genç, 2021). Another study conducted with different organic solvents (Hexane, Chloroform, Methanol) reported that leaf and flower extracts of *Inula viscosa* showed varying levels of antifungal activity against *Fusarium oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL), and *F. oxysporum* f. sp. *tuberosi* (FOT) (Omezzine et al., 2011). Additionally, Abu Jawdah et al. (2002) found that an oily extract of *I. viscosa* applied at a concentration of 2 ml/98 ml PDA inhibited the mycelial growth of *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis*, and *Verticillium dahliae* by 88%. Another study reported that *Inula viscosa* water extract inhibited the mycelium growth of the plant pathogen Pyrenophora teres, which causes blight in barley, by 70% (Karima et al., 2015).

In vitro Allelopathic activity of *Inula viscosa*: In a Petri dish study on allelopathy, different levels of allelopathic effects of *Inula viscosa* methanol extract were observed depending on the dose and the test plant. The results

obtained were statistically significant at the $p<0.05$ significance level. At the highest dose (4 mg/mL), *Inula viscosa* methanol extract completely inhibited the seed germination of *Amaranthus retroflexus* weeds, followed by *Portulaca oleraceae* with 93.75% inhibition and *Chenopodium album* with 87.32% inhibition. The seed germination of cultivated plants showed more tolerance to *Inula viscosa* extract. The extract inhibited the seed germination of *Lepidium sativum*, *Triticum aestivum*, and *Medicago sativa* by 31%, 12%, and 7.21%, respectively (Table 2, Figure 4). *Inula viscosa* methanol extract also exhibited a high inhibitory effect on the root and shoot elongation of test plants. Root and shoot growth of *P. oleraceae* and *A. retroflexus* were completely inhibited at a dose of 4 mg/mL. The extract inhibited root and shoot development of *Lepidium sativum*, *Triticum aestivum*, *Medicago sativa*, and *Chenopodium album* by 98.34% - 96.56%, 81.20% - 74.12%, 66.26% - 76.56%, and 79.45% - 76%, respectively (Table 2, Figure 4).

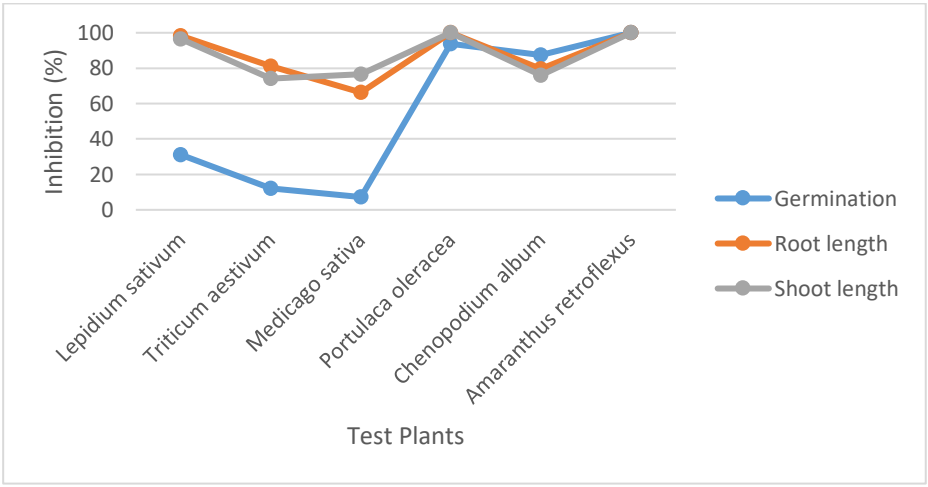

Figure 4. % Inhibition rate of 4 mg/mL dose on seed germination, root and shoot development of test plants.

Table 2. Effect of *Inula viscosa* methanol extract on seed germination and seedling growth of weeds and cultivated plants.

Test plants	Doses (mg/mL)	Germination (%)	Root (cm)	Shoot (cm)
Lepidium sativum	Control	98.00^{a*}±1.15	6.06^{a}±0.22	2.91^{a}±0.25
	0,5	97.0^{a}±1.0	5.96^{a}±0.42	2.04^{ba}±0.43
	1	96.0^{a}±1.63	5.00^{a}±0.73	1.43^{cba}±0.84
	2	81.0^{b}±4.72	0.59^{b}±0.41	0.98^{cb}±0.55
	4	67.0^{c}±7.00	0.1^{b}±0.02	0.1^{c}±0.07
Triticum aestivum	Control	100.0^{a}±0.0	7,66^{a}±0.36	7,38^{a}±0.12
	0,5	98.0^{a}±2.00	5,09^{b}±0.25	4,27^{b}±0.18
	1	98.0^{a}±1.15	4,47^{b}±0.34	3,66^{c}±0.14
	2	90.0^{b}±2.58	2,25^{c}±0.15	2,55^{d}±0.16
	4	88.0^{b}±4.32	1,44^{d}±0.06	1,91^{e}±0.14
Medicago sativa	Control	97.0^{a}±1.0	1.66^{b}±0.22	4.48^{a}±0.16
	0,5	93.0^{a}±3.0	2.05^{ba}±0.26	3.82^{ba}±0.40
	1	91.0^{a}±1.91	2.47^{a}±0.12	3.52^{cb}±0.27
	2	90.0^{a}±2.0	1.89^{b}±0.10	3.03^{c}±0.08
	4	90.0^{a}±2.58	0.56^{c}±0.09	1.05^{d}±0.14
Portulaca oleracea	Control	80.00^{a}±11.54	1.79^{a}±0.48	2.79^{a}±0.25
	0,5	72.00^{ba}±2.58	0.82^{b}±0.12	2.09^{b}±0.17
	1	70.00^{ba}±4.32	0.60^{bc}±0.05	1.93^{b}±0.11
	2	54.00^{b}±2.58	0.01^{c}±0.01	0.24^{c}±08
	4	5.00^{c}±3.0	0.0^{c}±0.0	0.00^{c}±0.0
Chenopodium album	Control	71.00^{a}±2.51	0.73b^{a}±0.06	1.25^{b}±0.02
	0,5	64.00^{a}±3.26	0.97^{a}±0.15	1.72^{a}±0.13
	1	46.00^{b}±2.58	0.55^{b}±0.05	1.46^{ba}±0.23
	2	21.00^{c}±4.44	0.27^{c}±0.04	0.78^{c}±0.12
	4	9.00^{d}±5.25	0.15^{c}±0.01	0.30^{d}±0.12
Amaranthus retroflexus	Control	77.00^{a}±1.91	0.73^{a}±0.01	1.34^{a}±0.15
	0,5	23.00^{b}±8.54	0.55^{a}±0.14	1.04^{a}±0.20
	1	7.00^{c}±1.91	0.50^{a}±0.10	0.98^{a}±0.05
	2	2.00^{c}±1.15	0.05^{b}±0.02	0.15^{b}±0.09
	4	0.00^{c}±0.0	0.00^{b}±0.0	0.00^{b}±0.00

* Means with different letters in the same column are different according to DUNCAN at $p<0.05$ significance level.

The allelopathic effect of the methanol extract from the above-ground parts of the *Inula viscosa* plant was investigated on various plant species including *Lepidium sativum*, *Triticum aestivum*, *Medicago sativa*, *Chenopodium album*, *Amaranthus retroflexus*, and *Portulaca oleraceae*. The

study found that the plant extract exhibited an allelopathic effect, with a greater impact on the seed germination of weeds compared to cultivated plants. This selectivity between weeds and cultivated plants suggests the potential of the *I. viscosa* extract to be utilized as a bioherbicide.

Previous studies on *I. viscosa* have also demonstrated its allelopathic effect. For example, the water extract obtained from the leaves of *I. viscosa* was found to negatively impact cell division in young roots of *Allium cepa* and inhibit root growth (Çelik and Aslantürk, 2010). Additionally, it was observed that the water extract reduced root length and suppressed root hair formation in lettuce seedlings (Levizou et al., 2002). Stephanou and Manetas (1995) and Staurianakou et al. (2004) reported that the water-soluble epicuticular material of the plant hindered seed germination and radical growth in certain weeds and cultivars. The susceptibility of cultivar seeds to water-soluble compounds varied depending on the species, suggesting the potential application of these compounds as selective herbicides for weed control (Stephanou and Manetas, 1995). The I. viscosa water extract exhibited efficacy against three weed species (*Vicia sativa* L., *Amaranthus retroflexus* L., and *Portulaca oleracea* L.) at different dosages (Kitiş et al., 2017).

The volatile components of the *I. viscosa* plant were found to cause oxidative damage, photoinhibition, and alterations in plant water status when tested on lettuce (Aranti et al., 2017). Omezzine et al. (2011) investigated the allelopathic effects of different extracts (water, hexane, chloroform, and methanol) obtained from various parts of *I. viscosa* (root, shoot, leaf, and flower) on seed germination and root-shoot elongation in *Lactuca sativa*, *Raphanus sativus*, *Peganum harmala*, and *Silybum marianum*. Dor and Hershenhorn (2012) demonstrated the allelopathic effect of I. viscosa leaf extracts on weed species (*Cuscuta campestris*, *Amaranthus palmeri* S. Wats, *Solanum nigrum* L., *Sinapis arvensis* L.) and cultivated plants (*Corchorus olitorius* L., *Lycopersicon esculentum* Mill., *Triticum aestivum* and *Cucumis melo* L.). The current study, along with previous research, confirms the allelopathic potential of *I. viscosa*, particularly in its ability to inhibit roots and induce phytotoxic effects and anatomical abnormalities in certain species (Çelik and Aslantürk, 2010). The biological activity of the *I. viscosa* plant is attributed to its active compounds. Various secondary metabolites, including

flavonoids (Grande et al., 1985), sesquiterpene lactones and acids (Camacho et al., 2000; Grande et al., 1992; Perez-Alanso et al., 1996), and triterpenoids (Grande et al., 1992; Simöes and Nascimento, 1990), have been identified in the plant. The leaf extracts of *I. viscosa* have been found to possess nematicidal properties (Oka et al., 2001; Oka et al., 2006), act as insecticides (Alexenizer and Dom, 2007; Mansour et al., 2004), and exhibit fungicidal activity (Mamoci et al., 2006; Maoz and Neeman, 2000).

CONCLUSION

In conclusion, the methanol extract of *Inula v*iscosa demonstrated significant antifungal activity against *Cercospora beticola*, a problematic pathogen in sugar beet cultivation. The extract also exhibited a noteworthy allelopathic effect on weed species. The findings of this study contribute to the literature and provide valuable insights for the development of alternative control strategies against *C. beticola* as well as the potential for new natural antifungal and herbicidal agents.

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CHAPTER 9

AMPELOGRAPHIC DESCRIPTION OF LOCAL GRAPE VARIETIES CULTIVATED IN GERCÜŞ (BATMAN) REGION

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INTRODUCTION

Plant genetic resources are one of the most crucial natural resources for humankind. They play an indispensable role and hold significant importance, particularly in meeting the basic needs of individuals, especially in terms of food production. However, plant genetic resources are rapidly declining or disappearing entirely due to various reasons. These reasons include pollution, climate change, continuous and improper usage, urbanization, and industrialization (Anonim, 2010a).

The conservation of these resources is of utmost importance in current and future breeding programs. Achieving the fundamental objectives of breeding programs necessitates harnessing the existing genetic diversity. Our country holds a unique position regarding plant genetic resources as it is located at the intersection of the Near East and Mediterranean gene centers, among the eight gene centers were identified by Vavilov (Ağaoğlu et al., 1995).

The geographical location of our country enables that to possess a rich plant genetic potential; however, it faces the loss of plant genetic resources due to various negative factors (Anonim, 2010a).

Viticulture, dating back thousands of years, holds a significant place in agricultural activities in our country. Globally, viticulture is practiced within the 10-52 degrees North latitude range in the Northern Hemisphere. The fact that our country is situated between 36-42 degrees North latitude indicates its highly favorable ecological conditions for viticulture (Oraman, 1970).

The collection, conservation, and development of grapevine genetic resources hold great importance for the development and restructuring of viticulture, a strategic agricultural sector in our country. These efforts play a critical role in the future of our viticulture applications (Çelik et al., 2005).

Despite the wide diversity found worldwide, factors such as the increase in international trade, the small-scale production of varieties in many countries, the limited production through cloning, the reduction of vineyard areas, and restrictive laws limiting the production of traditional varieties have led to a concerning level of genetic erosion in grape varieties. It is stated that in order to prevent this genetic erosion, each country should emphasize the protection

and conservation of its local varieties and wild vines in situ (Anonymous, 2010b).

According to the studies conducted in our country so far, it is reported that 1437 grapevine genotypes have been ampelographically identified and protected. Furthermore, molecular-level research conducted on these genotypes has determined that the actual number of varieties is approximately 870 (Uzun, 2015).

Due to changing in socio-economic structure throughout history, the Southeast Anatolia region holds a special position in terms of both cultivated and wild grapevine genetic potential (Karataş et al., 2007). Gercüş district almost have lost its former position in terms of vineyard areasand production. Insufficient productivity is observed due to the lack of preservation of existing vineyards. The reasons for the decrease in vineyard areas include the advanced age of vineyards, lack of knowledge of modern viticulture techniques, low yield and income, inability to economically utilize the products, and the absence of conscious viticultural practices in the region.

The aim of this study is to identify the local grape varieties cultivated in the Gercüş district according to international standards. Additionally, it aims to evaluate the current status of viticulture, analyze the technical and cultural practices employed, identify the encountered problems, and make a contribution to future studies in the field.

MATERIAL AND METHODS

This research was initiated as a preliminary study in 2018 with the objectives of identifying existing grape varieties, developing a study plan, and selecting research sites. The study was conducted in the vineyards of the Gercüş (Batman) district center in 2018. The ampelographic characteristics of the Binetahti, Dırejık, Reşe Mevişa, Zorava, Payizi Siyah, Mezrone, Hasani, Zeyti, Sinceri Zer, Hılsık, Hılsık Beyaz and Hılsık Reş varieties were determined. Data regarding the identified varieties was obtained through the interviews with grape producers, farmers, landowners, and local residents engaged in viticultural activities in the region, and the varieties were labeled according to their local names. Photographs of the sample materials were taken, and

observations, counts, measurements, weighing, and analyses were conducted, and the resulting data was recorded. While some of these procedures were carried out in the local area, other analyses were performed at the laboratories of Van Yüzüncü Yıl University. The ampelographic identifications of the varieties were conducted according to the "Descriptors for Grape" guidelines (Anonymous, 1983; 1997).

RESULTS and DISCUSSION

Ampelographic descriptions of the local grape varieties cultivated in the Gercüş region are provided in Table 1.

Table 1. Ampelographic descriptions of the examined grape varieties.

OIVCodes	Hasani	Zeyti	Sinceri Zer	Hilsik	Hilsik Beyaz	Hilsik Reş
001	3 Open	3 Open	3 Open	3 Open	3 Open	3 Open
002	2 All around	2 All around	2 All around	2 All around	2 All around	2 All around
003	7 Strong	5 Medium	7 Strong	9 Very strong	5 Medium	9 Very strong
004	3 Sparse	3 Sparse	1 Very sparse	3 Sparse	3 Sparse	3 Sparse
005	0 No	0 No	0 No	3 Sparse	0 No	0 No
006	3 Half upright	3 Half upright	3 Half upright	3 Half upright	3 Half upright	3 Half upright
007	2 Red striped Green	3 Red	2 Red striped Green	2 Red striped Green	1 Green	1Green
008	2 Red striped-Green	3 Red	2 Red striped Green	2 Red striped Green	1 Green	2 Red striped Green
009	2 Red striped Green	3 Red	2 Red striped Green	2 Red striped Green	3 Red	1 Green
010	2 Red striped-Green	3 Red	2 Red striped Green	2 Red striped Green	3 Red	2 Red striped-Green
011	0 No	0 No	0 No	0 No	0 No	0 No
012	0 No	0 No	0 No	0 No	0 No	0 No
013	0 No	0 No	0 No	3 Sparse	0 No	0 No
014	0 No	0 No	0 No	3 Sparse	0 No	0 No
015	5 Medium	9 Very strong	3 Weak	7 Strong	3 Weak	5 Medium
016	1 Segmented	1 Segmented	1 Segmented	1 Segmented	1 Segmented	1 Segmented
017	5 Medium (18.00±3.22)	7 Large (22.20±1.33)	5 Medium (17.00±4.15)	1Very Short (11.80±3.87)	3 Short (16.40±2.65)	7 Large (22.00±2.00)
051	2 Bronze-spotted Green	6 Copper-colored	2 Bronze-spotted Green	2 Bronze-spotted Green	2 Bronze-spotted Green	2 Bronze-spotted Green
052	5 Medium	9 Very strong	7 Strong	3 Weak	5 Medium	7 Strong
053	0 No	0 No	0 No	5 Medium	0 No	0 No
054	0 No	0 No	0 No	5 Medium	0 No	0 No
055	0 No	0 No	3 Sparse	7 Dense	3 Sparse	0 No
056	0 No	0 No	5 Medium	3 Sparse	0 No	0 No
065	5 Medium (228.39±40.98)	5 Medium (245.80±43.68)	5 Medium (188.02±25.47)	3 Small (156.84±21.48)	7 Large (283.95±38.77)	5 Medium (220.3±47.18)
066	5 Medium (15.63±3.37)	5 Medium (16.88±1.60)	5 Medium (14.06±1.20)	3 Short (13.27±1.15)	5 Medium (16.88±2.05)	5 Medium (15.20±.56)
067	3 Pentagonal	3 Pentagonal	3 Pentagonal	3 Pentagonal	2 Wedge	2 Wedge
068	3 Five	3 Five	3 Five	3 Five	3 Five	3 Five
069	7 Close-fittingk green	5 Green	7 Close-fittingk green	5 Green	7 Close-fittingk green	3 Light green
070	0 No	1 Very weak	7 Strong	0 No	0 No	0 No
071	0 No	1 Very weak	3 Weak	1 Very weak	0 No	0 No
072	+ Have	+ Have	+ Have	+ Have	+ Have	+ Have
073	0 No	1 Only on the stem	2 Usually	0 No	1 Only on the stem	0 No
074	1 Almost flat	3 Curved inward	2 Almost flat	2 Almost flat	3 Curved inward	3 Curved inward
075	5 Medium	3 Weak	5 Medium	3 Weak	5 Medium	5 Medium
076	2 Hit düz	2 Hit flat	2 Hit flat	2 Hit flat	2 Hit flat	2 Hit flat

Table 1. Ampelographic descriptions of the examined grape varieties (continued).

OIVCodes	Hasani	Zeyti	Sinceri Zer	Hilsik	Hilsik Beyaz	Hilsik Reş
001	3 Open	3 Open	3 Open	3 Open	3 Open	3 Open
002	2 All around	2 All around	2 All around	2 All around	2 All around	2 All around
003	7 Strong	5 Medium	7 Strong	9 Very strong	5 Medium	9 Very strong
004	3 Sparse	3 Sparse	1 Very sparse	3 Sparse	3 Sparse	3 Sparse
005	0 No	0 No	0 No	3 Sparse	0 No	0 No
006	3 Half upright	3 Half upright	3 Half upright	3 Half upright	3 Half upright	3 Half upright
007	2 Red striped Green	3 Red	2 Red striped Green	2 Red striped Green	1 Green	1Green
008	2 Red striped-Green	3 Red	2 Red striped Green	2 Red striped Green	1 Green	2 Red striped Green
009	2 Red striped Green	3 Red	2 Red striped Green	2 Red striped Green	3 Red	1 Green
010	2 Red striped-Green	3 Red	2 Red striped Green	2 Red striped Green	3 Red	2 Red striped-Green
011	0 No	0 No	0 No	0 No	0 No	0 No
012	0 No	0 No	0 No	0 No	0 No	0 No
013	0 No	0 No	0 No	3 Sparse	0 No	0 No
014	0 No	0 No	0 No	3 Sparse	0 No	0 No
015	5 Medium	9 Very strong	3 Weak	7 Strong	3 Weak	5 Medium
016	1 Segmented	1 Segmented	1 Segmented	1 Segmented	1 Segmented	1 Segmented
017	5 Medium (18.00±3.22)	7 Large (22.20±1.33)	5 Medium (17.00±4.15)	1 Very Short (11.80±3.87)	3 Short (16.40±2.65)	7 Large (22.00±2.00)
051	2 Bronze-spotted Green	6 Copper-colored	2 Bronze-spotted Green	2 Bronze-spotted Green	2 Bronze-spotted Green	2 Bronze-spotted Green
052	5 Medium	9 Very strong	7 Strong	3 Weak	5 Medium	7 Strong
053	0 No	0 No	0 No	5 Medium	0 No	0 No
054	0 No	0 No	0 No	5 Medium	0 No	0 No
055	0 No	0 No	3 Sparse	7 Dense	3 Sparse	0 No
056	0 No	0 No	5 Medium	3 Sparse	0 No	0 No
065	5 Medium (228.39±40.98)	5 Medium (245.80±43.68)	5 Medium (188.02±25.47)	3 Small (156.84±21.48)	7 Large (283.95±38.77)	5 Medium (220.3±47.18)
066	5 Medium (15.63±3.37)	5 Medium (16.88±1.60)	5 Medium (14.06±1.20)	3 Short (13.27±1.15)	5 Medium (16.88±2.05)	5 Medium (15.20±.56)
067	3 Pentagonal	3 Pentagonal	3 Pentagonal	3 Pentagonal	2 Wedge	2 Wedge
068	3 Five	3 Five	3 Five	3 Five	3 Five	3 Five
069	7 Close-fittingk green	5 Green	7 Close-fittingk green	5 Green	7 Close-fittingk green	3 Light green
070	0 No	1 Very weak	7 Strong	0 No	0 No	0 No
071	0 No	1 Very weak	3 Weak	1 Very weak	0 No	0 No
072	+ Have	+ Have	+ Have	+ Have	+ Have	+ Have
073	0 No	1 Only on the stem	2 Usually 0 No	0 No	1 Only on the stem	0 No
074	1 Almost flat	3 Curved inward	2 Almost flat	2 Almost flat	3 Curved inward	3 Curved inward
075	5 Medium	3 Weak	5 Medium	3 Weak	5 Medium	5 Medium
076	2 Hit düz	2 Hit flat	2 Hit flat	2 Hit flat	2 Hit flat	2 Hit flat

Table 1. Ampelographic descriptions of the examined grape varieties (continued).

OIV Codes	Binetahti	Direjik	Reşe Mevişa	Zorava	Payizi Siyah	Mezrone
077-1	3 Short (7.70±2.32)	7 Large (15.56±1.79)	5 Medium (12.50±2.55)	7 Large (14.40±2.40)	7 Large (16.66±3.49)	7 Large (13.62± 1.56)
077-2	3 Short (9.47± 1.17)	5 Medium (7.52±0.70)	5 Medium (9.50± 1.91)	5 Medium (10.80±1.90)	7 Large (17.24±2.33)	7 Large (19.00±4.47)
078-1	5 Medium (0.79±0.21)	5 Medium (1.02±0.10)	5 Medium (0.94±0.11)	7 Large (1.27±0.07)	5 Medium (0.88±0.13)	5 Medium (0.92±0.09)
078-2	5 Medium (0.77±0.05)	5 Medium (0.87±0.09)	5 Medium (0.77±0.10)	7 Large (1.09±0.08)	7 Large (0.93±0.12)	5 Medium (0.89±0.12)
079	3 Open	3 Open	5 Closed	3 Open	6 Lightly overlap	4 Partially open
080	2 V	1 U	2 V	2 V	2 V	2 V
081	1 No	1 No	1 No	1 No	1 No	1 No
082	3 Lightly overlap	3 Lightly overlap	5 Closed	3 Lightly overlap	3 Lightly overlap	4 Strongly overlap
083	2 V	1 U	1 U	2 V	2 V	2 V
084	1 Very sparse	1 Very sparse sessparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
085	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
086	1 Very sparse	3 Sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
087	1 Very sparse	3 Sparse	3 Sparse	1 Very sparse	1 Very sparse	1 Very sparse
088	0 No	0 No	0 No	0 No	0 No	0 No
089	0 No	0 No	0 No	0 No	0 No	0 No
090	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
091	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
092	5 Medium (13.44±3.65)	5 Medium (11.60 ±0.80)	3 Short (9.20±1.33)	3 Short (10.95±1.01)	5 Medium (12.32±1.39)	3 Short (9.72±2.24)
093	5 Equal (01.10±0.27)	5 Equal (0.97±0.24)	3 Shorter (0.79±0.12)	3 Shorter (0.73±0.10)	3 Shorter (0.88±0.06)	3 Shorter (0.82 ±0.16)
151	3 Hermaphrodite	3 Hermaphrodit	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite
153	2.1-1-2	1.1-2 Cluster	1.1-2 Cluster	2 1-3 Cluster	1.1-2 Cluster	2.1-3 Cluster
202	9 Very Large (250.02±119.2)	5 Medium (212.45±109.6)	3 Small (159.2±96.6)	3 Small (187.61±99.06)	5 Medium (214.02±111.2)	9 Very Large (395.5±124.4)
203	5 Medium (22.23±0.66)	5 Medium (23.25±4.42)	3 Short (15.1±2.12)	3 Short (16.53±3.59)	5 Medium (17.75±2.05)	7 Large (28.85±1.58)
204	7 Dense	1 Very sparse	7 Dense	3 Sparse	3 Sparse	5 Medium
205	7 Purplee (157.50±69.50)	1 Very Little (46.50±17.44)	3 Little (68.75±21.50)	3 Little (66.00±20.59)	3 Little (70.20±19.70)	3 Little (68.50±21.58)
206	1 Shorter (1.95±0.32)	3 Short (3.27±0.60)	3 Short (3.54±0.60)	1 Shorter (2.98±0.38)	3 Short (5.01±0.25)	3 Short (3.27±0.11)
221-1	5 Medium (21.36±2.48)	7 Large (25.96±2.23)	3 Short (16.77±1.38)	3 Short (16.33±1.27)	5 Medium (21.08±0.77)	3 Short (16.31±1.12)

Table 1. Ampelographic descriptions of the examined grape varieties (continued).

OIV Codes	Hasani	Zeyti	Sinceri Zer	Hilsik	Hilsik Beyaz	Hilsik Reş
077-1	5 Medium (13.40±2.27)	7 Large (16.58±2.07)	5 Medium (11.66±1.48)	5 Medium (13.2±2.02)	5 Medium (13.96±01.79)	7 Large (14.96± 1.52)
077-2	5 Medium (11.6± 2.75)	5 Medium (11.98±1.53)	3 Short (9.76±1.87)	5 Medium (11.4±21.90)	5 Medium (13.37± 1.98)	5 Medium (13.44 ±3.06)
078-1	5 Medium (1.01±0.10)	9 Very Large (1.46±0.14)	5 Medium (0.83±0.07)	7 Large (1.10±0.13)	5 Medium (0.99±0.08)	5 Medium (0.90±0.06)
078-2	5 Medium (1.03±0.13)	7 Large (1.13±0.16)	5 Medium (0.83±0.08)	7 Large (1.12±0.19)	5 Medium (0.93±0.06)	5 Medium (0.96±0.199)
079	5 Medium (13.40±2.27)	7 Large (16.58±2.07)	5 Medium (11.66±1.48)	5 Medium (13.2±2.02)	5 Medium (13.96±01.79)	7 Large (14.96± 1.52)
080	3 Open	2 Fully open	4 Partially open	3 Open	5 Closed	4 Partially open
081	2 V	1 U	2 V	1 U	2 V	2 V
082	1 No	1 No	1 No	1 No	1 No	1 No
083	3 Lightly overlap	3 Lightly overlap	3 Lightly overlap	3 Lightly overlap	3 Lightly overlap	1 Open
084	2 V	1 U	2 V	1 U	2 V	2 V
085	1 Very sparse	1 Very sparse	1 Very sparse	3 Sparse	1 Very sparse	1 Very sparse
086	1 Very sparse	1 Very sparse	1 Very sparse	3 Sparse	1 Very sparse	1 Very sparse
087	1 Very sparse	1 Very sparse	1 Very sparse	3 Sparse	3 Sparse	1 Very sparse
088	3 Sparse	1 Very sparse	1 Very sparse	3 Sparse	0 No	1 Very sparse
089	0 No	0 No	0 No	0 No	0 No	0 No
090	0 No	0 No	0 No	0 No	1 Very sparse	0 No
091	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
092	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse	1 Very sparse
093	3 Short (8.10±0.68)	3 Short (8.88± 2.11)	1Shorter (6.24± 0.91)	1 Shorter (6.76±0.07)	3 Short (10.24±2.32)	3 Short (9.52 ±0.98)
151	3Shorter (0.72±0.08)	3Shorter (0.71±0.09)	3Shorter (0.62±0.07)	3Shorter (0.69±0.07)	3 Shorter (0.83±0.11)	5 Equal (0.91±0.11)
153	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite	3 Hermaphrodite
202	1.1-2 Cluster	1.1-2 Cluster	2.1-3 Cluster	1.1-2 Cluster	2.1-3 Cluster	2.1-3 Cluster
203	7 Large (288.24±39.27)	9 Very Large (423.78±64.52)	3 Small (171.30±0.45)	5 Medium (206.89±106.6)	5 Medium (253.65±118.5)	7 Large (272.5±3.75)
204	5 Medium (15.60±0.60)	7 Large (25.50±0.20)	5 Medium (21.5± 1.40)	5 Medium (23.95±5.05)	5 Medium (21.28±2.95)	5 Medium (21.80±0.30)
205	7 Dense	5 Medium	3 Sparse	5 Medium	5 Medium	5 Medium
206	5 Medium (95.00±15.00)	7 Purplee (182.0±9.90)	1Very Little (36.67±4.71)	3 Little (65.95±20.09)	3 Little (77.80±28.05)	3 Little (68.33±6.24)
221-1	3 Short (3.51±0.55)	3 Short (5.03±0.67)	1 Shorter (2.13±0.64)	3 Short (3.38±0.97)	1 Shorter (2.47±0.89)	3 Short (4.16±0.81)

Table 1. Ampelographic descriptions of the examined grape varieties (continued).

OIV Codes	Binetahti	Direjik	Reşe Mevişa	Zorava	Payizi Siyah	Mezrone
221-2	5 Medium (15.76±0.70)	5 Medium (13.43±1.57)	5 Medium (14.3±0.90)	5 Medium (16.85±1.57)	5 Medium (16.45±0.82)	3 Close-fitting (14.80±0.71)
222	1 Uniform	1 Uniform değil	1 Uniform değil	1 Not uniform	1 Not uniform	1 Not uniform
223	4 Short ovals	9 Sickle	2 Round	2 Round	5 Cylindrical Short ovals	2 Round
224	2 Round	2 Round	2 Round	2 Round	2 Round	2 Round
225	1 Green-Yellow	1 Green Yellow	5 DarkRed-purple	2 Pink	5 DarkRed-purple	1 Green-Yellow
226	2 Uniform	2 Uniform	2 Uniform	1 Not uniform	1 Uniform değil	1 Not uniform
227	3 Weak	3 Weak	5 Medium	5 Medium	5 Medium	5 Medium
228	1 Very Thin	3 Thin	3 Thin	3 Thin	3 Thin	3 Thin
229	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced
230-231	0 Uncolored	0 Uncolored	1 Less colored	1 Less colored	1 Less colored	0 Uncolored
232	+ Watery	+ Watery	+ Watery	+ Watery	+ Watery	+ Watery
233	9 Very high (73.67±1.25)	9 Very high (76.33 ±4.03)	9 Very high (70.00±0.82)	9 Very high (70.00±8.49)	9 Very high (71.00±0.82)	9 Very high (71.00±0.83)
234-235	5 Medium	5 Medium	3 Low	7 High	7 High	3 Low
236	0 No	0 No	0 No	0 No	0 No	0 No
238	3 Short (6.89±0.83)	3 Short (9.16±1.45)	3 Short (7.40±1.15)	3 Short (7.37±0.80)	3 Short (10.97±1.38)	3 Short (6.95±0.73)
239-240	3 Hard	5 Medium	7 Easy	5 Medium	3 Hard	5 Medium
241	2 Have	2 Have	2 Have	2 Have	2 Have	2 Have
242-1	7 Large (6.95±0.44)	7 Large (7.31±0.45)	7 Large (7.22±0.38)	7 Large (7.25±0.41)	7 Large (6.95±0.44)	7 Large (6.54±0.33)
242-2	7 Dense (4.09±0.37)	7 Dense (3.82±0.22)	9 Very Dense (4.62±0.65)	9 Very Dense (4.72±0.50)	7 Dense (4.09±0.37)	9 Very Dense (4.36±0.14)
243	7 High (50.60±5.85)	5 Medium (45.67±1.62)	7 High (57.44±6.63)	9 Very high (63.10±6.89)	7 High (52.80±5.95)	7 High (51.90±6.76)
244	0 No	0 No	0 No	0 No	0 No	0 No
301	03.04.2018	05.04.2018	02.04.2018	05.04.2018	10.04.2018	12.04.2018
302	26.05.2018	16.05.2018	17.05.2018	18.05.2018	23.05.2018	27.05.2018
303	24.07.2018	13.07.2018	12.07.2018	21.07.2018	27.07.2018	02.08.2018
304	23.08.2018	15.08.2018	15.08.2018	16.08.2018	25.08.3018	26.09.2018
502	7 Large (695±111.2)	3 Small (219.75±31.55)	3 Small (265.00±54.73)	3 Small (186.50±34.50)	3 Small (212.0 ±60.0)	5 Medium (472.25±26.90)
503	5 Medium (3.63±0.52)	5 Medium (3.46±0.74)	3 Small (2.60±0.47)	3 Small (2.58±0.65)	5 Medium (3.66±0.51)	3 Small (2.82±0.41)
504	4.26±0.41	3.29±0.10	3.22±0.12	3.98±0.81	3.18±0.90	7.08±1.22
505	5 Medium (%18)	7 High (%20)	7 High (%21)	7 High (%21)	5 Medium (%19)	5 Medium (%19)
506	3 Low (4.21)	1 Very Low (2.76)	3 Low (4.26)	3 Low (4.24)	3 Low (4.12)	1 Very Low (2.16)

Table 1. Ampelographic descriptions of the examined grape varieties (continued).

OIV Codes	Hasani	Zeyti	Sinceri Zer	Hilsik	Hilsik Beyaz	Hilsik Reş
221-2	3 Close-fitting (16.36±1.81)	7 Dense (18.71±1.11)	3 Close-fitting (14.74±1.23)	3 Close-fitting (13.55±0.79)	7 Dense (17.58±2.27)	5 Medium (15.04±1.03)
222	1 Not uniform	1 Not uniform	1 Not uniform	1 Not uniform	1 Not uniform	1 Not uniform
223	6 Dense yumurta	4 Short ovals	6 Dense yumurta	3 Wide ovals	4 Short ovals	2 Round
224	2 Round	2 Round	2 Round	2 Round	2 Round	2 Round
225	1 Green yellow	1 Green yellow	1 Green yellow	1 Green yellow	1 Green yellow	5 Dark Red purple
226	1 Not uniform	2 Uniform	1 Not uniform	1 Not uniform	1 Not uniform	1 Not uniform
227	3 Weak	1 Very weak	3 Weak	5 Medium	5 Medium	3 Weak
228	1 Very Thin	3 Thin	3 Thin	3 Thin	3 Thin	3 Thin
229	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced	1 Slightly pronounced	2 Pronounced	1 Slightly pronounced
230-231	0 Uncolored	1 Less colored	0 Uncolored	0 Uncolored	0 Uncolored	1 Less colored
232	+ Watery	+ Watery	+ Watery	+ Watery	+ Watery	+ Watery
233	5 Medium (63.00±0.82)	9 Very high (70.67±1.25)	7 High (66.00±0.82)	7 High (68.00±0.82)	9 Very high (77.33±4.19)	7 High (69.00±0.82)
234-235	5 Medium	5 Medium	5 Medium	5 Medium	5 Medium	3 Low
236	0 No	0 No	0 No	0 No	0 No	0 No
238	3 Short (7.59±0.37)	3 Short (9.01±0.96)	3 Short (7.13±0.87)	1 Shorter (3.80±0.38)	3 Short (7.27±0.90)	3 Short (8.73±0.59)
239-240	7 Easy	3 Hard	5 Medium	3 Hard	1 Very Hard	7 Easy
241	2 Have	2 Have	2 Have	2 Have	2 Have	2 Have
242-1	9 Very Large (8.10±0.42)	7 Large (7.21±0.45)	7 Large (6.73±0.19)	7 Large (7.33±0.23)	7 Large (6.95±0.43)	7 Large (7.13±0.47)
242-2	9 Very Dense (4.89±0.17)	7 Dense (3.82±0.24)	9 Very Dense (4.26±0.26)	9 Very Dense (4.45±0.11)	9 Very Dense (4.19±0.39)	9 Very Dense (4.13±0.30)
243	9 Very high (73.10±13.83)	7 High (57.25±9.26)	7 High (50.60±6.83)	7 High (55.70±6.66)	5 Medium (58.00±12.44)	5 Medium (47.30±5.04)
244	0 No	0 No	0 No	0 No	0 No	0 No
301	05.04.2018	08.04.2018	07.04.2018	08.04.2018	10.04.2018	04.04.2018
302	22.05.2018	25.05.2018	29.05.2018	26.05.2018	25.05.2018	18.05.2018
303	23.07.2018	17.07.2018	02.08.2018	28.07.2018	20.07.2018	27.07.2018
304	21.08.2018	13.08.2018	05.09.2018	26.08.2018	05.08.2018	21.08.2018
502	5 Medium (395.50±70.50)	7 Large (659.5±128.02)	1 Very Small (92.50±2.50)	3 Small (272.0±93.77)	7 Large (497.43±79.8)	5 Medium (317.0±23.0)
503	5 Medium (3.68±0.82)	5 Medium (3.87±0.56)	3 Small (2.83±0.45)	3 Small (2.07±0.36)	5 Medium (5.34±0.98)	5 Medium (3.21±0.51)
504	5.93±2.10	10.43±3.80	3.16±0.50	4.08±1.01	7.46±1.83	4.75 ± 2.08
505	5 Medium (%19)	5 Medium (%17)	7 High (%23)	5 Medium (%17)	5 Medium (%19)	5 Medium (%19)
506	3 Low (4.50)	3 Low (4.54)	3 Low (2.46)	1 Very Low (2.79)	1 Very Low (2.64)	3 Low (3.25)

It has been reported that the color of healthy shoot tips is an important character for determining differences among varieties (Morton, 1979). In the

studies conducted on the evaluated varieties, it has been determined that the distribution and intensity of anthocyanins in the shoot tips are "very strong" in the Hılsık and Hılsık Reş varieties. The distribution and intensity of anthocyanins in the shoot tips, categorized into different classes as determined in our study, are consistent with the findings of other researchers' studies (Regner et al., 1999; Asensio et al., 2002; Santiago et al., 2007; Çelik et al., 2008; Güler, 2007; Uyak, 2010; Uyak et al., 2011a-b; Doğan et al., 2017). The color of mature leaves is an important characteristic in terms of ampelographic features. It is stated that this feature can vary due to vine nutrition and other factors (Anonymous, 1983). Regarding the coloration of the main veins on the upper and lower surfaces of mature leaves, different varieties have been classified into various groups. The coloration of anthocyanins on the upper surface and the lower surface of the leaves has been categorized as "very strong" in the Payizi Siyah and Mezrone varieties. In the study conducted by Ünal (2000), the anthocyanin coloration in the main veins of the upper surface was determined as "strong," while in the lower surface, it was determined as "weak." In the study by Kılıç (2009), the anthocyanin coloration in the main veins of the lower surface was evaluated as "absent," while on the upper surface, it was assessed as "very weak." In terms of the density of prostrate hairs on the shoot tips of the evaluated varieties, Sinceri Zer is observed as "very sparse," while all other varieties fall into the "sparse" class. The absence of erect hairs in the majority of the varieties highlights the importance of prostrate hairs in grapevines. Similar findings are also reported in studies conducted by Kara (1990), Altın (1991), Diri (1996), Kılıç (2009), Uyak (2010) Yağcı (2013) and Arslan et al., (2018).

Leaf size is another measurement-based characteristic used in the classification of grape varieties. Various methods have been employed to determine leaf areas in these varieties, including the use of a Planimeter or Area Meter, computer-based analysis, and the utilization of leaf area coefficients. In this study, leaf areas were measured using the length-width multiplication method as described by Anonymous (1983). Accordingly, four varieties were classified as "large" in terms of leaf size, five varieties as "medium," Dırejık and Payizi Siyah varieties as "very large," and the Hılsık variety as "small." Uyak (2010) found in his study, that leaf size in varieties differed in different

years, while Güler (2007) classified leaf sizes as "very small," "small," and "medium." Uyak (2010) observed that leaf sizes in one variety were "small" in the first year and "medium" in the second year. Although both studies used the same method, the observed differences may be explained by Morton's (1979) notion that leaf size varies even within the same variety because of some factors such as soil fertility, growth vigor, training systems, and climatic conditions. Oraman (1972) stated that the location of grapevines can cause variations in leaf shape, clusters, and berries.

In terms of the number of lobes on mature leaves, all varieties were determined to be "five-lobed." According to Uyak (2010), leaf shape and lobing were considered as definitive characteristics for classifying varieties. Demir (1987) stated that leaf shape and lobing were related to vine development and soil structure, while Gider (1995) expressed that the number of lobes in clones or the same varieties was a character less influenced by environmental conditions.

The length of the first flower cluster was determined to be "medium" in two varieties, "short" in nine varieties, and "very short" in Zorava variety. In Uyak's study (2010), it was found that varieties exhibited different classifications in both years. There appears to be a regression between the length of the first flower cluster and the length of the grape cluster. Grape cluster lengths were observed to have higher values than the length of the first flower cluster. Regarding the size of the grapes within the clusters, all evaluated varieties were as "non-uniform." After the green color of the berries dropped, it transformed into a variety-specific color. The berry skin color was determined to be "green-yellow" in eight varieties, "pink" in the Zorava variety, and "dark red-purple" in the other three varieties. The color of the fruit pulp was classified as "very light-colored" in five varieties and "colorless" in the remaining varieties. The observation that the fruit pulp is colorless aligns with the idea of an independent relationship between the skin and pulp color (Morton 1979; Uyak 2010). Out of the examined varieties, three have a "typical" skin color, while the other varieties do not. Although berry color is considered a characteristic specific to each variety, variations in color and intensity within the same vineyard are influenced by environmental conditions and the required and accumulated heat (Fidan, 1985).

It has been determined that there are significant variations in grain shape, with varieties classified as "sickle-shaped," "short oval," "cylindrical," "broad oval," "wide egg," and "egg-shaped." The transverse section of the seeds in all varieties was classified as "round." It is noted that grain shape is a variety-specific characteristic but can also be influenced by soil structure, climatic factors, and the applied technical and cultural practices (Fidan, 1975; 1985; Uyak, 2010). Additionally, it is stated that the shape and the size of seed kernels have an effect on grain shape (Barış and Gürnil, 1991). In all varieties, the presence of kernels was determined to be "present." Kernel lengths were mostly classified as "long," while varieties classified as "very long" were also observed. In the evaluated varieties, kernel widths were found to be in the categories of "wide" and "very wide." Regarding kernel weights, varieties were classified as "medium," "high," and "very high." It was observed that the number and shape of kernels also influenced grain size.

The dry matter content in the must was found to be "high" in four varieties and "low" in the other eight varieties. The acidic levels of the musts were classified as "very low" in five varieties and "low" in the remaining varieties. Uyak (2010) determined that the dry matter content in the eight examined varieties differed in both years, but no significant variation was observed in the other varieties. In another study, Uyak found differences in acidic levels among varieties, while variations in the dry matter and acid content in the must could be attributed to climate conditions and variations in harvest times. However, according to Kara (1990), the ratio of acid in the dry matter of the grapes can vary based on the variety, presence, and size of the kernels, and climate conditions according to other researchers' findings.

Although bud break and flowering times of grapes grown in the same region are close to each other, variations in the ripening periods of different varieties have been reported by various researchers, indicating that the main differences among grapes begin with veraison and become fully evident during the maturity stage (Ergenoğlu, 1985; Özışık, 1991; Anonim, 1992). In the conducted study, phenological differences among varieties were observed, with a 10-day difference in bud break, a 9-day difference in full bloom, a 19-day difference in veraison, and a 52-day difference in terms of maturity. It can be

concluded that the varieties have similar bud break and full bloom periods, while they fall into the "late" category in terms of harvest time.

The evaluated varieties Zeyti, Hılsık Beyaz, and Mezrone, are considered as table grapes in the region due to their attractive and productive nature, good cluster sizes, medium-sized berries within the cluster, and uniform berry color. Among these varieties, especially Zeyti deserve special attention. Additionally, the Binetahti variety, with its dense and showy berries and high juice yield, should be separately considered as a wine-making or juice-making variety. Apart from being consumed as table grapes in the region, varieties with abundant juice content are used in the production of grape molasses and other products derived from grape juice. Even to a limited extent, wine producers from outside the region demand specifically Mezrone grape variety.

The cluster and mature leaf photographs of the examined grape varieties are provided in Figure 1-6.

Figure 1. Cluster and mature leaf photographs of Binetahti and Dırejık varieties

Figure 2. Cluster and mature leaf photographs of Reşe Mevişa and Zorava varieties.

Figure 3. Cluster and mature leaf photographs of Payizi Siyah and Mezrone varieties.

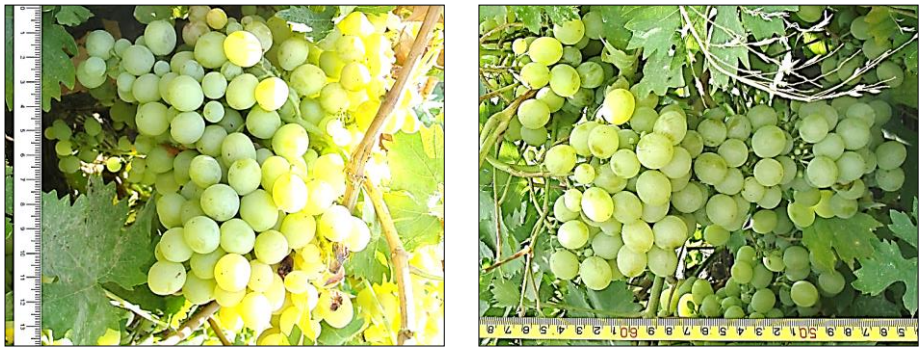

Figure 4. Cluster and mature leaf photographs of Hasani and Zeyti varieties.

Figure 5. Cluster and mature leaf photographs of Sinceri Zer and Hılsık varieties.

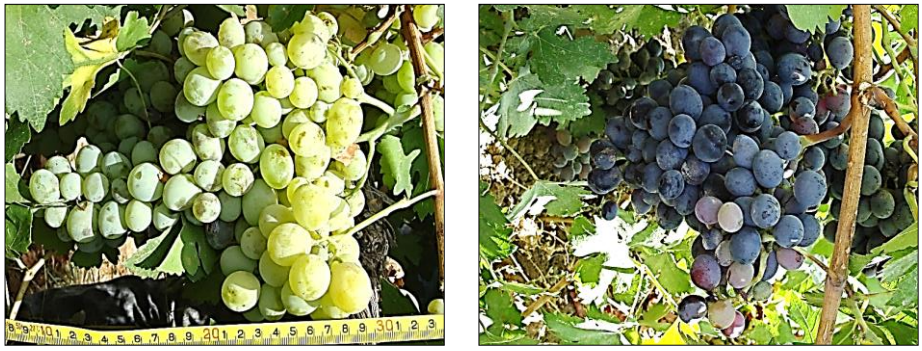

Figure 6. Cluster and mature leaf photographs of Hılsık Beyaz and Hılsık Reş varieties.

CONCLUSION

The development of viticulture and the preservation of local varieties are of great importance in order to contribute to the social and economic development of the Gercüş district. Studies should be conducted on the varieties with superior characteristics to increase productivity and enhance economic returns. One issue with the existing vineyard areas is the continuation of outdated viticultural practices. Adequate soil cultivation, fertilization, pruning, and disease and pest management are not being carried out in areas where old viticultural techniques are still employed. The irregularity of row spacing and inter-row spacing leads to difficulties in implementing cultural practices.

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CHAPTER 10

BIOREMEDIATION OF TOXIC LEVEL PLANT MICRO NUTRIENTS IN WASTEWATERS FOR IRRIGATION

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1. INTRODUCTION

Water is the key of life. Although 71% the earth is covered in water, only about 1% of water of earth is drinkable. Agricultural use of water for irrigation of the crops constitutes more than 80% of the drinkable water usage all over the world. Besides this, industrial requirement for freshwater is outrageous, which in advance result in contamination and pollution of the already scarce and valuable water sources. Human activities including daily life, urbanization, industrialization and agriculture require and use, and also pollute the water sources. Many industrial applications yield wastewaters highly contaminated with organic and inorganic substances. The wastewater treatment systems firstly concentrate on the elimination of organic loads of the wastewaters. There are both biological and non- biological systems used for the treatment of wastewaters, which can also eliminate heavy metals or other toxic substances alone or with additional processes. Besides the industrial wastes, agricultural applications such as pesticides and fertilizers may contaminate the soil and finally the water sources. Application of the absolute plant nutrition elements like iron, copper, zinc and manganese together with excessive fertilization in order to increase the productivity of the non-fertilized soil as a result of conventional agriculture also cause the pollution of water sources. Moreover, when these nutrition elements (heavy metals, at the same time) are applied in excess via pesticides, they both harm plants and pollutes water sources. Since the highest proportion of freshwater is utilized for irrigation of agricultural crops, evaluation of wastewaters for agricultural purposes is significantly considered and suggested as an important option to manage water shortage. Reduction of nitrogen and phosphorus is at the heart of remediation of wastewaters, however, removal of iron, copper, zinc and manganese heavy metals, which are also absolutely required by plants, is significant for the recovery and reuse of wastewaters for irrigation. Reuse of treated wastewaters is especially very appealing to be used for dry regions with low availability of freshwater sources for irrigation. Treated wastewaters can also be evaluated for industrial reuse, street cleaning, firefighting, recreational uses, however, agricultural irrigation is suggested to be most acceptable way for evaluation of wastewaters due to the high and inevitable water need in agriculture (Rizzo et al., 2020). Reducing water usage together with ameliorating the wastewaters

for reuse are important to save water sources and hence the whole environment and ecosystems. Remediation of environmental elements such as the water and soil are at the very heart of future and sustainability of agriculture.

2. BIOREMEDIATION

The wastewaters are good candidates for irrigation water after efficient treatment processes. Remediation of soil and water can be carried out by conventional non-biological methods such as adsorption, electro-dialysis, precipitation and ion-exchange (Kapahi & Sachdeva, 2019). Moreover, biological techniques which employ plants or microorganisms can also be used for remediation. Bioremediation is the technique which employs organisms to remove, convert toxic substances into a less toxic form. Organisms may also degrade organic materials and can mineralize organics into carbon dioxide, nitrogen gas, etc. While phytoremediation is the method of using plants for cleaning up wastes, bioremediation is the method of using microorganisms such as yeast, fungi, algae and bacteria to ameliorate wastewaters and soils. Microorganisms use up the contaminants as nutrient or energy sources, or they can convert, adsorb or modify the contaminants into a less toxic forms. They can act in biosorption, bioaccumulation, bioconversion, and precipitation (either inside the cells or in between the cells) (GM, 2000; Mosa et al., 2016). Bacteria, archaea and fungi are the primary microorganisms that have bioremediation capability. These organisms are very effective in biosorption of heavy metals such as Cu, Cd, Cr, Fe, Zn, Mn, Ni, and Pb. They can develop resistance to heavy metals in the areas highly contaminated with heavy metals. Moreover, metabolic actions of microorganisms are at the heart of the bioremediation process. They employ their enzyme systems and energy producing strategies to degrade pollutants and hence produce energy and biomass. Microorganisms can initiate mobilization or immobilization of metals by their redox reactions. They also increase the solubility of ions such as Fe (III) by reducing them [to Fe (II) in this example]. Bio methylation of heavy metals is another important action of microorganisms that modify toxicity, volatility, and mobility of heavy metals (Bolan et al., 2014). The efficiency of bioremediation depends on factors such as the characteristics of the pollutants, presence of other toxic compounds, pH, temperature, moisture content, oxygen

concentration, availability of other nutrients for microorganisms, species, population size of the microbial strains primarily capable of bioremediation of a certain pollutant, and genetic composition of the microorganisms (Abatenh et al., 2017).

Microorganisms have been extensively used for the treatment of wastewaters as they efficiently decrease the organic load of the wastewaters (H. Li et al., 2020; Vítězová et al., 2020; Waldrop, 2021). Besides this, they can be employed for the decrease of heavy metal in the wastewaters which occur directly from many industrial applications such as mining, dyeing, tannery and electroplating, waste treatment plants, and in municipal wastewaters (Kapahi & Sachdeva, 2019). Bioremediation of nonbiodegradable heavy metals such as Cr, Hg, Cd, Cu Fe, Mn and Pb is an eco-friendly, cost effective and efficient and also alternative to the conventional methods (Igiri et al., 2018).

In order to be considered for irrigation, the heavy metal concentrations should be lowered in wastewaters as they can be accumulated in the crops and related food chains. Moreover, and more importantly, the concentrations of some metals which are also nutrient elements for plants should be taken into consideration. Metals such as Fe, Cu, Mn and Zn are micronutrients absolutely required by plants for healthy growth and metabolism. These metals can be applied to soils in fertilizers. Therefore, the concentrations of these micronutrient metals can get significantly high in the soil if wastewaters will be used for irrigation where different fertilization regimes are applied.

3. SOME PLANT MICRO NUTRIENT ELEMENTS

Plant nutrient elements can be simply categorized as macro- and micro-nutrient elements. Macro nutrient elements are N, Mg, Ca, K and P. They are vital for growth and development of plants. They are required by plants in higher concentrations than the micro elements such as Fe, Cu, Mn, Zn, B, Mo and Cl. Micronutrients constitute less than 1% of the dry weight of most plants (Welch & Shuman, 2011). Since these nutrient elements are vital for plant development, fertilization aiming for the deficient elements can be performed for crop production in case of scarcity in soil. The elements Fe, Cu, Mn and B are also heavy metals which can be abundant in wastewaters, especially

industrial and municipal wastewaters. If the wastewaters are directly applied to soil after a primary treatment to lower organic materials, the heavy metals can be highly concentrated in soil and this can toxify the plants. That is why the heavy metals should be remediated from the wastewaters to a certain level. The remediated wastewaters with a acceptable amount of heavy metals can indeed reduce the need for fertilization applications towards micronutrients. In the following sections, bioremediations of certain heavy metals which are micro nutrient elements for plants are reviewed.

3.1 BIOREMEDIATION OF IRON PLANT NUTRIENT ELEMENT

Iron is an abundant element on earth with a low solubility. Being a central component of electron chains and a cofactor of many crucial enzymes makes iron an essential element for every life form. In plants it is an absolute micronutrient required for chlorophyll synthesis and photosynthesis metabolism (M. Broadley et al., 2011; Karaman et al., 2012; Schmidt et al., 2020). Therefore, insufficient iron levels in plants cause growth retardation in plants. Sufficient levels in plants are important to fight against iron deficiency anemia in humans. Iron fertilization can be performed to maintain a sufficient iron level in soils and the plants, as more than one third of the arable lands are iron deficient. But, iron fertilizers are costly and not environmentally friendly. On the other hand, in excess iron can cause toxicity in cellular and organismal levels (Schmidt et al., 2020). That is why when wastewaters are intended to be used for agricultural irrigation, the iron levels in the wastewaters should be taken into consideration. If in an acceptable level, wastewater irrigation can replace iron fertilizer applications. For short term usage, Fe in irrigation water is suggested to be 20 mg/L, while if long term evaluation of wastewaters for irrigation is considered, the level is suggested to be maximum 5mg/L (Hashem & Qi, 2021). (Lokeshwari and Chandrappa 2006) used sewage-fed lake water for irrigation and investigated the heavy metal levels in crops such as rice. They resulted that heavy metal levels, including iron, were beyond acceptable limits, and this caused heavy metal accumulation in crops. Some plants have the ability to tolerate and accumulate heavy metals more in their bodies. Such plants can clean up the soil and can grow and develop, however, their nutritional or other

characteristics may change. In a study, we have found that excess iron in the soil caused by iron fertilization is taken up by basil can decrease the antibacterial activity of basil (Gürgan & Adiloğlu, 2021). Such as the remediation of soil by plants, the wastewaters should be remediated for heavy metals before application as irrigation water.

Wastewaters containing heavy metals are resulted from several industries such as dyeing, tannery, electronics, cosmetics industries, and from pesticide and fertilizer applications, municipal sewage sludge, use of personal care products and cosmetics, etc. (Kapahi & Sachdeva, 2019). Iron heavy metal is present in the wastewaters of especially mining, steel and iron industries, fertilizers and herbicides (Corral-Bobadilla et al., 2019). The studies have revealed some species of bacteria, algae and fungi are capable of remediate iron from the wastewaters.

For example, chemolithotrophic and acidophilic bacteria such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* can carry out metal leaching in order to obtain energy from Fe (II). This results in solubilizing of metals and formation of Fe (II). This process changes the pH of the environment and hence other metals solubilize, too (Gadd, 2019)This process has been used industrially for bio-mining processes, after which the heavy metal contents of the wastewaters can be reduced (Kamizela et al., 2021).

In a study carried out with influent and effluent of a municipal wastewater in Poland, two species of algae were employed for removal of heavy metals Fe, Zn and Mn. The iron concentrations in the influent and effluents of this treatment plant were 158.27 and 1560.0 µg/L, respectively. Iron could be more efficiently removed by *Scenedesmus armatus* than *Chlorella vulgaris* (Kwarciak-Kozłowska et al., 2014).

Performances of fungi were investigated for remediation of Fe, as well. A species of Fungi, *Pleurotus ostreatus* was found to be s a good biosorbent agent for heavy metals in lab scale experiment. A removal efficiency of more than 80% was achieved for an initial 272.05 mg/L Fe (II) concentration. The effects of different parameters such as pH, temperature, agitation were investigated in the study (Arbanah et al., 2012). In another study, six fungal species were tested for the biosorption of different heavy metals. Among them

Aspergillus flavus and *Sterigmatomyces halophilus* were most effective for the removal of heavy metals, with the highest efficiency for Fe and Zn (Bano et al., 2018).

3.2 BIOREMEDIATION OF COPPER PLANT NUTRIENT ELEMENT

Copper is a redox active transition element. Being a micro nutrient element in plants, it plays important roles in photosynthesis, respiration, carbon and nitrogen metabolisms, and protection against oxidative stress. There are a lot of copper containing proteins in plants, examples are the proteins taking place in photosynthesis, Cu-bound chaperons, Cu-containing oxidase enzymes such as superoxide dismutase, cytochrome c oxidase, diamine oxidases and polyphenol oxidases (M. Broadley et al., 2011). When above the tolerable limits, Cu can inhibit shoot and root development. Copper is utilized in several industries such as production of industrial machinery and products, electronic products, transportation equipment, etc. The copper containing wastewaters should be remediated before being used as irrigation water. For copper, the maximum levels in the irrigation water are suggested to be 0.5 and 20 mg/L in terms of long term and short-term usage, respectively (Hashem & Qi, 2021).

There are various studies on the bioremediation of copper using bacteria, algae and fungi species. On example of bacterial bioremediation of copper was employing *Bacillus* species. These bacteria were found to efficiently absorb Cu ions from aqueous solutions (Tunali et al., 2006). Besides using suspended organisms, immobilized bacteria have been tried for removal of copper from aqueous solutions. *Arthrobacter* species were investigated with being immobilized on polysulphone. The immobilized biomass was successful for removing copper with a maximum of 89.6% performance (Hasan & Srivastava, 2009). Different *Pseudomonas* species such as *P. putida*, *P. putida* CZ1 and *P. fluorescens* were also tested for their ability to remove copper. It was found that living cells of bacteria can remove more heavy metals due to both accumulation in the cells and passively binding the metals (Chen et al., 2005; López et al., 2000; R et al., 2003). Copper from sewage wastewater could also be removed by cyanobacteria *Anabaena subcylindrica* and *Nostoc muscorum, Nostoc* PCC 7936, *Cyanospira capsulate,* and *Tolypothrix ceytonica* (Idi et al).

The microalgae *C. vulgaris* was investigated in the bioremediation of some heavy metals. It was found that this organism performs a remediation of Cu with an efficiency of > 97% under the optimal conditions. This alga was suggested to be an environmentally friendly biosorbent for Cu and some other heavy metals from aqueous solutions. (Goher et al., 2016). The algae *Palmaria palmata* was investigated in laboratory conditions for the sorption of heavy metals in water samples. The affinity of this algae species was highest for Cu, followed by Zn and Mn (Rajfur & Kłos, 2013). Different red, brown and green algal species were tested to remove different heavy metals from aqueous solutions. The results revealed that the best performance was carried out by the brown algae *Fucus spiralis* (Romera et al., 2007). This species was best at removing, then Cd and Cu with equal efficiencies, followed by Zn and Ni.

Besides bacteria and algae, some fungi were shown to successfully remove copper from wastewater environments. Among many fungi tested by (Bano et al. 2018), A*spergillus flavus* and *Sterigmatomyces halophilus* had the best performance to remediate copper with efficiencies more than 80%. In other study, three fungi species, *Schizophyllum commune*, *Ganoderma lucidum* and *Pleurotus ostreatus* were tried on copper and other heavy metals resulting from electroplating industrial effluents. All the tested fungi performed well with an order of *S. commune*, *G. lucidum* and *P. ostreatus* (Javaid & Bajwa, 2008). Additional study showed the importance of using microorganisms which are already tolerant and even resistant to the heavy metals for better removal performance. *Talaromyces helices* was trained with high copper levels and this way it became tolerant to Co, Pb and Cd by the activated biochemical mechanisms. Moreover, the copper adaptation of mycelium was transferred to the spores, which doubled the copper removal efficiency (Romero et al., 2006).

3.3 BIOREMEDIATION OF ZINC PLANT NUTRIENT ELEMENT

One of the other micronutrients required by plants and also a heavy metal often encountered in wastewaters is zinc. It is the second most abundant transition metals found in the organisms after iron. It does not take part in redox reactions, but there are a lot of zinc including or zinc binding proteins in organisms. These proteins play roles in DNA metabolism, gene expression

regulations they also play roles in detoxification of superoxide radicals, and membrane integrity, and also production of the phytohormone indole acetic acid in plants (M. Broadley et al. 2011). Zinc also contributes to stress tolerance of plants in their environments. Zinc toxicity can be observed in zones of mining activities, and when sewage sludge is applied to the soil (M. R. Broadley et al., 2007). Moreover, zinc is encountered in the wastewaters of metal coating, mining and pigment industries. Zinc toxicity causes chlorosis and therefore reduction in photosynthesis and also inhibition of root elongation. The maximum suggested levels of Zn in wastewaters used for irrigation are 2 mg/L and 10 mg/L for long term and short-term usage, respectively (Hashem & Qi, 2021). The wastewaters can be highly contaminated with zinc and therefore should be remediated for this metal to reduce to acceptable limits (Shamuyarira & Gumbo, 2014).

In order to remove zinc from wastewaters, microorganisms can be used. For instance, the influent of municipal wastewater plant contained 119.56 µg/L zinc which was more efficiently removed by *Scenedesmus armatus* than *Chlorella vulgaris*. However, when the zinc concentration was 323 µg/L in the effluent of wastewater plant, the latter algal species could perform better in cleaning up the zinc (Kwarciak-Kozłowska et al., 2014). Another algal species tested for the remediation of zinc was *Chlorella kessleri* which was immobilized onto alginate. This unicellular green alga was efficient for the bisorption of both zinc and copper from the aqueous solution (Horváthová et al., 2009).

In another study to investigate the metal contaminated water, soil and sediment of an industrial area, the zinc concentration in water was found to be varying from 39.8 to 310 mg/L in water samples. Different bacterial species were tested for the removal of zinc and the results showed that some *Bacillus* species were highly resistant to zinc, which are suggested to be employed to remove zinc from the industrial wastewaters (Krishna et al., 2013). When bacteria and other microorganisms expose to certain substances such as organics and heavy metals, they can generate tolerance and resistance, and can grow on concentrations of those materials which most other organisms cannot survive. A study carried out the purple non sulfur bacterium *Rhodobacter sphaeroides* revealed that although zinc and cadmium could inhibit growth of

this bacterium, it could remediate a wastewater polluted with Cd and Zn under ambient temperature and pH conditions with an efficiency higher than 97% through biosorption and precipitation of heavy metals (X. Li et al., 2017).

Fungi can also be employed to take up zinc from water environments. Among many *Aspergillus* species tested, *Aspergillus flavus* performed the best for removal of zinc, together with another halophilic fungus *S. halophilus* with an efficiency of more than 80% (Bano et al., 2018). In Another study, *Pleurotus ostreatus* was applied as a bio sorbent for Cu, Zn, Fe and Pb. The optimum conditions for removal of zinc were pH 4.0 and 25°C. However, this fungus was more effective for Cu and Fe than for Zn and Pb (Arbanah et al., 2012). Removal of Zn from electroplating industrial effluents could be achieved by was lower than *S. commune, G. lucidum* and *P. ostreatus*, although with a lower efficiency than other heavy metals such as Ni, Cr and Cd (Javaid & Bajwa, 2008)

3.4. BIOREMEDIATION OF MANGANESE PLANT NUTRIENT ELEMENT

One of the heavy metals important for plants as a micronutrient element is manganese. There are many enzymes activated by Mn^{+2} in plants, but only a few enzymes directly contain Mn. Mn-containing superoxide dismutase, Mn-protein in photosystem II, and oxalate oxidase are the examples for enzymes directly related with the Mn element in their structure. However, besides the significant metabolisms of such proteins, being a cofactor of about 35 enzymes makes Mn important for the plant metabolism. (M. Broadley et al., 2011). Steel production, mining processes, mineral processing and other human activities lead to release of manganese with the wastes (Das et al., 2015). Excess Mn can cause impairments in plasma membrane and increases Ca+2 influx, resulting in formation of callose and brown speckles on the leaves (Führs et al., 2010). For manganese, the maximum levels in the irrigation water are suggested to be 0.2 and 10 mg/L in terms of long term and short-term usage, respectively (Hashem & Qi, 2021).

The study with the municipal wastewater treatment plant in Poland revealed that *Scenedesmus armatus* was an efficient algal species for the remediation of Mn whose concentration was 133.08 µg/L in the effluent. The

concentration of the element increased to 320 μL/g in the effluent where the performance of *Chlorella vulgaris* was higher. The performance of algae for remediation of heavy metals were higher at the beginning of the treatment processes (Kwarciak-Kozłowska et al., 2014). Another algal species, *Spirogyra sp*. was tested for the removal of manganese from surface water. The microorganisms could sorb the heavy metals proportionally to the amount of this metal ions in the solution (Rajfur et al., 2010).

Among the bacteria tested to be effective for manganese removal, the manganese oxidizing bacterium *Brachybacterium* sp. strain Mn32 which was highly resistant to Mn (II) and it was shown to be used for treatment of Mn-contaminated water (Wang et al., 2009). Another study showed that M could be recovered from water by *Staphylococcus epidermidis* by 80% (Das et al., 2012). Fungi, moreover, can be used to treat Mn from wastewaters. Different obligate halophilic fungi species, namely *Aspergillus flavus*, *Aspergillus gracilis*, *Aspergillus penicillioides*, *Aspergillus penicillioides, Aspergillus restrictus* and *Sterigmatomyces halophilus* were tested to remove metals including manganese. All these species were efficient at different levels to remove heavy metals (Bano et al., 2018)

CONCLUSION

The fast industrialization and urbanization, besides their benedictions, resulted in production of wastewaters which end up with the contamination of water sources. Water sources are very critical for the continuation of life, and consumed mostly for agricultural irrigations. The evaluation of municipal and industrial wastewaters as irrigation water for the agriculture is suggested to be a very important alternative to using freshwater. The wastewaters should and at present undergo a treatment before reuse, especially for the removal of heavy metals. Heavy metals in the wastewaters have been treated by many chemical processes which are expensive and not environmentally friendly. Therefore, biological methods are gaining attention for the remediation of wastewaters. Some heavy metals such as Fe, Cu, Mn, and Zn are required by plants as absolute micronutrient elements. These heavy metals are often present in several different wastewaters. Containing a certain level of heavy metals which are added to the soil in fertilizers, is therefore can be an advantage for reuse the

wastewaters for irrigation. However, when the levels of heavy metals are high, they can cause toxicity in plants. That is why heavy metals should be removed from the wastewaters up to a level and using microorganisms for this purpose is a highly environmentally friendly and cost-effective method. Different bacteria, algae and fungi species have been tested for many different wastewaters for their heavy metal remediation efficiencies, and studies regarding to find more efficient microorganisms for bioremediation of wastewaters are still ongoing.

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CHAPTER 11

A TRADITIONAL BEVERAGE: BEET KVASS

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INTRODUCTION

Fermentation is a metabolic process that occurs when microorganisms (lactic acid bacteria and yeasts) convert carbohydrates in food into acids or alcohols. The history of fermentation, known as one of the oldest methods used for food preservation, dates back to 6000 BC. The pH that decreases with fermentation naturally extends the shelf life of the food. Fermentation is preferred not only to extend the shelf life of food, but also to improve organoleptic properties, protect bioactive components and improve health properties. Metabolic by-products formed during fermentation (biologically active compounds such as diacetyl, acetoin, polyols, hydrogen peroxide, antifungal and antibacterial peptides, ethanol, organic acids, fatty acids, carbon dioxide) increase the positive effects of fermented food on health (Gogigeni et al., 2013; Egan et al., 2016; Jakubowski, 2017; Bell et al., 2018; Mathur et al., 2020; Barcenilla et al., 2022). Many different food groups can be fermented, including dairy products, vegetables, legumes, grains, starchy roots, fruits, meat and fish (Bell et al., 2018). Lactic acid bacteria (LAB) are used in the production of fermented foods such as yogurt, cheese, pickles, sourdough, kimchii, kombucha, beetroot kvass. LAB are known to be a part of the gut microbiota and is the most commonly used probiotic in the food industry (Tannock, 1997; Messens ve De Vuyst, 2002; Oppegard et al., 2007; Barcenilla et al., 2022).

The word probiotic means "for life" in Greek. The existence of probiotic microorganisms, which have many benefits for human health when consumed in sufficient quantities, was first identified by the Nobel Prize-winning Russian scientist Elie Metchnikoff in the early 1900s (Metchnikoff, 1908; FAO/WHO, 2001; Gupta and Garg, 2009; Soccol et al., 2010; Hill et al., 2014; Pandey et al., 2015; Plaza-Diaz et al., 2019). The definition of "probiotic" was first used by Lilly and Stilwell in 1965 (Lilly and Stillwell, 1965). Specific probiotic bacterial strains are *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Leuconosto*c, *Pediococcus*, *Streptococcus* bacteria and *Saccharomyces* yeast (Alvarez-Olmos and Oberhelman, 2001; Gupta and Garg, 2009; Fijan, 2014). *Bifidobacterium* and *Lactobacillus* strains are the most commonly used in foods (Martins et al., 2014).

In many countries, the interest in natural foods, which are naturally preserved and have functional properties, is increasing day by day due to the increase in the consciousness level of consumers (Singh and Hathan, 2014; Clifford et al., 2015). Beetroot (*Beta vulgaris* L. ssp. *vulgaris*) (known as beet, red beet, spinach beet) is a valuable vegetable widely used in the food industry (canned food, juices, food coloring etc.) as well as fresh consumption (Singh and Hathan, 2014). Beet kvass, obtained by lactic acid fermentation of red beet (*Beta vulgaris* L. ssp. *vulgaris*), is a probiotic beverage traditionally consumed in Poland. During fermentation, the compounds in the beet pass into the liquid, resulting in a nutritious mixture. Beet kvass, which has therapeutic effects such as antioxidant and anti-inflammatory, is also a valuable drink rich in minerals, vitamins, betalain, phenolic compounds. In addition, the probiotic microorganisms in its content are effective on the solution of digestive problems (Mueller, 2014; Jakubowski, 2017). However, there are unfortunately very few studies with beet kvass using spontaneous or lactic cultures (Vanajakshi et al., 2016). In this study, it is aimed to emphasize the importance of beet kvass by mentioning the benefits of beet kvass in terms of consumers. In addition, this study is aimed to be a guide for researchers who are considering working on this subject.

1. OVERWIEV OF THE IMPORTANCE OF FERMENTED PROBIOTIC PRODUCTS

Probiotic microorganisms are live organisms that can reproduce at 37°C, are not affected by digestive system conditions, regulate intestinal health and balance, have positive effects on diarrhea, cardiovascular health, immune system, and have anticarcinogenic effects (Fijan, 2014; Staniszewski and Kordowska-Wiater, 2021; Yadav et al., 2022). Intestinal microflora is a habitat for many types of microorganisms with different effects (beneficial, neutral and harmful). The number of microorganisms that have beneficial effects (probiotic microorganisms), less or more than harmful microorganisms (pathogenic microorganisms), affect human health, and it is known that human health improves by ensuring this balance (Al-Nabulsi et al., 2014). Consumption of fermented foods is potentially beneficial for gut microbiota and host metabolic health (Taylor et al., 2020). Taylor et al. (2020) found a relationship between fermented food consumption and gut microbiome using a combination of

ohmic-based analyzes.

Fermented foods (kefir, yogurt, tempeh, miso, sauerkraut, kimchii, kombucha, pickles, beet kvass, etc.) contain live probiotic bacteria, yeast and prebiotic fibers (Fijan, 2014; Mueller, 2014; Taylor et al., 2020; Gasmi et al., 2021; Staniszewski and Kordowska-Wiater, 2021; Yadav et al., 2022). These microorganisms are not affected by the digestive conditions in the stomach and proceed to the intestine and regulate intestinal health (Fijan, 2014; Staniszewski and Kordowska-Wiater, 2021; Yadav et al., 2022). Fermented foods are more easily digested by humans as probiotic bacteria and yeasts use carbohydrates in foods during fermentation. Therefore, fermented foods can be considered "pre-digested". In addition, due to the metabolites produced by these bacteria, foods with high nutritional value are obtained. In the meantime, it is a great advantage that these products can be easily consumed in a controlled manner by people with lactose intolerance and food allergies (Fijan, 2014; Mueller, 2014; Singh and Hathan 2014; Abdo et al., 2020; Taylor et al., 2020; Gasmi et al., 2021; Staniszewski and Kordowska-Wiater, 2021; Yadav et al., 2022). Consumption of fermented foods is also known to strengthen immunity and improve memory impairment (Januario et al., 2017).

Another advantage of fermentation is that the lactic acid produced by LAB is naturally preserved by lowering the pH of the food. Thus, the shelf life of the food is extended without the use of any chemical preservatives (Singh and Hathan 2014; Abdo et al., 2020). Due to all these benefits increasing consumer awareness, the commercial production of fermented functional foods produced by controlled fermentation is increasing day by day (Vaithilingam et al., 2016; Abdo et al., 2020).

2. BEETROOT: BIO-COMPOUNDS AND HEALTH BENEFITS

Red beet (*Beta vulgaris* L. ssp. *vulgaris*) is a biennial herbaceous plant, a member of the *Chenopodiaceae* family. It is known by different names such as beet, red beet, spinach beet. It has different uses and is a widely consumed vegetable. It can be consumed raw as well as used as a valuable raw material in

the production of canned food, fruit juices and natural pigments (betanin) used for food coloring (Klewicka et al., 2015; Kale et al., 2018; Abdo et al., 2020). The composition of beet contains 87.3% moisture, 9.1% carbohydrates, 1.6% protein, 1.1% ash, 0.8% fiber and 0.1% fat (Yoon et al., 2005). In Figure 1, bioactive components of beet except dietary fiber are given schematically.

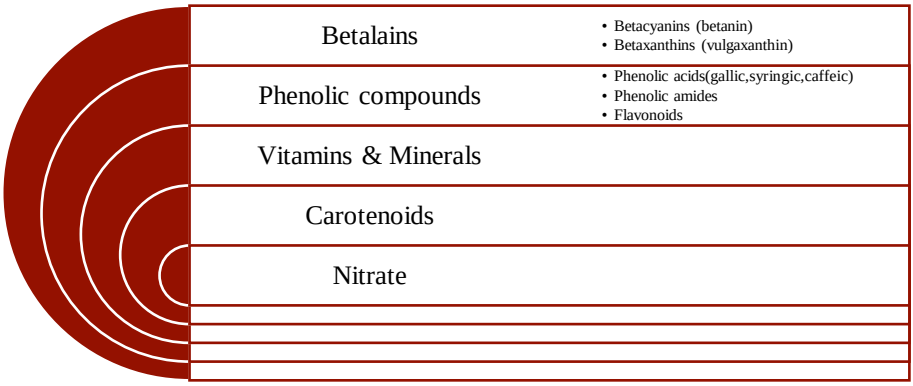

Figure 1. Bioactive compounds of beet

References: (Strack et al., 2003; Singh and Hathan, 2014; Clifford et al., 2015; Klewicka et al., 2015; Kale et al., 2018; Abdo et al., 2020; Punia Bangar et al., 2022)

Beetroot is grown in many countries of the world and has an intense red color due to its high betalain content. Betalains are water-soluble nitrogen-containing pigments, and divided into 2 subgroups as red-violet betacyanins and yellow-orange betaxanthins. The most common of the betacyanins is betanin (betanidin-5-O-β-glucoside) and is used as a natural coloring agent (E162) in the food industry (Kujala et al., 2002; Strack et al., 2003; Clifford et al., 2015; Esatbeyoglu et al., 2015). The high antioxidant, anti-inflammatory, hepatoprotective and anticancer activity of betalain has made beet more popular in the health sector compared to the food sector. Beetroot is among the ten most powerful vegetables in terms of antioxidant properties (Strack et al., 2003; Georgiev et al., 2010; Clifford et al., 2015; Kale et al., 2018). Although the information about the use of beet as a natural medicine dates back to the Roman period, scientific studies have been carried out in the last few decades (Clifford et al., 2015). Today, beetroot is used in the medical world for different purposes such as antidepressant, antifungal, anti-inflammatory, antimicrobial, antioxidant, diuretic, expectorant and carminative (Singh and Hathan, 2014;

Kale et al., 2018; Abdo et al., 2020; Janiszewska-Turak et al., 2022; Punia Bangar et. al., 2022; Thiruvengadam et al., 2022).

Beetroot is a rich source of vitamins B1, B2, B3, B6, B7, B9, B12 and C, and minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc (Dambalkar et al., 2015; Vaithilingam et al., 2016). It is also thought to have an important effect in the prevention and treatment of cardiovascular diseases, as it is a good source of nitrates (Lundberg et al., 2008; Clifford et al., 2015; Abdo et al., 2020). In addition, beetroot is a nutrient rich in phenolics and flavonoids that help hematological, immunological, kidney and liver functions. Carotenoids (1.9 mg/100 g), abundant in beets, are powerful antioxidants that play a key role in disease prevention. They also show anticarcinogenic and immunostimulant effects (Punia Bangar et. al., 2022).

Beets are harvested seasonally and there is a risk of loss of nutritional components during storage. Therefore, it must be processed to preserve biologically active components (Klewicka et al., 2015). Heat treatment of beet leads to loss of vitamins, minerals and bioactive components. And this leads to a significant reduction in health benefits (Abdo et al., 2020). Ramos et al. (2017), examined the effects on bioactive components by applying various cooking methods to beetroot. For this purpose, they treated beet with 4 different heat applications (baking in an oven, boiling in water, pressure cooking and steam cooking). They reported that antioxidant activity, total phenolic and anthocyanin content of the samples did not change after heat treatment, but the flavonoid and betalain content of all samples decreased compared to raw beetroot (Ramos et al., 2017). Controlled lactic acid fermentation can be given as an example for the method that ensures the preservation and enrichment of the biologically active components of beet. After fermentation, betanin and isobetanine concentrations decrease and their aglycones, betanidine and isobetanidine, are formed. Betanidine is the compound with the highest antiradical activity among the betalains (Klewicka et al., 2015; Abdo et al., 2020). Klevicka and Cyzowska (2011) determined that the antimutagenic activity of fermented beet juice was preserved for 30 days. Fermentation improved the nutritional value of the beet and the acceptability of the product. By including beet in commercial fermented product production, a product with

high nutritional value and improved organoleptic properties will be obtained. In addition, this new product will greatly contribute to human health (Abdo et al., 2020).

3. BEET KVASS

Natural nutrients found in fruits and vegetables that positively affect human health attract the attention of consumers and researchers every day (Rakin et al., 2007). Vegetable juices are suitable substrates for lactic acid bacteria due to the carbohydrates they contain. Lactic acid bacteria synthesize vitamins and antimicrobial compounds during fermentation and increase these contents in fermented products. Thus, while the nutritional properties of the products increase with lactic acid fermentation, their shelf life and organoleptic properties are improved (Cleveland et al., 2001; Rakin et al., 2007; Taylor et al., 2020).

Beet kvass is a nutritious non-alcoholic probiotic beverage with a sour and salty taste, obtained by slicing and fermenting (lactic acid fermentation) beetroot (*Beta vulgaris* L.). It is commonly made and consumed in Russia and Eastern European countries (Mueller, 2014). It is traditionally consumed especially in Poland and is included in the Traditional Products List of the Polish Ministry of Agriculture and Rural Development. It is rich in betalains, oxalic acid, phenolic acids. It also has therapeutic effects such as antioxidant and anti-inflammatory activities (Jakubowski, 2017; Janiszewska-Turak et al., 2022).

Beetroot is a good source of carbohydrates for LAB, and when fermentation is over, a decrease in carbohydrate level is observed. When the caloric value of 100 g beetroot (43 cal) and 100 ml beet kvass (22 cal) is compared, it has been observed that kvass has lower calories (Jakubowski, 2017). Beet kvass can be said to be the most beneficial beverage compared to other fermented beverages due to its probiotic quality as well as its vitamin and mineral density (Mueller, 2014).

3.1. Preparation of Beet Kvass

The only ingredients required for the fermentation of vegetables are raw chopped vegetables, sterile water and salt. Different herbs and spices can be used to add flavor, as well as starter cultures (whey, kefir, cultured vegetable juice, stale bread, dried fruits such as raisins) to accelerate fermentation. 1 cup of whey or 1 packet (about 5 grams) of freeze-dried starter per 1 gallon is sufficient for the fermentation process. With the use of starter culture, the fermentation time is shortened and the amount of salt required is also reduced (Mueller, 2014).

There are three basic types of fermentation. These; spontaneous fermentation caused by the natural microflora of the raw material; a controlled fermentation culture that involves the addition of a starter culture and the addition of a sterile culture to the raw material as a starter (Singh and Hathan, 2014). When controlled fermentation will be carried out in beet kvass production, the microorganisms commonly used to accelerate the fermentation process are *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Candida boidinii*, *Saccharomyces cerevisiae*, *Saccharomyces boulardii* and *Wickerhamomyces anoma* (Yoon et al., 2005; Janiszewska-Turak et al., 2022).

Traditionally, for the preparation of beet kvass, beets are first peeled and sliced. Sliced beets are placed in a 2 liter glass jar with 1 tablespoon of salt and 1/4 glass of whey and the jar is filled with water until it is over. The mouth of the jar is covered with cheesecloth. After the mixture is kept at room temperature for 4-14 days, it is filtered and the liquid part is kept in the refrigerator and consumed (Ekin and Deliorman Orhan, 2020; Mueller, 2014).

Increasing the temperature of the room and adding starter culture shortens the fermentation time. Fermentation time (4-14 days) and temperature (26–42 ◦C) should also be in optimum conditions. Less or more fermentation time or temperature causes a decrease in the number of bacteria. In addition, salt concentration has an effect on the number of bacteria in the production of beet kvass. Addition of salt at a rate of 0.5–1% contributes positively to the

development of LAB. All these conditions also indirectly affect the biomass yield (Mueller, 2014; Janiszewska-Turak et al., 2022).

3.2. Biological and Nutritional Properties of Fermented Beet Juice

According to Rakin et al. (2007) fermented beet juice enriched with brewer's yeast autolysate by inoculating it with *Lactobacillus acidophilus* culture. After fermentation, moisture, protein, carbohydrate, lipid and ash contents were determined as 90.06 ± 0.08, 4.05 ± 0.03, 4.13 ± 0.07, 1.32 ± 0.03 and 0.33 ± 0.02 g/100 ml, respectively. The amounts of Ca, Mg, Na, K, Fe and P were 0.180 ± 0.01, 0.236 ± 0.02, 0.625 ± 0.04, 1.88 ± 0.06, 7.22 ± 0.03 and 0.595 ± 0.03 g/l, respectively. Betanin percentage was 0.384 ± 0.05, B1, B2, B6, vitamin C and β-carotene amounts were reported as respectively 1.77 ± 0.09, 1.84 ± 0.02, 0.26 ± 0.02, 103 ± 1.05 and 1.30 ± 0.03 mg/l. (Rakin et al., 2007).

Yoon et al. (2005) obtained beet juice by pressing red beet and added *L. acidophilus, L. casei, L. plantarum, L. delbrueckii* lactic starter cultures, and carried out the fermentation process. As a result of the study, it was determined that the pH value of all fermented beet juices was less than 4.5 and contained a significant amount of lactic acid bacteria (10^9 cfu/ml). They reported that *L. acidophilus* was less durable than other lactic acid bacteria cultures (*L. plantarum, L. casei,* and *L. delbrueckii*) during storage at +4 °C.

Betalains found in beetroot are commonly known as food colorings, but are also compounds with potent antiradical and antioxidant properties that may protect against oxidative stress-related disorders in vivo (Ninfali and Angelino, 2013).

Klewicka and Czyzowska (2011) stored beet juice, fermented with *L. brevis* and *L. paracasei*, at 4°C for 180 days. At the end of the 7th day, they found that the betalain content decreased to 88% and maintained this level for 30 days, 32% on the 90th day and 25% on the 180th day. In addition, they reported that the antimutagenic effect was maintained for 30 days and decreased

on the 90th and 180th days. They determined that the count of lactobacilli bacteria was 9.11 log cfu/ml after fermentation, 8.15 log cfu/ml on the 30th day, and 6.80 log cfu/ml on the 180th day and stated that the optimum storage period in terms of biological activity was 30 days.

3.3. Health Benefits of Beet Kvass

Beet kvass, which is a rich source of antioxidants, has effects that heal cells, protect from damage, and even help reverse DNA and tissue damage. In addition, it regulates the digestive system in individuals who consume it regularly, and greatly reduces inflammation in the body by breaking the vicious cycle of intestinal damage and dysbiosis. The probiotic microorganisms it contains (for example, *L. plantarum*) significantly help prevent the development of *Candida* genus yeast, which causes diseases such as inflammation, abdominal pain, gas formation, depression, and brain fog (Dearie, 2016). Beet kvass helps cleanse the liver. It can also be used to treat kidney stones. Betacyanin, which is concentrated in beets, increases the amount of oxygen that blood cells can carry and is known to clean the blood (Mueller, 2014).

Klewicka et al. (2015) produced fermented beet juice with starter cultures of *L. brevis* and *L. paracasei*. They found that adding fermented beet juice to the diet of mice reduced ammonia levels by 17% in the group treated with N-nitroso-N-methylurea. In addition, positive modulation of intestinal microflora and metabolic activity was observed in groups of rats fed diet supplemented with fermented beet juice. They reported that in the rat group treated with a mixture of fermented beet juice and N-nitroso-N-methylurea, the antioxidant capacity of the blood serum aqueous fraction increased by approximately 69% compared to the group treated without adding fermented beet juice.

Vaithilingam et al. (2016) obtained fermented beet juice with starter cultures of *L. acidophilus* and *L. plantarum*. They compared raw and fermented juices after 24 and 48 hours of fermentation. They stated that beet juice fermented for 48 hours showed a significant antibacterial effect against *Listeria*

monocytogenes. In addition they found that doxorubicin, a widely used drug against human liver cancer Hep G2 cells, showed 86% cytotoxic activity, while fermented beet juice had 64% cytotoxic activity. They also reported that beet juice fermented for 48 hours had a higher amount of polyphenol (0.104 mg/ml) and organic acid content.

Klewicka (2010), investigated the effect on antimutagenic activity by performing spontaneous and controlled lactic acid fermentation process. At the end of the study, it was determined that the antimutagenic activity was preserved with controlled lactic acid fermentation. Klevicka and Cyzowska (2011) found that the antimutagenic activity of fermented beet juice was preserved for 30 days. They stated that it gradually decreased after 90 and 180 days.

The relationship between beetroot and vascular system is related to high nitrate (NO_3) (>2500 mg/kg fresh weight) content of beet. The health benefit of nitrate is related to its reduction to nitric oxide. 25% of dietary nitrate ion is converted to nitrite (NO_2) ion in saliva and upper gastrointestinal tract (Ninfali and Angelino, 2013; Clifford et al., 2015). Some of the ingested nitrite is reduced to nitric acid (NO) in the acidic environment of the stomach. NO has the potential to lower blood pressure (Ninfali and Angelino, 2013). It is also highly effective in improving athletic performance and treating cardiovascular disorders (Lundberg et al., 2008; Ninfali and Angelino, 2013; Clifford et al., 2015; Abdo et al., 2020). Excessive nitrate accumulation in vegetables both negatively affects their nutritional value and limits their durability (Czapski et al., 1998). The Acceptable Daily Intake (ADI) for nitrate is 3.7 mg/kg body weight/day (Ninfali and Angelino, 2013). Excess nitrate and nitrite in vegetables is difficult to remove. Nitrate extraction is not possible, especially in finely chopped products such as puree and vegetable juice (Czapski et al., 1998). However, it is known that nitrate concentration can be significantly reduced by fermentation strategies in industrial applications (Ninfali and Angelino, 2013).

Fermented beet juice obtained by Klewicka et al. (2012) using cultures of *L. brevis* and *L. paracasei* reduced the number of aberrant crypt foci (ACF),

one of the earliest changes that can lead to colon cancer, by approximately 60% in rats treated with N-Nitroso-N-methylurea (MNU). Significant cytotoxic and genotoxic effects in Caco-2 cells of MNU fed rats were abolished by adding fermented beetroot juice to the diet. They found that the presence of fermented beet juice in the diet adhered to the colon epithelium of many microorganisms, including *Lactobacillus*/*Enterococcus* bacteria. They reported that by adding lactofermented beet juice to the diet, protection against pre-cancer diseases can be provided and the cytotoxic and genotoxic effects of fecal juice can be reduced.

According to Kapadia et al. (2003) investigated the effectiveness of betamine for long-term local suppression of skin and liver tumors. They found that regularly consumed betanin acts as an effective cancer chemopreventive agent in mice. Their most interesting observations are that the cancer chemopreventive effect was demonstrated even at a very low dose (0.0025%) used in the study. For this reason, they reported that more research should be done for possible human applications in the malignancy control of beet.

4. CONCLUSION

More than 70 probiotic products are commercially consumed worldwide. In addition to the contribution of lactic acid bacteria to the quality and safety of food, adding functional properties to food makes probiotic products even more attractive. Due to the increasing awareness of consumers, the demand for natural and functional foods is increasing day by day. Considering this demand, it is important for the producers to increase the variety of products by taking advantage of the opportunities offered by nature and for scientists to contribute. Beetroot is a very valuable food due to its nutritional value and beneficial effects on health. It is also very easy to reach because it is an abundant and cheap food source. Beet kvass, a functional beverage with better organoleptic properties, is obtained by fermentation of beets. Studies show that beet kvass can be consumed as a functional beverage. There are few studies on fermenting beet juice, but there are almost no studies on beet kvass. For this reason, studies should be conducted on the structural characterization and evaluation of functional properties of beet kvass. It will be important to understand beet

kvass, a traditional probiotic product, in depth.

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CHAPTER 12

DETERMINATION OF ENTREPRENEURSHIP TENDENCIES OF FACULTY OF AGRICULTURE STUDENTS: KIRŞEHİR AHİ EVRAN UNIVERSITY

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INTRODUCTION

Entrepreneurship plays a crucial role in the economic and social development of modern societies, contributing to innovation, creativity, and risk-taking initiatives. Entrepreneurs conceive projects across various fields, establish companies, and engage in activities that have a significant impact on the market. In today's dynamic world, consumer demands can undergo changes influenced by fundamental factors such as socio-economic and cultural shifts, technological advancements, and economic progress. Entrepreneurial activities are instrumental in effectively and successfully incorporating changes in consumer demands into production processes and economic activities. Accordingly, studies aimed at fostering entrepreneurial traits among the young and dynamic population, including university students, through educational initiatives, are increasingly gaining significance. The provision of theoretical entrepreneurship courses at universities makes critical contributions in this regard.

The idea of entrepreneurship encompasses an entrepreneur embracing a way of life through the establishment and administration of a financially self-sustaining business, along with organizations that generate profits by offering marketable products and services (Özdemir and Toker, 2018). Entrepreneurial individuals evaluate business opportunities by generating new ideas and strive to establish successful businesses. To thrive in the business world, developing innovative ideas and challenging the status quo are key characteristics of entrepreneurs. Furthermore, these individuals adeptly manage creative thinking processes, results distinct solutions to problems and gaining a competitive advantage. Another notable trait of entrepreneurial individuals is their willingness to take risks and propose successful solutions in uncertain situations (Bozkurt and Alparslan, 2013). Moreover, these individuals possess strong communication and collaboration skills, maintaining a consistent demeanor. The financial acumen and managerial expertise of entrepreneurs are also crucial, as the establishment, management, and sustainability of a business depend on the nature of the entrepreneurial endeavor (Ballı, 2017), all while pursuing profitability. Making sound and informed decisions regarding budgeting, cash flow management, and financial analysis is vital for commercial activities. Additionally, adaptability and flexibility, which are

indicators of intelligence, play a significant role in monitoring market trends and making decisions amidst changing conditions. The adaptability of entrepreneurial individuals is also closely tied to their capacity for learning and development. Staying abreast of rapidly advancing technology and incorporating its implications into their business activities greatly benefits entrepreneurial individuals in competitive processes. Alongside the aforementioned traits, the socio-demographic backgrounds of entrepreneurial individuals can give rise to additional distinct and effective characteristics (Bozkurt and Alparslan, 2013). Determining whether an individual possesses entrepreneurial qualities is a complex undertaking. Entrepreneurial inclination can emerge based on variables such as genetic makeup, personality traits, abilities, experiences, and environmental factors. Presently, researchers employ scales and various survey studies to examine individuals' entrepreneurial tendencies (İşcan and Kaygın, 2011; Uluyol, 2013; Salik and Kaygın, 2016).

Determining the entrepreneurial tendencies of young individuals, especially university students, and supporting them in this field is one of the important research and education areas. Ekici and Turan (2017) in their study, it was examined whether personal attitude, social norm and perceived behavioral control, which are the elements of 'Planned Behavior Theory', as well as entrepreneurship education have an impact on students' entrepreneurial tendencies. In the study conducted with the students of the 3rd and 4th grade business department who received entrepreneurship education at Osmaniye Korkut Ata University and Kahraman Maraş Sütçü İmam University, it was found that the perceived behavioral controls of the university students had the highest positive effect on the entrepreneurial tendency. Korkmaz (2012) in her study, Bülent Ecevit University aimed to determine whether the students of the department of business administration have an entrepreneurial personality and to determine what psychological, demographic and family factors are effective in entrepreneurial tendencies. Results show that there are significant relationships between psychological, demographic and family characteristics, which are effective in students' seeing themselves as an entrepreneur and their desire to start a business in the future. Örücü et al., (2007) In their study, they examined the relationship between various familial factors such as upbringing, family income and having an entrepreneurial family, which are factors affecting

entrepreneurial tendency, and entrepreneurial tendencies of university senior students. In the research conducted with Balıkesir University senior students, it was determined that the entrepreneurial tendencies of the individuals were affected by their family income. In addition, it has been determined that the upbringing style and whether there is an entrepreneur in the family have no effect on the entrepreneurship tendency of university senior students and male students tend to be more entrepreneurial than female students. Arslan (2002) in his study, the role of physical, social and economic environment in the formation of entrepreneurial tendencies and professional preferences of Haliç University students was examined. The results report the expected positive relationship between the income level of the students' families and the low number of children in the family and entrepreneurship. Karakaş (2012), In this study, it is aimed to determine the factors affecting the entrepreneurship tendency of university students. For this, various demographic variables were included in their research. Results show that business department students have higher values than engineering department students in their 'feeling of internal control, need for achievement, tendency to take risks, tolerance for uncertainty, self-confidence and being innovative'. In addition, it was concluded that the business administration education program affects the entrepreneurship tendency at higher rates compared to the education programs related to engineering departments. Çelik et al., (2014) in their study, they examined the relationship between entrepreneurial intentions and familial factors of students studying at the Faculty of Economics and Administrative Sciences of Mersin University. According to the results of the analysis, it was determined that there is a relationship between entrepreneurial intention and having an entrepreneurial personality, being an entrepreneur in the family and the degree of participation in the decisions made in the family.

In this study, it is aimed to determine the entrepreneurship tendencies of university students. A five-point Likert-type questionnaire was conducted to determine the levels of success-oriented, risk-taking and innovativeness, and entrepreneurial tendency in order to evaluate entrepreneurial activities.

MATERIAL AND METHOD

The sample of the study consists of 147 students studying at Kırşehir Ahi Evran University, Faculty of Agriculture in the 2021-2022 academic year. Sample size in the study; The power of the test was determined as 0.80, effect size 0.25, and type 1 error level 0.05 in the light of power analysis data. In this study, the data collection tool was prepared by using the questionnaire study developed by Melike Ubuz and Prof. Dr. Veysel Bozkurt (Ubuz, 2019) in order to determine the entrepreneurial tendencies of university students. A five-point Likert-type questionnaire was conducted to determine the levels of success-oriented, risk-taking and innovativeness, and entrepreneurial tendency in order to evaluate entrepreneurial activities. The questionnaire form used in the study consists of two parts. In the first part, there are questions about determining the demographic characteristics of the students. In the second part, there are scale expressions of success-oriented, risk-taking, innovativeness and entrepreneurial tendency of the agriculture faculty students who participated in the survey. Descriptive statistics, frequency tables and graphical representations were used of the analysis. Analyzes were carried out with SPSS (Statistical Package for Social Science) 26.0 statistical package program.

RESULTS

In this study, the entrepreneurial tendencies of university students were tried to be evaluated in terms of various scales. For this purpose, demographic characteristics of the students who participated in the survey, including gender, age, marital status, and various socio-economic information, were included in the results. In order to investigate the entrepreneurship tendencies of university students, success-oriented, innovative, risk-taking behavior and entrepreneurial tendency scales were used. Table 1 contains information about the demographic characteristics of university students. According to the results of gender distribution, 65.3% of the 147 students participating in the survey were male and 34.7% were female. It is seen that the participating male students constitute most of the sample's percentage and frequency.

Table 1. Demographic characteristics of university students.

Variables		Frequency	%
Gender	Male	96	65.3
	Female	51	34.7
Living Place	Village	20	13.6
	Town	5	3.4
	District	42	28.6
	Province	79	53.7
Marital Status	Married	16	10.9
	Single	129	87.8
	Divorced	1	0.7
	Widow	1	0.7
Department	Agricultural Economics	54	36.7
	Field Crops	7	4.8
	Horticulture	19	12.9
	Plant Protection	67	45.6
Class	2	53	36.1
	3	39	26.5
	4	55	37.4
Foreign Language Level	Low	76	51.7
	Medium	63	42.9
	Good	8	5.4

It was determined that the youngest of the students participating in the study was 19 years old and the oldest was 53 years old. In the study, the average age of the students was calculated as 23.61. When the places where the students participating in the survey live are examined, it is seen that five students live in the town, 79 students live in the province, 20 students live in the village and 42 students live in the district. When the distribution of the marital status of the students participating in the survey was examined, it was determined that 87.8% of the students were single. calculated.

Table 2. Characteristics of university students' families.

Variables		Frequency	%
Sibling rank	1-5	52	29,9
	>6	95	64,7
Mother's Education Status	Illiterate	7	4,8
	Literate	11	7,5
	Primary school	54	36,7
	Middle school	31	21,1
	High school	28	19,0
	College	1	0,7
	University	10	6,8
Mother Working Status	Not working	115	78,2
	Own Workplace	14	9,5
	Public Sector	6	4,1
	Private Sector	9	6,1
Father's Education Status	Illiterate	2	1,4
	Literate	3	2,8
	Primary school	30	20,4
	Middle school	32	21,8
	High school	42	28,6
	College	5	3,4
	University	30	20,4
Father Working Status	Not working	26	17,7
	Own Workplace	52	35,4
	Public Sector	36	24,5
	Private Sector	28	19,0

When examining the frequency and percentage distributions of the departments of the students participating in the survey, it can be observed that 67 students (45.6%) are from the Plant Protection Department, 54 students (36.7%) are from the Agricultural Economics Department, 19 students (12.9%) are from the Horticultural Department, and 7 students (4.8%) are from the Field Crops Department. In addition, 53 students (36.1%) are in their second year, 39 students (26.5%) are in their third year, and 55 students (37.4%) are in their fourth year, all studying at the Faculty of Agriculture. The grade point average of the 147 students from the Faculty of Agriculture who participated in the survey was calculated as 2.65. When examining the frequency distribution of foreign language levels among university students, it is evident that the majority of students define their foreign language proficiency as low or medium. Specifically, 51.7% of students described their foreign language level as low,

42.9% as medium, and only 5.4% as good. Regarding the household structure of the surveyed students, it was found that the number of households ranged from a minimum of 1 to a maximum of 9. The average number of people per household was calculated as 4.25.

Table 2 contains information about the characteristics of university students' families. In the answers given by the university students to the question about the number of children in the family, the frequency of being between the first and fifth children was 52 (29.9%), and the frequency of being sixth or more was 95 (64.7%). It was determined that the most observed frequency values in the educational status of the mothers of the students were 54 (36.7%) in primary school, 31 (21.1%) in secondary school and 28 (19.0%) in high school. It was observed that the highest frequency values in the educational status of the fathers of the students were 30 (20.4%) in primary school, 32 (21.8%) in secondary school, 42 (28.6%) in high school and 30 (20.4) in university. In the results related to the working status of the mothers of the students, it was observed that the mothers of 115 students (78.2%) were not working, the fathers of 26 students (17.7%) were not working and the fathers of the 52 students (35.4%) were working in their own workplace.

Table 3. Some opinions of university students about entrepreneurship.

Variables		Frequency	%
Employment status of university students during their undergraduate education.	Yes	82	55,8
	No	63	42,9
Entrepreneur(s) presence status in the family or close circle of university students.	Yes	77	52.4
	No	70	47.6
Status of university students taking entrepreneurship-related course(s).	Yes	79	53.7
	No	65	44.2
Have the lessons you learned changed your ideas about being an entrepreneur in a positive way?	Yes	73	49.7
	No	44	29.9
Do you think you can reach the necessary financial resources to become an entrepreneur?	Yes	71	48.3
	No	74	50.3

Table 3 shows the answers of university students to some questions about entrepreneurship. The status of university students working at a job during their undergraduate education was reported as positive with 82 frequency values (55.8%) and negative with 63 frequency values (42.9%). 77 of the students (52.4%) reported that there were entrepreneurs in their families or in their environment, and 47.6% of them did not. 53.7% of the students took entrepreneurship-related courses, while 44.2% did not. According to the answers given by the students who participated in the survey, 73 (49.7%) people stated that the courses they took changed their ideas about being an entrepreneur in a positive way, while 44 (29.9%) people expressed the opposite opinion. According to the results, it shows that university students taking lessons in this subject during their education process and directing them consciously will make significant contributions to the professional lives of our students in the future. The students expressed a positive opinion of 48.3% and a negative opinion of 50.3% of the students about reaching the necessary financial resources in order to be an entrepreneur in the future.

Table 4. The level of economic self-evaluation of university students.

Variables		Frequency	%
The level of economic self-evaluation of university students	Very low	21	14,3
	Low	12	8,2
	Average	82	55,8
	Good	26	17,7
	Very Good	5	3,4

Table 5. Students' career goals after graduation.

Variables		Frequency	%
Students' career goals after graduation	Start Your Own Business	47	32.0
	Private Sector	23	15.6
	Public Sector	41	27.9
	Academic Career	22	15.0
	Other	11	7.5

Table 4 contains information about the level of economic self-evaluation of university students. The results are summarized as follows: 21 students are very low (14.3%), 12 students are low (8.2%), 82 students are moderate

(55.8%), 26 students are good (17.7%), and 5 students are very good (3.4%). The students were asked a question about what they would like to do after graduating from school as a career goal. As can be seen in Table 5, the number of students who want to start their own business is 47 (32.0%), the number of students who want to work in the private sector is 23 (15.6%), the number of students who want to work in the public sector is 41 (27.9%), and the number of students who want to become an academic is 22 (15.0) and the number of students who marked the other option was determined as 11 (7.5%). Results show that the number of students who want to start their own business is higher than other options. In this case, it can be interpreted that the entrepreneurship tendencies of the students of the faculty of agriculture are at positive levels. In addition, the number of students who want to work in the public sector was found to be higher than other options. It can be interpreted that the students who prefer the public sector are slightly more distant from the entrepreneurial tendency and prefer a more stable order in their professional life.

Table 6 shows the results related to the achievement-oriented feature scale of university students. In the statement “It is important for me to be successful in life”, 92 out of 147 students gave the answer that I strongly agree. 4 people gave the answer they do not agree. In the statement "I try to be the best in what I do", the frequency value for the strongly agree option is 96 people and the frequency value for the disagree option is 2. Another remarkable result of the scale examined was that 90 students chose the option "I strongly agree" and 1 student "I disagree" in the statement "I believe that people should have goals". In general, the results of the scale indicate that university students are conscious of success and find themselves focused on success. The majority of the students participating in the research believe that they will be successful with hard work and agree that it is necessary to use time wisely in order to be successful. It can be commented that students' awareness levels are at positive levels on time management, which is one of the important characteristics of entrepreneurial individuals.

Table 6. Achievement-oriented trait scale results.

Achievement-oriented trait scale	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
Being successful in life is very important to me.	9	4	7	34	92
I try to be the best at what I do.	9	2	11	28	96
My friends think I'm lazy.	67	29	21	11	18
I always do the most important things first.	6	3	22	50	63
I focus on results in my work.	8	6	13	44	74
My friends think I am an ambitious person.	9	20	37	34	46
I believe we shouldn't waste our time mindlessly.	16	6	20	38	66
For my future success, I can give up my comfort today.	9	7	29	39	61
I believe that for the person who works hard, nothing is impossible.	9	6	11	41	79
I believe that I am a success-oriented person.	6	6	24	50	59
If I waste time, I will be unhappy.	8	10	35	43	48
I have always loved taking responsibility.	11	11	28	43	53
I believe people should have goals.	6	1	3	47	90
I believe that if I work hard, I will be successful.	8	3	9	38	89
People must use time wisely to be successful in life.	7	3	9	39	89

Table 7 presents the results related to the risk-taking feature scale. The scale comprises 10 different expressions associated with risk-taking. The majority of the surveyed individuals indicated a preference for taking risks, particularly if there is a potential for high earnings. Notably, the statements in the scale regarding the preference for known safe routes over engaging in risky behaviour attracted attention due to the balanced marking structure in the 5-point Likert-type response distribution. It can be inferred that university students are inclined to take risks, possibly due to their lack of experience in the business world or their relatively new status. However, they also demonstrate a moderate approach by opting for safe and familiar paths.

Table 7. Risk-taking trait scale results.

Risk-taking trait scale	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
I like to take reasonable risks.	10	8	31	44	54
If there is a higher profit opportunity, I take more risks.	9	7	41	34	55
I always avoid taking risks.	54	41	29	15	8
I always choose known safe paths in life.	11	24	34	39	36
The exciting lifestyle is more attractive to me than the safe lifestyle.	14	21	41	28	42
My friends believe that I take too many risks for an exciting life.	19	29	49	20	30
I always prefer safe routes, not risky alternatives.	21	27	34	40	24
The more risk I take, the more profit I get.	15	34	53	20	23
I believe people should be able to take risks without thinking too much about the consequences.	20	26	46	27	28
I usually take more risks than other people.	18	18	39	38	34

Table 8 presents the results related to the scale of innovativeness. The scale includes 16 different expressions associated with innovativeness. The frequency distributions indicate that participants hold negative opinions about performing the same job every day while expressing positive views on seeking new ways in their work. Moreover, students who exhibited a highly positive attitude towards innovative ideas also displayed significant engagement in leading a life filled with innovations. In this regard, it is crucial to engage in activities that guide the young population's dynamics towards the right channels and provide them with the appropriate information. This approach can be seen essential for harnessing the energy they possess and transforming it into beneficial outcomes.

Table 8. Innovativeness scale results.

Innovative Feature	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
The status quo never satisfies me, I always seek innovation.	7	11	34	43	47
I have always loved innovation.	5	4	18	60	58
I always look for new ways in what I do.	3	7	25	53	56
If I do the same (routine) tasks every day, I get bored.	9	8	17	41	69
My mind is always busy with new plans and projects.	8	11	40	44	41
I believe I am a creative person.	10	9	28	43	52
My friends believe that I am an innovative person.	9	11	36	48	40
I always had original ideas.	3	9	31	57	45
I always try to make a difference in what I do.	5	7	27	50	55
New ideas excite me.	5	4	22	44	68
My mind is constantly filled with new plans to make.	6	9	38	40	49
I always strive to create new things.	7	12	38	49	38
My head is usually busy with new ideas.	5	12	44	44	39
Others think I am an energetic person.	6	6	30	46	55
My approach to problems is unique and different from other people.	4	13	34	47	45
I want a life full of innovation.	5	4	20	38	77

Table 9 shows the results related to the entrepreneurship tendency feature scale. Most of the students stated that they prefer to be their own boss and that they plan to establish their own businesses. In general, a significant majority of the students who participated in the survey thought of being independent and owning their own business. In addition, they stated that job security is important to them. The number of students who can choose to work in a paid job in their professional life is also quite high. It has been observed that there are students who want to work in government institutions and practice their profession with regular salary income rather than risk.

Table 9. Entrepreneurial propensity trait scale results.

Entrepreneurial propensity trait	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
I prefer to be my own boss.	7	5	21	26	86
Entrepreneurship fascinates me.	7	8	30	46	53
I plan to start my own business one day in the future.	7	5	26	29	77
Job security is very important to me.	4	5	15	35	83
I would rather have a paid job than start my own business.	53	22	41	14	13
I would rather be independent and own my own business than work for others.	6	8	19	31	81
I prefer to work in a paid job in a large organization.	38	12	44	23	27
I don't want to take orders from others in my job.	11	11	27	27	69
I am seriously considering starting my own business after graduation.	10	9	38	26	62

CONCLUSIONS

Today, economic developments and the competitive environment brought about by globalization have led to increased research and applications in entrepreneurship. The concept of entrepreneurship is closely linked to tendencies towards success-oriented, innovative, and risk-taking behaviours. To adapt to the ever-evolving and transforming world order, an increase in entrepreneurial tendencies provides individuals with the opportunity for sustainable development at the individual level, thereby enabling them to succeed within their organizational structures. This study aims to determine the entrepreneurial tendencies of university students studying at Kırşehir Ahi Evran University Faculty of Agriculture. Within this context, the results obtained from scales measuring success-oriented attitudes, risk-taking propensity, innovation, and overall entrepreneurial tendencies were evaluated. The research results indicate that university students exhibit a highly positive approach towards entrepreneurship. They believe that hard work is essential for success and acknowledge the importance of time management. Additionally, it was observed that while they possess a tendency for risk-

taking, they may still prefer to follow safe and familiar paths. The students displayed a strong inclination towards innovative ideas, expressing a desire for independence and owning their own businesses. Furthermore, job security was identified as a significant situation for them. Properly guiding and structuring the dynamics of the young population in our country is crucial for economic development and the quality of employment.

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CHAPTER 13

A REVIEW ON THE REGENERATIVE AGRICULTURE AND THE RELATED MECHANIZATION PRACTICES

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INTRODUCTION

Maintaining ecological balance of our planet is a must to survive from recent issues such as climate change and pandemics, and support the wellbeing of humanity (Özdemir et al. 2022).

Soil can be accepted as a living organism that has a complex structure consists of biological, chemical and physical characteristics. Climate, flora, fauna, topography, time and behavior of human beings affect the soil. It is important to preserve the health of this living life form, to ensure the sustainability of our world (Katsuyuki, 2021).

Although conventional farming practices are carried out in order to preserve and increase soil fertility, to prevent soil compaction (Önal, 2017), spread of less harmful methods are needed to protect flora, fauna and biodiversity in the soil. Making the least possible physical intervention that will have a negative impact on the soil can be favorable (Giller et al. 2021). On the other hand, with the introduction and use of holistic approaches that allows better resource allocation water, energy and food nexus brings better understanding of complex systems and provide more effective solutions. This kind of systems thinking is crucial to provide openness to the new practices that is necessary in order to be able to say that sustainable agriculture has been done (Ertuğrul et al. 2022; Degirmecioglu et al. 2019). Sustainable agriculture aims to meet food requirement while being environmentally friendly by providing the most efficient use of non-renewable and on-farm resources and sustain natural biological cycles. Economic viability of farm operations is also important to guarantee and enhance the well-being of farmers (Evcim et al. 2012).

Regenerative agriculture (RA) is an emerging term to describe a new farming system approach and covers relatively narrow meaning in comparison to sustainable agriculture (Schreefel et al. 2020). RA can be considered as part of sustainable agriculture. RA is a natural farming system to increase soil biological activity, maintain and enhance soil health by improving nutrient cycling, while producing required goods (Khangura et al. 2023). The feature that distinguishes RA from sustainable agriculture; while sustainable

agriculture aims to "protect" the ecosystem as much as possible, regenerative agriculture aims to "improve" it. In RA, providing a healthy carbon cycle in plants, improving soil health, providing a healthy nutrient-photosynthesis cycle with effective crop management are the main goals. Basically, it aims to improve the ecosystem we live in while farming, a farm management approach designed with the thought that practices that will make the soil, which is losing its health, healthy again. Although it is limited, RA contributes to reverse climate change by increasing soil carbon storage (Rui et al. 2022). Increasing soil organic matter and soil biodiversity by RA practices improves soil fertility and water cycle, and increases the amount of carbon that can be extracted from the atmosphere. In agriculture, production continues mostly with a monoculture farming method, which is generally a single species cultivation system called monoculture. Although monoculture can provide greater yield at a lower cost, producing single crop intensively can greatly reduce the amount of nutrients and humus that keeps the soil fertile and on a very large scale limits important functions that nature provides and reduces biodiversity (Salaheen and Biswas, 2019). In regenerative agriculture, which is a polyculture farming system, it can be seen that biodiversity is preserved as it is, and water use can be reduced by 75%. Practices involved in regenerative agriculture include no-till/reduced tillage techniques, use of cover crops, crop rotations, proper use of compost and animal manures, soil reclamation and grazing management with compost or compost extracts to restore soil microbial activity. To conduct the RA practices, mechanization tools such as direct seeding machines, mulching tools, weed crushers, and strip tillage tools can be used in the regenerative farming system. In this study, it is aimed to give information about the "regenerative" agriculture system, which is observed to be a new phenomenon for Turkish Agriculture, and the mechanization practices used in this system (Özdamar et al. 2022).

MATERIAL AND METHOD

In this chapter, published articles about regenerative agriculture around the world, open sources of research institutions operating in this field and private sector institutions that invest or prepare to invest in this field were

examined via the internet, all positive/negative ideas about the regenerative agriculture method and the applications being carried out. Consequently, the tools and equipment used in the regenerative agriculture practices are summarized by scrutinizing.

RESULTS AND DISCUSSION

Although there are short-term benefits of intensive tillage, it harms the nature when it is unconsciously applied. With the practice of burning or removing the plant residues left in the field after harvest, which is still common today, an increase in environmental damage, especially erosion, occurs (Allam et al. 2022; Pelosi et al. 2014). Roger-Estrade et al. (2010) states that soil complexity can be characterized by based on organism size, the structure of soil food webs and function of soil organisms. Those features can be affected by practices specific to the crop management, such as mechanization, no-till or the chemical weed control practices. Tillage methods have significant effect on soil physicochemical structure, in short time periods. To summarize the known tillage practices;

1. Conventional Tillage: It is a mould board plough centered method in which 85% of plant residues are buried at a depth of 25-30 cm with a the plough. Disc harrows are also among the most preferred equipment to break the bigger aggregates and mix the fertilizers to the soil. With this kind of intensive tillage method, it can be said that the risk of erosion is very high compared to other methods due to the low rate of plant residues remaining on the surface and the deteriorated soil structure (Çelik et al. 2019).
2. Conservative Tillage: This is actually a group of methods, developed with the search for a solution to the erosion problem, is based on leaving 30% or more plant residues on the soil surface (Köller, 2003). Conservative tillage methods can be classified as follows;
    - *Reduced tillage* is an energy-saving method, as tools are used that damage the soil much less than the conventional tillage method and require less traction power. Chisel or disc tools can be used for primary tillage, disc tools or cultivators can be used for secondary tillage and seedbed preparation.

- *Strip or ridge tilling* is carried out by cultivating only one third of the soil surface in strips with a width of 5-30 cm depending on the type of crop to be planted, mostly using disc tools. Plant residues are left in untreated areas.
- *Mulch tillage* can be carried out with tools such as chisels, cultivators, disc harrows, in order to take precautions against the cream layer and reduce erosion without removing plant residues on the soil surface.
- *No-till* farming, zero tillage, is also called direct seeding. No-till is actually not a new method as it is thought. Baeumer and Bakermans (1973) mention that in 1927 Garber successfully carried out the first attempt at direct seeding by planting a legum variety on turf. Today, the efforts of many countries to adapt the no-till method to their agricultural production systems have intensified (Derpsch and Friedrich, 2009). In no tillage, planting is made directly to the furrows opened on the plant residues by specially designed furrow openers. The seeds covered with soil and plant residues and will be protected from the problem of emergence caused by the surface crust.

There are many studies on efficiency comparing zero tillage with other tillage methods. When these studies are examined, it is emphasized that even in different crop rotations, no significant differences are observed between no-till and conventional tillage in the short term in yield and soil structure, and it is emphasized that the time and energy savings provided by no-till are high. In the long term, it has been observed that the amount of organic matter and nitrogen in the soil is determined to be higher in the fields with zero tillage compared to traditional tillage, and it is concluded that this situation will have a positive effect on soil improvement and crop yield (Claudia et al. 2003). Confirming the stated before, Gao et al. (2004) and Kladivko (2001) underlines the benefits of no-till practices to protect the all type of organisms in the soil. Mathew et al. (2012) determined that no-tillage and reduced tillage methods make it possible to have significantly higher soil organic matter contents. This idea is supported by Pareja-Sánchez et al. (2017) as well.

In terms of Regenerative Agriculture Methods are examined in the literature, 4 basic applications are encountered;

1. No-till or conservative tillage practices that contribute to soil production, improvement/remediation, soil fertility and preservation of health (Reicosky, 2021),
2. Use of compost and crop rotation practices that provide water infiltration, water retention and clean/safe water cycling (Tan and Kuebbing, 2023),
3. Practices where various plants that increase biodiversity and ecosystem health and resilience are grown together and cover crops are used (Newton et al. 2020),
4. Practices that include environmentally friendly grazing methods that reduce carbon emissions as a result of existing agricultural practices (McLennon et al. 2021).

Mechanization tools used in Regenerative Agriculture Applications

"Zero" tillage (no-till) reduced or conservative tillage methods are accepted as a part of regenerative agriculture. In no-till, direct seeding machines that can sow stubble are used (Figure 1).

Figure 1. Direct seeder

In conservative tillage methods, the aim is the to use less or surface tillage practices than conventional tillage practices, by using surface tillage tools that are capable to till vertically (Figure 2).

Figure 2. Vertical coulters

There are applications such as the use of materials that protect the soil surface from external factors, such as cover crops, mulch, and taking measures against erosion. Mulchers capable of crushing the stubble can be used to cover the soil surface with chopped stubble (Figure 3).

Figure 3. Stubble Mulcher

Strip-till and surface till equipment are well known to partial tillage practices (Figure 4). Thus, tillage is reduced and the harm to the soil is minimized.

Figure 4. Strip-till equipment

Special seeders designed for planting cover crops between rows are favorable for protecting soil from external factors and increases the effectiveness of weed control (Figure 5).

Figure 5. An interseeder equipment

Weed crushers and specific hoe equipment for hoeing weeds can be used as well for weed control purposes (Figure 6).

Figure 6. A weed crusher

CONCLUSION

Regenerative agriculture is an agricultural production method that focuses on the sustainability of soil vitality, supports biodiversity, can reduce the cost of agricultural operations in the right place and time, if applied correctly, and can positively affect food quality and supply security in the long run by restoring the health of the ecosystem. Recently, the importance of agricultural activities in harmony with nature has been increasing, and accordingly, many researchers and organizations put regenerative agriculture on their agenda.

In this study, the importance of regenerative agriculture, the tools and machines used in the practices were reviewed, and it was thought that the inclusion and spread of regenerative agriculture practices in the farming systems with all their good aspects would have a positive contribution.

In order to disseminate the regenerative agriculture approach:

1. There is no one size fits for all approach! Regional or basin-based assessment should be made, “What reparative measures might work best for each region/basin?” The

answer to the question must be sought; relevant public institutions and farmers should work together on this issue.

2. It is necessary to start the implementation of visible regenerative measures by the farmers: For farmers, seeing is believing - with smart on-site trial design, it will encourage farmers to see in their fields what the impact of ecologically friendly practices can be.
3. Government support or easily accessible financing options should be provided to enable farmers to implement regenerative farming practices that are cost-effective.
4. Stronger-practical network platforms should be established to increase farmer's awareness and facilitate learning, and the opportunity to exchange ideas between farmers on important implementation challenges should be provided. Practical training on important regenerative applications should be given.
5. It is important to make soil health a priority as much as yield. Monitoring soil health consistently at regular intervals with low-cost or free soil analyzes would be effective.
6. Comparing and sharing the results of regenerative practices with conventional practices publicly can be effective to build trust and start a regenerative farming movement.
7. Regenerative agriculture can be made more attractive to farmers by developing new income models.

Although there is a long way to go in order for regenerative agriculture to become widespread, the implementation of the regenerative practices can be possible by cooperation of universities, governmental institutions, agricultural enterprises, marketing sector and industrial sector.

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CHAPTER 14

CARBON NANOSTRUCTURE FOR AGRICULTURAL APPLICATIONS

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INTRODUCTION

Today, nanotechnology makes its presence felt in every aspect of life. Especially with the methods developed in the last forty years, nano-sized materials have become a part of daily life, not only in research laboratories. Nanotechnology focuses on the substance's atomic structure to increase its strength, hardness, durability, etc. At the nanoscale scale, characteristics are dramatically impacted (10^{-9}m). It has highly unique and unusual features that may be used for our practical issues because of its tiny size and high surface-to-volume ratio. The fundamental requirements of today's industry are to create a system with better efficiency and fewer losses (Prakash Sharma et al., 2018). Nanomaterials are intended to be used in R&D studies in various fields. It is already used to increase efficiency in applications of some fields. Environment, electronics, biomedical, agriculture, energy, and food applications can be given as examples of these fields(Khan et al., 2019). Examples show that the unstable

Today, the variety of nanomaterials obtained with the developing technology has also increased. The reason for this is the use of more controlled methods in the procedures and the development of more sensitive characterization techniques. Generally, nanomaterials are prepared with top-down and bottom-up approaches. Particle structure forms an important part of nanomaterials. Nanomaterials consist of materials such as metallic, polymeric, lipid, silica, magnetic, and carbon(Saleh, 2020). In this study, carbon-based nanomaterials and their current-planned applications in agriculture will be explained.

1. Carbon Basen Nanomaterials

1.1 History and Properties Carbon-Based Nano Structures

Carbon is an extraordinary nonmetal element with the astonishing capacity to form a wide range of compounds and structures on both a large and nanoscale. More than 95% of all known chemical substances are carbon-based. Carbon has four valence electrons, two from the 2s orbital and two from the 2p orbital, which actively engage in bonding, producing single, double, and even triple bonds. Carbon also quickly interacts with more electronegative and electropositive atoms, resulting in stable compounds. The resultant collection

of chemicals and nanostructures has a wide range of chemical, physical, and biological characteristics. As a result, carbon has become one of the most thoroughly investigated elements in materials science and research (Slepičková Kasálková et al., 2021; Speranza, 2021).

As compared to conventional materials, carbon-based nanomaterials (NMs) demonstrate superior capabilities in terms of optics, electricity conduction, mechanical strength, and surface area-to-volume ratio. As a result, both the scientific community and several companies have paid close attention to these materials. Because of their special characteristics, carbon-based NMs have great promise for cutting-edge uses in a variety of industries, including tissue engineering, energy studies, capacitors, wastewater treatment, gas membranes, heterogeneous catalysis, bioimaging and sensors (Onyancha et al., 2022)(a)

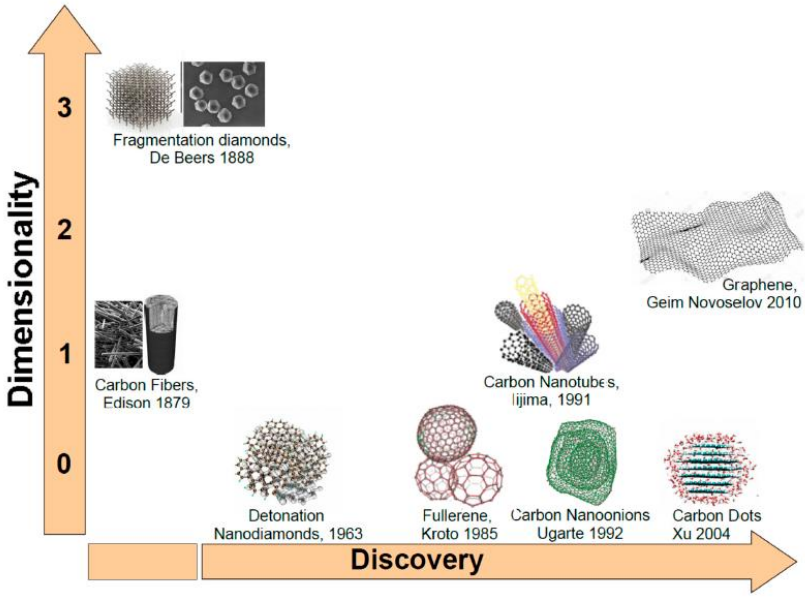

Figure 1. Summary of the historical development of carbon nanostructures.
References(Speranza, 2021)

With the development of materials science, the synthesis of carbon-based nanostructures has increased and species have become richer. This nanostructure, carbon nanotubes fullerenes, graphene, nanodiamonds, carbon

quantum dots, etc. can be exemplified. Carbon nanostructures can be geometrically 2D or 3D. The first 3D and 1D structured particles were discovered in the 1880s as fragmentation diamonds and carbon fiber. However, 0D carbon nanostructures, which are the most used and functional today, were prepared after the 1980s. In 1985, Kryto et al. synthesized fullerene for the first time, then Lijima et al. discovered carbon nanotubes in 2004, and In 2010, Geim and Novoselov were awarded the Nobel Prize in Physics for th eir work on graphene isolation (Porto et al., 2020; Speranza, 2021). Figure 1 summarizes the historical development of carbon nanostructures.

1.2 Types of Carbon Nanostructure

Carbon nanostructures are classified according to their size, carbon arrangement, and geometry. The types of carbon nanostructures are summarized in Figure 2. It is expected that the types of carbon nanostructures will be enriched and increased with the studies carried out. The properties and synthesis of some carbon nanostructures are given below.

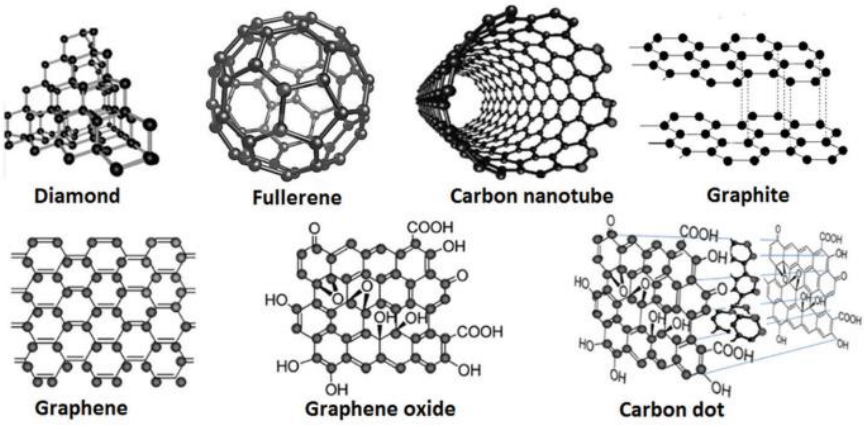

Figure 2. Types of carbon-based nanostructures
References (Yan et al., 2016)

1.2.1.Nanodiomands

Nanodiamond (ND), a carbon-based substance, has emerged as a fascinating and innovative candidate for biomedical applications due to its remarkable physical and chemical properties. Moreover, this nanomaterial exhibits minimal toxicity and high biocompatibility, further enhancing its potential for use in various biomedical searches (Hui et al., 2010).

Although they normally have a hard structure, they can easily be surface modified. In this way, they become functional for many applications. Thanks to their strong structure, they are hard and can be used as cutting tools. In addition, solid structures are resistant to fluorescent rays. On the other hand, just like other materials, nano size enriches the properties of the material. (van der Laan et al., 2018). In the historical process, nano-sized particles of 4-5 nm were produced for the first time. Later, alternative fluorescent nanodiamonds to semiconductor QDs were produced and used for biological imaging. In the following processes, nanodiamonds took place in sensor studies and biological applications thanks to their modified surfaces. Nowadays, more pure, harmless, and cost-effective nanodiamonds can be obtained with environmentally friendly methods. It is even called the least toxic material among carbon-based nanostructures(Mochalin et al., 2012)

Along with the developing technology, nanodiamond preparation methods have also been varied. Some of the methods that are used for nanodiamond synthesis are dynamic synthesis using detonation techniques, chemical vapor deposition (CVD), high-temperature and pressure conditions, and laser ablation. While the detonation method is more specific than the others, the natural diamond formation from the high temperature and pressure method is combined. On the other hand, high-yield and high-quality nanodiamonds were produced by laser ablation and chemical vapor deposition methods. Laser ablation is an environmentally friendly method. The chemical vapor method is more classical and widely used(Mochalin et al., 2012).

1.2.2 Fullerenes.

Fullerenes have attracted a lot of attention since they were synthesized in 1985. Fullerene called a carbon molecule, is an allotropic form of carbon and

has a cage structure(Porto et al., 2020) More than 20 carbon atoms are connected by carbon bonds in sp2 hybrid form and are in the form of a network of symmetrical hexagonal and pentagonal rings (Zaytseva & Neumann, 2016).

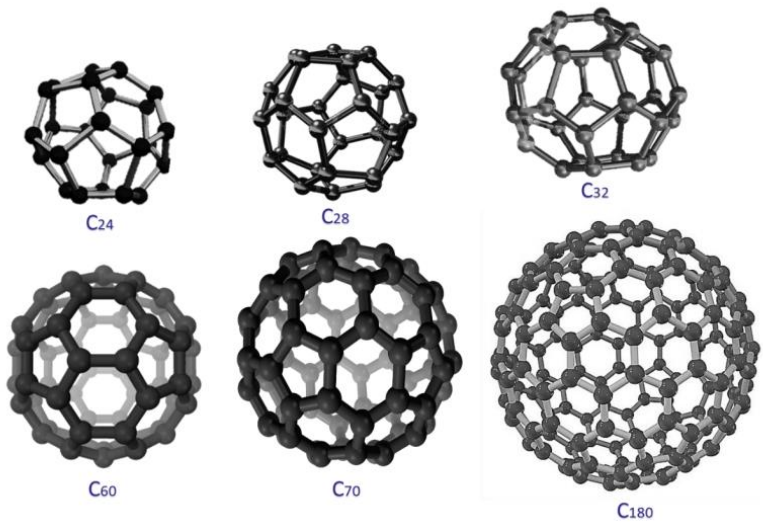

Figure 3. Types of fullerene nanostructures
References (Kausar et al., 2023)

Generally, fullerene C60 has the most ideal spherical form that has a 07, nm diameter. There are 12 pentagons and 20 hexagons on its surface (similar to a classical sewn soccer ball). The hexagon is composed of a system of alternating single and double bonds, whereas the pentagons are built of single covalent bonds(Zemanova et al., 2013). Fullerenes were originally manufactured by laser vaporization of carbon in an inert environment, however, this approach yielded relatively little fullerenes. Nonetheless, considerable amounts of fullerene C60 were later produced by arc-heating graphite and laser-irradiating polyaromatic hydrocarbons (PAHs). Synthesis by Electric Arc Synthesis by electric and resistive arc heating of graphite are other preparation methods of fullerenes (Nimibofa et al., 2018).

Fullerenes are preferred in many fields of application due to their spherical geometry (large surface area), lattice structures, surface

modification possibility, fluorescence radiation under certain conditions, superconducting and semiconducting properties, and finally low toxic effects. These areas are ;

optoelectronics, solar cells, sensors, agricultural and environmental applications, biomedical studies and electronic devices, etc (Fan et al., 2020)

1.2.3. Carbon Nanotubes

Carbon nanotubes (CNTs) are small materials made by rolling one or more sheets of graphene into cylindrical forms along their axis. This method produces tubular objects with diameters in the nanoscale range and lengths ranging from micrometers to centimeters. Carbon nanotubes (CNTs) are recognized as one of the greatest materials for the creation of next-generation composite materials since they have a large surface area, high aspect ratio, and outstanding material qualities, including mechanical strength, thermal conductivity, and electrical conductivity (Dubey et al., 2021a; Porto et al., 2020)

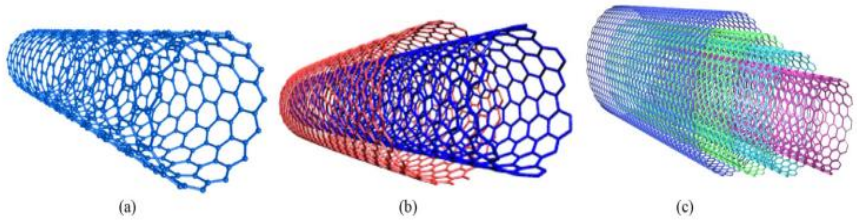

Figure 4 Scheme of a.) Single-walled CNTs, b) Double-walled CNTs, and c.) Multiwalled CNTs
References (Sobamowo et al., 2021)

Carbon nanotubes are classified into two types: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (MWCNTs). These nanotubes consist of cylindrical structures made by rolling up graphene sheets. The walls of the nanotubes are composed of interconnected carbon hexagons, and at the curved ends of the tube, there may be carbon

pentagons present.(Dubey et al., 2021b) Figure 2 indicates the shape of carbon nanotube types.

Because of the carbon-carbon sp2 bond, CNTs exhibit a high degree of hardness and axial strength. They have the highest measured Young's modulus of 1.4 TPa of any fibers. Its elongation to failure is 20–30%, and when paired with their hardness, they have a very high tensile strength of over 100 GPa. CNTs' distinct electrical properties are derived from their 1D features and graphite's unusual electronic structure. They have a very low electrical resistance. electrical resistance. Moreover, they have been shown to be superconducting even at low temperatures. Due to these features, they are highly preferred in electronic applications (Dubey et al., 2021b) .

The carbon bond's stiffness aids in the transmission of vibrations throughout the nanotube, resulting in superior heat conductivity. Carbon nanotubes have an unusually high melting point because each carbon atom is covalently linked to three other carbon atoms (Safdar et al., 2022).

Specially SWCNTs exhibit distinct optical features, including as intense fluorescence emission in the near-infrared (NIR) spectral range, primarily between 900 and 1600 nm, and a wide absorption spectrum in comparison to organic molecules. These properties make them attractive for intracellular fluorescence imaging (Hendler-Neumark & Bisker, 2019).

One of the most important disadvantages of carbon nanotubes is their very low water solubility. This situation creates difficulties, especially in biological studies. To overcome this problem, surface functionalization is performed. Another disadvantage of CNT is toxicity which is another significant hurdle. The size of the nanotubes can influence how hazardous CNTs are. The particles smaller than 100 nm may be more hazardous to the lungs, evade the body's usual phagocytic defenses, change the structure of proteins, trigger inflammatory and immune reactions, and maybe redistribute from where they were initially deposited (Eatemadi et al., 2014) .. Another disadvantage is that it is expensive and difficult to remove impurities at the production stage(Pitroda et al., 2016).

Several approaches have been developed to synthesize high-quality carbon nanotubes with varying structures and shapes. CNTs are often manufactured using three methods: laser ablation, chemical vapor deposition (CVD), and arc discharge. Of the most popular and traditional methods of CNT synthesis is arc discharge. Regardless matter whether a CNT is a SWCNT or a MWCNT, it might be difficult to preserve its shape, including its diameter and the layer count or sheets. This is because arc discharge uses a high temperature of about 1700°C, and the procedure is more difficult than other preparation methods. Green synthesis is a bottom-up strategy, comparable to chemical reduction, in which a costly reducing agents agent is replaced with a natural product extract. Green synthesis employs eco-friendly and sustainable reagents as reducing agents, lowering the possibility of harmful residues being released into the environment(Patel et al., 2020; Salah et al., 2021) .

For instance, Tripathi et al. successfully demonstrated green catalyst-assisted bulk CNT growth Green catalyst, produced from plant extracts of neem (Azadirachta indica), wall nut (Juglans regia), rose (Rosa), and garden grass (Cynodon dactylon), has been used to develop CNTs (Patel (Kumar) et al., 2022).In addition, Hakim et al. prepared carbon nanotubes from coconut shells by the quenching method in accordance with the green chemistry approach(Hakim et al., 2018).

1.2.4 Graphane-Graphane Derivatives

A carbon allotrope with a two-dimensional structure is called graphene. It has a honeycomb-like hexagonal lattice shape and is made of a material. For other carbon-based materials including fullerene, CNTs, and graphite, graphene serves as a fundamental building component(Onyancha et al., 2022) . The C-C bond is sp2 hybridized and has a high bond energy inside the graphene sheet. A weak contact force called the van der Waals force holds the layers of graphene together. Graphene is thought to be an ideal contender for lowering friction, wear, and enhancing lubrication due to its high Young's modulus and outstanding self-lubricating capabilities(Sahu et al., 2021a; Sun & Du, 2019) Because of its sp2 covalent bonds and the electrons' mobility, graphene offers many useful, catalytic, mechanical, thermal, optical and physical, features in several fields of science and industry. Extremely high mechanical strength and

flexibility, a variable bandgap, high optical transmittance superb thermal and electrical conductivity are just a few of graphene's exceptional qualities. These characteristics elevate graphite above diamond and steel as one of the strongest, lightest, and most conductive materials(Castelletto & Boretti, 2021).

The challenging handling requirements and limited supply of clean graphene serve as a counterpoint to its remarkable features. In reality, nanographite and a few layered materials are defined as graphene in a lot of literature. Several materials have been suggested as solutions to the problems with the usage of actual neat graphene. Graphene oxide is the first substitute (GO). GO is a derivative of oxidized graphene that has a lot of oxygen functions. Epoxide and hydroxyl groups make up the majority of residual groups on the GO basal lattice, whereas carbonyl and carboxylic groups are more prevalent on the margins. In contrast to pure plain graphene, the methods used to produce GO have a significant impact on its structure(Catania et al., 2021a).

There were differences between Graphene and Graphene oxide and the usage preference may vary according to the purpose. Graphene has high electrical conductivity and is easy to function, while graphene oxide is cheaper and has a higher water solubility, and is easier to work with in processes(Sahu et al., 2021b).

The production of graphene and its derivatives has given rise to a variety of synthetic techniques. The technique of exfoliating both graphite and graphite derivatives to make nano-sized graphene sheets is known as the top-down synthesizing strategy, also known as the destruction approach. This is the first of the terms, which are classified as The top-down and The bottom-up technique. Mechanical and liquid phase exfoliation, carbon nanotube unzipping, oxidative exfoliation reduction, and arc discharge, are a few top-down approaches (CNT). Little fragments of graphene were combined to form a different strategy known as the bottom-up methodology, which is also known as a building method. Some examples of the building technique are substrate-free gas-phase synthesis (SFGP), chemical vapor deposition (CVD), epitaxial growth, template approach, and organic synthesis. The bottom-up method has certain benefits over the top-down method since it can create graphene that is

almost flawless and has a large surface area. But different techniques can be used for the purpose(Catania et al., 2021b; Sahu et al., 2021b; Sastry et al., 2021; Sun & Du, 2019).

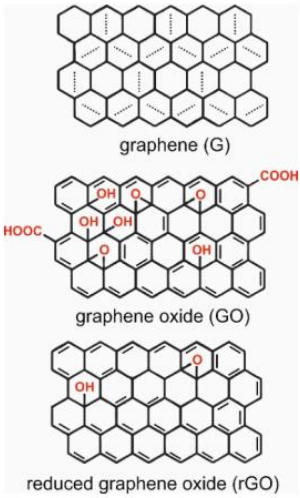

Figure 4 Scheme of graphene and graphene derivatives
References (Tadyszak et al., 2018)

1.2.5. Carbon Quantum dots

Carbon dots (CDs), often referred to as carbon quantum dots (CQDs), are brand-new zero-dimensional fluorescent nanostructures made of carbon. Because of their superior optical qualities, eco-friendliness, water solubility, low toxicity, biocompatibility, and straightforward synthesis methods, CQDs have garnered a great deal of interest from all over the world (Yadav et al., 2023). The arc-discharge method, microwave pyrolysis, hydrothermal process, and electrochemical synthesis are just a few of the several methods that may be used to easily and affordably create CQDs (X. Wang et al., 2019).

Because of their benign, numerous, and economical nature, carbon-based quantum dots with fascinating properties have slowly evolved as a novel nanocarbon component Carbon is a typical black substance that was previously thought to have little water solubility and weak fluorescence.

Because of their high solubility and powerful luminosity, carbon-based quantum dots have received a lot of interest, earning them the moniker "carbon .nano lights (Y. Wang & Hu, 2014).

2. Agricultural Applications of Carbon-Based Nanomaterials

According to FAO research (FAO, Rome, Italy, 2020), the world produced 9.2 billion tons of primary crops in 2018, which was around 50% more than it did in 2000. Yet, the usage of agrochemicals, such as pesticides and fertilizers, is crucial for agricultural output and is not sustainable. In 2018, 4.1 million tons of insecticides and 188 million tons of fertilizers were used, respectively. Overuse of fertilizers and pesticides results in soil deterioration and environmental contamination in addition to raising the cost of producing agricultural goods(Zhu et al., 2022).

Throughout plant growth, both abiotic and biotic loads are present. As a result, finding solutions to aid plants in responding to pressure is critical for innovative and reasonable farming, as well as reducing the heavy reliance on chemical medications. Nanotechnology is increasing, and nanomaterials have demonstrated promise in horticulture, particularly in improving crop sustenance, decreasing pests and illnesses, expanding pressure toughness, and assessing plant physiological status. The fundamental use of nanotechnology in farming integrates nano-compost and nano-pesticides to filter things and supplement levels to improve production without cleaning terrains and streams, as well as provide protection against a variety of bug troubles and microbiological diseases(Bartholina, 2022).

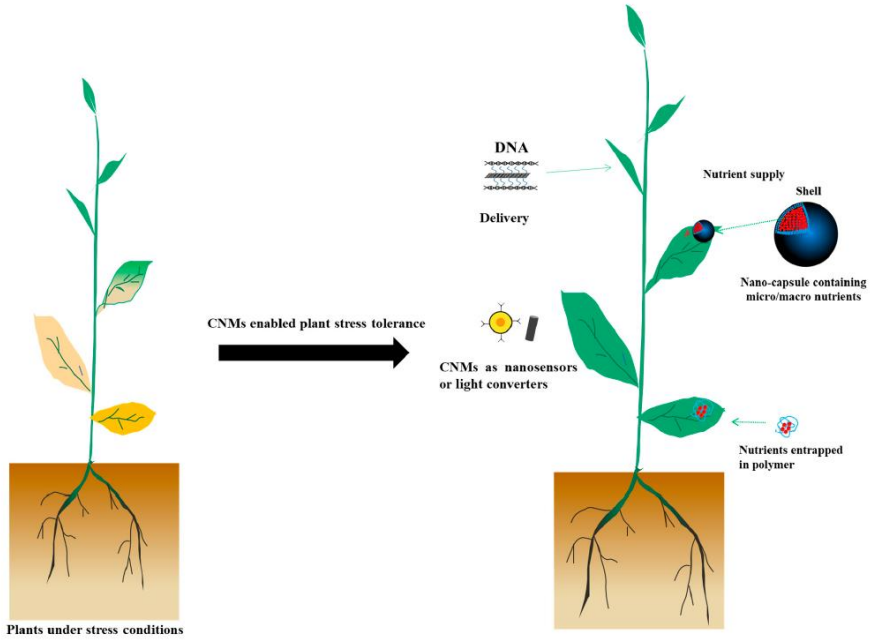

Figure 5 Function of carbon nanomaterials at plants
References (Zhu et al., 2022)

A variety of metallic polymer-based oxide nanoparticles and nanocomposites have shown good impacts on plant growth in agriculture. Nevertheless, various studies have found that the phytotoxicity of nanocomposites, metal nanoparticles, and oxides limits their use in agriculture. Last thirty decades, carbon-based nanomaterials have shown to be extremely useful in agriculture as nutrient transporters, pesticide sorbents, plant growth regulators, nanosensors for insect detection, and pest control agents(Aacharya & Chhipa, 2019).

At this part, some of application examples will be given about carbon-based nanostructures for plant growth.

Ratnikova et al. subjected tomato seedlings to ultrasonic irradiation after feeding them two types of 50mg/L $C_{60}(OH)_{20}$ (fuller) and multi-walled nanotubes (MWNTs). Seed treatments with US at 30 or 60 minutes in the

presence of MWNTs physically damaged the seed coat; nevertheless, the semipermeable layer's integrity was not compromised. The addition of MWNTs increased germination %, seedling length and weight, however C60(OH)20 had no effect. The combination of seed exposure to carbon nanomaterial and ultrasonic irradiation revealed information on the nanoparticle-seed interaction and may serve as a delivery strategy for boosting seed germination and early seedling development.(Ratnikova et al., 2015).

Kabiri et al. described the creation of a novel transporter platform based on graphene oxide (GO) sheets that can deliver huge loadings of plant micronutrients with a controlled gradual release. To demonstrate this notion, two micronutrients, zinc (Zn) and copper (Cu), were loaded onto GO sheets, and thus a GO-based micronutrient fertilizer was created. When GO-based fertilizers were employed in a pot experiment, wheat absorption of Zn and Cu was greater than when normal zinc or copper salts were utilized. This is an important study on the agricultural performance of a slow-release fertilizer based on GO(Kabiri et al., 2017).

Li et al investigated the photosynthetic effect of carbon quantum do fluorescence radiation in plants. The effect of carbon quantum dots on photosynthesis was then studied further on rice plants, resulting in a considerable boost in plant growth(Li et al., 2021).

Khodakovskaya et al investigated the impact of carbon nanotubes on plant growth and fruit number. Tomato plants cultivated in CNT-enriched soil generate twice as many blossoms and fruit as those produced in control soil(Khodakovskaya et al., 2013).

CONCLUSION

Increasing world population, environmental pollution, and climate change caused by developing industry have made the world's resources insufficient for living things. In recent years, the scientific world has focused its focus on sustainability to overcome this challenge. Nanoscience, which is one of the most important science fields of the last century, is advancing in line with the same goal to develop sustainable technologies in the fields of energy, environment, health, agriculture, food, etc. The sustainability approach is based

on high-efficiency production without harming the existing one. For this reason, the technology produced should not be harmful to the environment and health.

Agriculture is an area that needs to be addressed for the world as mentioned in the previous section. Decreases in irrigation, decrease in fertile soil by erosion, increase in fertilizer need, increase in the amount of spraying for crop yield, decrease in agricultural lands due to urbanization and decrease in crop yield due to these developments have had dire consequences in agricultural practices and have caused food supply to become dangerous. In this study, carbon-based nanomaterials developed by nanoscience for increasing productivity (number of products, product size, pesticide effect, etc.) in agricultural applications are mentioned. The relatively low toxicity of these materials and the fact that they do not leave hazardous waste if they deteriorate have made these materials preferable. In the production of properties, sustainability needs to apply the green chemistry approach more than other nanomaterials (such as metallic, polymeric).

The examples given in the last part of this study showed that when various carbon-based nanomaterials were used as fertilizer, capsul material, light converter for photosynthesis, drug, gene and fertilizer carrier, the total yields of the plants obtained were higher than the control groups.

The studies carried out in this area will provide more efficiency by using less resources in the following years and will eliminate the danger of a possible shortage.

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CHAPTER 15

TREE RESILIENCE IN URBAN AREAS: *CLIMATE CHANGE AND GENETIC CONSERVATION PERSPECTIVES*

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1. INTRODUCTION

As urban areas contend with the challenges posed by multiple stressors, resilience has emerged as a top priority for cities around the globe. Trees and forests play an important role in the urban ecosystem, providing numerous benefits to city residents and contributing to the resilience of the larger social-ecological system. These advantages include mitigating stormwater discharge, providing shade and cooling, and increasing human well-being (Huff et al., 2020). Due to fragmented landscapes, challenging site conditions, altered climatic conditions, and anthropogenic and ecological disturbance patterns, urban trees and their genetic background face different growing conditions (Savolainen et al., 2007; Escobedo et al., 2011; Scharenbroch et al., 2017). It is very clear that biodiversity, including genetic diversity, and urban green areas are located in at heart of nature based solution models for urban areas, as it is shown in the system thinking approach map of Gómez Martin et al. (2020) study (Figure 1).

The strength of genetic resilience in urban trees resides in its capacity to improve adaptability to the unique challenges of urban environments, such as pollution, limited space, and climate change. (Nowak et al., 2008) Genetic diversity within urban tree populations promotes resilience, allowing trees to better withstand stressors and flourish in urban settings. These challenges can be met by understanding the genetic diversity, local adaptation, and phenotypic plasticity of tree genotypes (Savolainen et al., 2007; Franks et al., 2014), maximizing population sizes, and selecting genetically diverse genotypes suitable for future environments (Sgrò et al., 2011), as well as expanding *ex-situ* conservation areas like arboreta, botanical gardens, and common gardens (Fady and Rihm, 2022; Kevin et al., 2020). This chapter focuses on the effects of climate change on urban trees, the genetic resilience of urban trees, and conservation strategies for all.

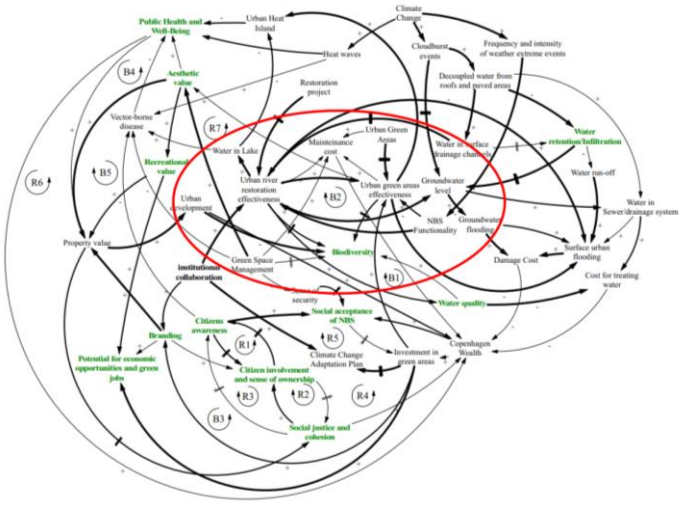

Figure 1: Map showing the importance of urban green with system-based approach by analyzing the Nature-based solution in urban areas.
Reference: Gómez Martin et al., 2020

2. What is resilience theory and genetic resilience in urban tree?

Resilience theory (Folke, 2006; Walker et al., 2004) covers the study and application of techniques to improve a system's ability to endure and recover from disruptions while retaining its fundamental structure, function, and identity (Huff et al.,2020). In urban environments frequently characterized by stressors such as pollution, a lack of available space, heat island effects, and climate change, the theory of resilience is used to create and maintain the resilience of trees.

Resilience theory also emphasizes the importance of genetically adaptive techniques, such as recording changes in environmental conditions, evaluating tree health, and modifying management practices as needed (Pickett et al., 2004). The capacity of tree populations to adapt to and resist urban challenges such as pollution, a lack of space, pests and diseases, and fluctuating climate conditions is known as genetic resilience in urban trees. The increased genetic diversity of urban tree populations facilitates their adaptation to stresses and

survival in urban environments. The study by Nowak et al. (2008) examined the contribution of urban trees and vegetation to the reduction of air pollution in the United States. This kind of research emphasized the importance of genetic diversity among tree species for their ability to effectively reduce air pollution in urban environments. By applying resilience theory to urban tree management, cities can create healthier, more genetically resilient urban forests that benefit urban communities in multiple ways, including the environment, society, and economy (Nowak et al., 2008; Gómez-Baggethun et al., 2013; Shanahan et al., 2016).

3. Adapting to a Changing Climate: Improving Tree Resilience Against Ecological Challenges

As a consequence of climate change, urban trees are subject to changes in temperature, precipitation patterns, and severe weather, which pose a number of challenges (Franks et al., 2014). In light of these climatic changes, it is crucial to increase tree resilience to ensure their survival and capacity to provide essential ecosystem services. Researchers emphasize the importance of selecting and promoting resilient tree species that can withstand changing climatic conditions in urban environments (Zhang and Brack, 2021). Due to adaptation traits such as heat, drought, and insect resistance, these species can survive and even flourish in harsh urban environments. By prioritizing the adoption of resilient tree species, cities can create urban forests that are better able to withstand the effects of climate change and continue to provide a variety of benefits to residents.

Green infrastructure and nature-based solutions (NBS) can also enhance the resilience of urban trees. NBS provides numerous benefits for the resilience of urban trees, such as the development of green spaces, urban forests, and tree corridors. By providing shade, reducing the effects of heat islands, regulating stormwater runoff, and enhancing biodiversity, they improve tree health and adaptability (Zhang and Brack, 2021; Janowiak et al., 2014). These nature-based strategies, when combined with proper management techniques, can generate resilient urban landscapes that support the long-term survival and

functionality of urban tree populations in the face of environmental changes caused by climate change.

For urban tree populations to be more resistant to climate change, species selection and conservation of genetic diversity are crucial. In their 2014 study, Janowiak et al. highlighted the importance of genetic diversity in nurturing the resilience of urban trees. Genetic diversity enables trees to adapt to changing environmental conditions by providing a wider spectrum of genetic characteristics and variations. It increases the resistance of urban trees to stressors such as parasites, increased precipitation, and heatwaves. By preserving genetic diversity and encouraging the use of genetically robust individuals in conserved areas, urban forests are better able to adapt to climate change and maintain ecological stability.

4. Unveiling the Power of Genetic Resilience: Improving the Adaptability of Urban Trees

To adapt to the unique and frequently harsh conditions of urban environments, urban trees must surmount numerous obstacles. However, genetic resilience holds immense promise for enhancing urban tree adaptation. Genetic resilience is the capacity of tree populations to thrive in urban environments in spite of disturbances by utilizing their genetic diversity and adaptive characteristics. Vanden Broeck et al. (2018) demonstrate the importance of genetic diversity in urban tree populations because it lays the groundwork for enhanced adaptability. Thanks to genetic diversity, which provides a greater variety of genetic features and variants, urban trees may be better able to withstand urban stressors such as pollution, space constraints, and shifting climatic conditions.

To completely comprehend the potential for genetic resilience, one must have a comprehensive understanding of the genetic makeup and characteristics of urban tree populations. Nowak et al. (2008) emphasize the significance of genetic factors in determining an urban tree's adaptability to its environment. Important for the adaptability of urban trees are characteristics such as disease

resistance, drought tolerance, and growth patterns, which are influenced by genetic diversity. By identifying and promoting genetically resilient individuals and species, urban tree managers can increase the resilience of urban tree populations and ensure their long-term survival and functionality against urban challenges.

It has been repeatedly demonstrated that genetic diversity, including the presence of rare alleles, is essential to the survival of a variety of biological populations due to the development of resistance following exposure to a variety of selective forces (Schaberg et al., 2008). Examples of how people are changing evolutionary trajectories include the rising resistance of microorganisms to drugs, insects to pesticides, herbaceous weeds to herbicides, and tree species to air pollution. (Palumbi, 2001; Schaberg et al., 2008). These examples also demonstrate that, when the three conditions for evolution by natural selection are met (i.e., when a species is genetically variable), evolution occurs. A trait can influence the survival or reproductive success of the species, and if there are disparities, then genetic variability provides the basis for adaptation and long-term resilience within a population.

When working to make urban trees more adaptable, it is essential to consider the significance of genetic diversity conservation. One way to identify genetic loss or gain is by using microsatellite markers in urban trees, as shown with *Tilia* species by Andrianjara et al. 2021 (Figure 2). Rare alleles detected by the markers facilitate population adaptation and survival in response to environmental change (Schaberg et al., 2008). Numerous studies have found a positive correlation between genetic diversity and various measures of fitness (Arcade et al., 1996; Aravanopoulos and Zsuffa, 1998; Mosseler et al.,2003). By preserving and increasing genetic diversity, urban tree populations have access to a broader range of genetic resources and adaptive abilities. This can be achieved through strategies such as preserving diverse urban tree populations, establishing seed banks, nurseries with breeding programs, botanical gardens, and arboretums. To create urban forests that can withstand the stresses of urbanization, climate change, and other stresses, it is essential to utilize the power of genetic resilience to increase the adaptability of urban trees.

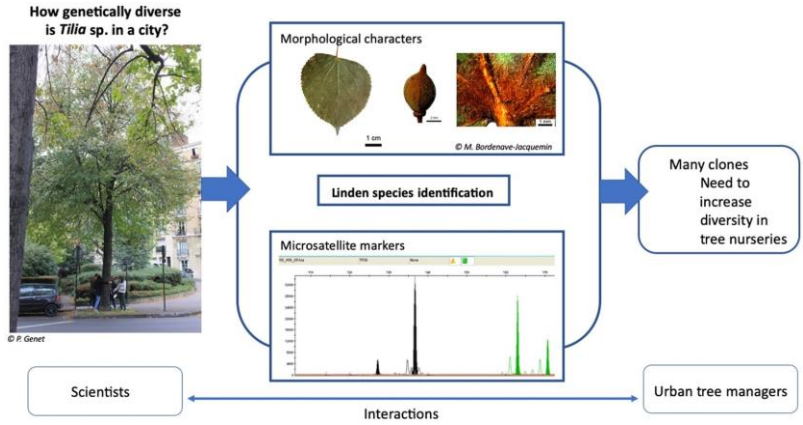

Figure 2: One case study showing the evaluation of the genetic diversity of *Tilia* sp. in an urban area.
Reference: Andrianjara et al., 2021

5. Preserving Urban Tree Heritage: The Importance of Genetic Conservation through Botanical Gardens, Arboretums, and Nurseries

To maintain biodiversity, adaptability, and resiliency in urban landscapes, it is necessary to preserve the genetic heritage of urban trees. The genetic preservation of urban tree populations relies heavily on arboretums, botanical gardens, and nurseries. These facilities serve as storage facilities for various tree species and provide a controlled environment for the propagation and preservation of genetic material. Fady and Rihm (2022) highlight the importance of *ex-situ* conservation for maintaining the genetic diversity of urban trees, such as those found in botanical gardens and arboretums. By serving as living gene banks, these conservation zones allow for the long-term preservation of tree genotypes and the potential future recovery of rare or endangered species.

There are numerous benefits to genetic conservation through botanical gardens, arboretums, and nurseries. These conservation platforms provide a protected and managed environment that permits the controlled cultivation and propagation of tree species. This allows for the preservation of genetic diversity and the possibility of future reintroductions and restoration initiatives (Fady

and Rihm, 2022). These also contribute to informed decision-making and sustainable urban greening practices through collaborations with scientists, horticulturists, and urban planners.

Moreover, the efforts of botanical gardens, arboretums, and nurseries to conserve genetic material contribute to the resilience and adaptability of urban trees. The material preserved in all is a valuable resource for selecting and propagating trees with desirable traits such as disease resistance, tolerance to urban stressors, and enhanced urban performance by maintaining genetically diverse collections (Kevin et al., 2020). These resources of knowledge about the genetic diversity and adaptability of urban tree species can inform urban greening initiatives and contribute to the development of resilient urban forests. Especially to increase the diversity of genera and species, it is suggested that local urban trees be mined and used in urban gardens rather than exotic ones (Sjöman et al., 2012). Some other recently published research demonstrates that tree nurseries play a crucial role in supplying trees with diverse genetics (Andrianjara et al., 2021). A Mediterranean fir plantation in France's common garden can be used to provide new genetic material under changing climate conditions (Figure 3). There are some other examples, such as the common garden of oak seedlings in the National Botanical Garden of Türkiye, which was established to understand the future potential of environmental adaptation by using genomic architecture (Figure 4). Or, the obtained results constitute a successful example of integrated research for *Platanus orientalis* germplasm conservation in Central Italian historical gardens (Ciaffi et al., 2018). Besides, on those days, rather than monoculture plantations, mixed plantations in urban nursery areas are suggested as being more resilient and able to adapt to these changes in social and environmental conditions (Urgoiti Otazua and Paquette, 2018).

Figure 3. A partial view of a common garden in Saint Lambert, France, that tests different provenances of *Abies cephalonica* Loud., a rarely used exotic.
Reference. Fady and Rihm, 20221

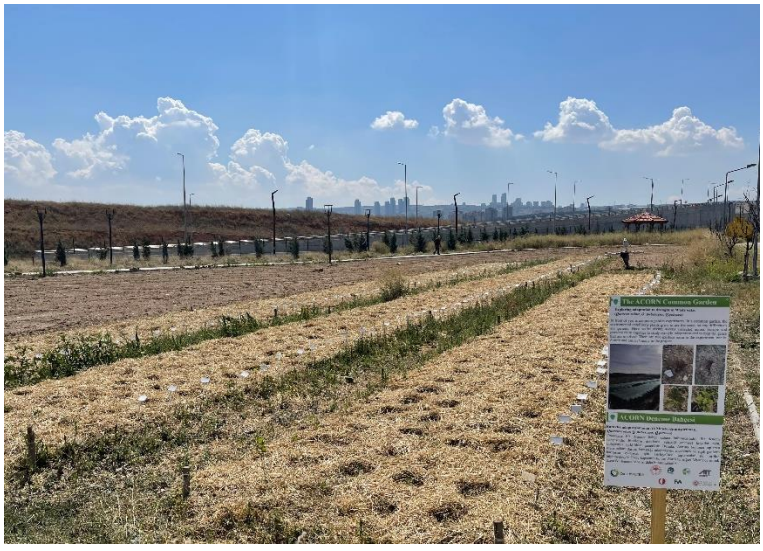

Figure 4. The newly established Acorn common garden to understand the effects of climate change on *Quercus* sp. in the National Botanical Garden of Türkiye, Ankara, Türkiye.
Reference. BiodivClim-931, Identifying seed sources for highly adaptable oak forests in a changing climate, with the acronym 'ACORN' (Ongoing Project of Biodiversa Network).

In conclusion, preserving urban tree heritage through genetic conservation in botanical gardens, arboretums, common gardens, and nurseries is essential for sustaining biodiversity, promoting adaptation to climate change, and enhancing tree resilience. These conservation areas serve as important repositories of genetic diversity, also contribute to research and education, and play an essential role in promoting sustainable urban greening practices. Genetic diversity research in urban conserved areas that prioritizes resilience can contribute to the development of more resilient and adaptable ecosystems for urban green in the face of climate change.

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CHAPTER 16

SIDERITIS

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Introduction

Medical plants are a significant source of natural remedies that have been used for the treatment of diseases by people since ancient times. These plants contain bioactive substances that exhibit biological activity. In medical treatment, either the whole plant or certain parts of it are used. In the past, various parts of plants were utilized, such as the whole plant (herba), root (radix), rhizome (underground stem), tuber (swollen underground stem), bulb (onion), bark (cortex), wood (linum), leaf (folia), flower (flos), fruit (fructus), and seed (semen). With the advancement of chemistry, the active constituents present in plants began to be used in medical treatments. Over time, these active compounds have served as models for the development of synthetic drugs.

Medicinal and aromatic plants are used in both traditional and modern medicine for the prevention of diseases, maintenance of health, and treatment of illnesses. These plants serve as biological, cultural, and industrial resources, finding applications in various fields. The demand for these resources has significantly increased in recent years and continues to grow (BAKA, 2012).

Currently, around 900 medicinal and aromatic plant species are commercially cultivated worldwide (Arslan et al., 2015). When examining the global production figures and major producer countries for certain medicinal and aromatic plants, as reported by the Food and Agriculture Organization (FAO), it can be observed that countries rich in plant species such as China and India lead in production. In our country, it is estimated that at least 1,000 species are utilized in various forms, with around 400 of them being traded (Arslan, 2014). Parallel to the consumption of medicinal and aromatic plants in various sectors and industries, the global trade volume of these plants is increasing every day. The growth in trade volume and the increasing demand have led to intensified efforts to enhance the production capabilities of these plants. Likewise, there has been an increase in the collection rates from natural sources. Generally, medicinal and aromatic plants are predominantly traded in dried form, but there is also some trade in essential oils and, to a lesser extent, in fresh form. Globally, approximately half a million tons of dried medicinal and aromatic plants are traded internationally each year, and there is also a

significant amount of trade in these plants on local and national markets, the exact quantities of which are not fully known (Acıbuca et al., 2018).

Medicinal and aromatic plants, which provide raw materials and inputs in various sectors and hold an important position in global trade, have shown significant growth in import and export figures, attracting attention. Based on statistical data obtained from the International Trade Center (ITC) and the Turkish Statistical Institute (TÜİK), the figures reflecting trade related to medicinal and aromatic plants have also shown significant increases. The export value of medicinal and aromatic plants and their derived products worldwide increased from $48.7 billion in 2001 to $207.5 billion in 2019. The import value followed a similar upward trend, increasing from $48.9 billion in 2001 to approximately four times that amount, reaching $205.9 billion in 2019. The increasing trend observed globally is also seen in Turkey's foreign trade. The export value of medicinal and aromatic plants and their products in Turkey increased from $143.6 million in 2001 to $1.02 billion in 2019, while the import value rose from $282.7 million in 2001 to $1.36 billion in 2019 (Boztaş et al., 2021).

There are multiple aspects to consider when evaluating medicinal and aromatic plants, encompassing a wide range of plants, active compounds, and areas of consumption. In this regard, although there is no standardized classification system in place today, they can generally be categorized based on their families, active constituents, modes of consumption and usage, utilized plant parts, and pharmacological effects (Faydaoğlu and Sürücüoğlu, 2011).

Thanks to its geographical location, rich plant diversity, climate, and ecological structure, Turkey possesses a diverse flora of medicinal plants. Lamiaceae is one of the families that contribute to this flora. The members of this family are plants of great importance in terms of traditional medicine, spices, and food, dating back to ancient times. They hold significant medicinal and economic value.

Lamiaceae Family

From ancient times to the present day, species of the Lamiaceae family have been used by the public for medicinal and culinary purposes. Out of the

plants mentioned in Dioscorides' work "Materia Medica", around 40 of them belong to the Lamiaceae family (Baytop, 2000).

The Lamiaceae family is a cosmopolitan family that includes herbaceous, shrubby, or tree-like plants, and it holds significant economic importance in many parts of the world (Suddee, 2001). The Lamiaceae family is represented by approximately 230 genera and around 7,100 species worldwide (Stagos et al., 2012). Despite being most abundant in Turkey and the Mediterranean basin in terms of natural distribution, members of the Lamiaceae family can be found in almost all habitat types and elevations, with very few regions in the world where they are absent. They are distributed from the Arctic regions to the Himalayas, from Southeast Asia to Hawaii, as well as in Australia, throughout Africa, and along the northern and southern regions of the Americas (Watson and Dallwitz, 1978; Davis et al., 1982; Heywood, 1996; Kaya, 1997).

In Turkey, the Lamiaceae family is represented by 46 genera, 577 species, and a total of 755 taxa (including 246 infraspecific taxa and 23 hybrids). New species are periodically added to the flora (Özhatay and Kültür, 2006; Dinç and Doğan, 2006; Dirmenci, 2005; Hamzaoğlu et al., 2005).

The Lamiaceae family, which is rich in endemic plants, has a high yield of essential oils. Some of well-known and important genera within this family are *Thymbra, Thymus, Origanum, Satureja, Mentha, Teucrium, Ballota, Stachys, Salvia, Ajuga, Prunella, Melissa, Lamium, Marrubium*, and *Sideritis*. This family is widespread in the mountainous areas of Turkey's Mediterranean region, and the endemism rate of the family is reported to be 42.2%. The genera with the highest number of species in this family in Turkey are *Salvia* L., followed by *Stachys* L. and *Sideritis* L. (Kocabaş and Karaman, 2001; Özkan, 2007).

Sideritis

Sideritis, in Greek literature, is translated as "iron" (sideros). The plant was known to the ancient Greeks, particularly Dioscorides and Theophrastus. Dioscorides described three species belonging to this genus. In ancient times, *Sideritis* was a general reference to plants that could heal wounds caused by

iron weapons during battles. However, some believe that the name of the plant originated from the shape of its leaves, which resemble the tip of a spear. In Turkey, *Sideritis* species, widely used as folk medicine and herbal tea, have various regional names such as "dağ çayı," "yayla çayı," "sarıkız çayı," "kuyruk çayı," and "adaçayı" (Ayaz, 2008).

Sideritis species belonging to the Lamiaceae family in our country are plants that are distributed in subtropical and temperate climatic regions. The genus *Sideritis* is widespread in the Mediterranean basin, particularly in a large area ranging from Turkey to Spain, Morocco, Greece, Syria, and Italy, represented by over 150 species and divided into two subgenera. These are subgenus *Sideritis* and subgenus *Marrubiastrum* (Moench) Mendoza-Heuer. The Marrubiastrum subgenus, endemic to Macaronesia, is divided into three sections [Marrubiastrum (Moench) Bentham, Empedocleopsis Huynh, Creticae P. Perez & L. Negrin], and the entire subgenus was revised by Perez De Paz and Negrin Sosa in 1992, with a total of 24 species in these three sections. The *Sideritis* subgenus, widespread in the Mediterranean, has four sections: two with perennial plants [*Sideritis, Empedoclia* (Rafin) Bentham] and two with annual plants [*Hesiodia* (Moench) Bentham, *Burgsdorfia* (Moench) Briquet] (Duman et al., 2005; Zeki A. et al., 2000).

In Turkey, the genus *Sideritis* is represented by three sections: Hesiodia (Moench) Bentham, Burgsdorfia (Moench) Briquet, and Empedoclia (Rafin) Bentham. There are 44 Sideritis species in Turkey, and when including subspecies, this number reaches 53 (Davis, 1982; Davis, 1988; Duman, 2000). Out of these species, 38 (71.7%) are endemic to Turkey (Table 1).

In Turkey, the annual *Sideritis* species are found within the sections Hesiodia (Moench) Benth. and Burgsdorfia (Moench) Briq., while all perennial species are classified under the section Empedoclia (Rafin) Benth. (Duman et al., 2005).

Due to the high rate of endemism within the *Sideritis* genus, Turkey is one of the two main genetic centers for this genus. The other genetic center for the *Sideritis* L. genus is the Iberian Peninsula region in southwestern Europe, where approximately 50 species belonging to the *Sideritis* section are found (Kırımer et al., 2001).

The genus *Sideritis*, a member of the Lamiaceae family, is highly notable in the Flora of Turkey due to its remarkable endemism rate of 78.2% (Davis, 1988; Başer, 1998).

Morphological features

Sideritis species are herbaceous or small shrubs with erect and ascending stems. They have four-angled stems that are rarely glabrous and usually covered in pilose or tomentose hairs. They lack glandular structures or glandular hairs. The leaves are simple, either petiolate or sessile, and can be either entire or crenate-dentate along the margins. Vertasillastrum inflorescences are (4-) 6 (-10)-flowered and can be sparsely or densely arranged. Bracteoles are absent. Bracts resemble leaves, broad and hiding the base of the calyx tube. The calyx is tubular to bell-shaped, sometimes bilabiate, with 5-10 veins and 5 spiny teeth. The teeth are equal or the upper ones are wider than the lower four. The corolla is usually yellow, occasionally white or red. The corolla tube is enclosed within the calyx, bilabiate, with the upper lip nearly erect and either entire or two-lobed (trifid), while the middle lobe is wider and deeper. The stamens are didynamous, with the lower stamens longer than the upper ones. The anthers are 2-loculed and often distorted in shape. The style is cylindrical, gynobasic, bifid, with the upper lobe blunt at the tip and the lower lobe broad and yellowing the upper lobe. The ovary is superior, 4-loculed, with ovate nucules and a blunt, rounded apex, and it is often glabrous (Davis, 1982).

Table 1. General information about Turkey's endemic *Sideritis* species

Plants name	Annual/ Perennial	Flowering period	Habitat	Altitude	Distribution in Turkey
Sideritis romana* subsp. *romana	Annual	5-6	Cultivated fields,road sides, wastelands	0-0	Northwest. Turkey
Sideritis hololeuca	Perennial	6-8	Dry hill slopes, chalky rubble	900-1300	South Anatolia
Sideritis phlomoides	Perennial	7-9	Mountain slopes	1800-2550	South Anatolia (Anti-Taurus mountain)
Sideritis erythrantha* var. *erythrantha	Perennial	7-8	Rugged rocky slopes, Pinus nigra	1220-1525	Southwest Anatolia

			and Cedrus forests		
Sideritis erythrantha* var. *cedretorum	Perennial	7-8	Limestone slopes, Pinus nigra and cedar forests	1220-1525	South Anatolia
Sideritis brevidens	Perennial	6-8	Chalky rocks, mushroom shrubbery	950-950	South Anatolia
Sideritis stricta	Perennial	5-8	Coastal cliffs, cork oak forests	0-915	Southwest Anatolia
Sideritis vulcanica	Perennial	7-8	Volcanic peaks, rocky slopes	1100-2200	West Anatolia
Sideritis condensata	Perennial	5-7	Pine forests, firigana,road edges	0-1600	South Anatolia
Sideritis cilicica	Perennial	6-7	Open Pinus brutia forests, limestone slopes, maquis	600-950	South Anatolia
Sideritis niveotomentosa	Perennial	6-6	Open Pinus brutia foresti Quercus shrubland	960-970	South Anatolia
Sideritis arguta	Perennial	6-8	Open pine woodlands, mushroom shrublands, vineyards	830-1100	South Anatolia
Sideritis lycia	Perennial	5-8	Rocky,Pinus brutia forests, and maquis	0-80	Southwest Anatolia
Sideritis leptoclada	Perennial	6-7	Pinus brutia forests, serpentine rocks, conglomerate ledges, and scree.	0-800	Southwest Anatolia
Sideritis brevibracteata	Perennial	5-8	Rocky limestone slopes	30-80	South Anatolia
Sideritis albiflora	Perennial	5-7	Conglomerate scree and limestone cliffs	0-800	South Anatolia

Sideritis rubriflora	Perennial	4-6	Rocky limestone slopes, maquis vegetation, fallow fields	0-470	South Anatolia
Sideritis argyrea	Perennial	7-8	Rocky slopes with Pinus nigra and Pinus brutia	1000-1000	South Anatolia
Sideritis bilgerana	Perennial	6-8	Chalky slopes with Pinus nigra forests, shrub of mushrooms, and steppe vegetation	650-1400	South Anatolia
Sideritis hispida	Perennial	7-9	Limestone slopes, steppe vegetation	1400-1350	South Anatolia
Sideritis dichotoma	Perennial	7-8	Rockylimestone, open cedar forests	900-2500	Northwest Anatolia and northern Anatolia
Sideritis trojana	Perennial	7-8	Rocky mountain slopes	1500-1770	Northwest Anatolia
Sideritis phrygia	Perennial	6-7	Rocky slopes	1100-1500	Central Anatolia
Sideritis amasiaca	Perennial	7-7	Dry limestone cliffs, vineyards	300-600	Northern and central Anatolia
Sideritis galatica	Perennial	7-9	Pinus nigra forests, arid slopes	1000-1400	Central Anatolia
Sideritis gulendamiae	Perennial	7-8	Gypsum and marl steppes	880-950	Central Anatolia
Sideritis armeniaca	Perennial	7-9	Rocky slopes,step pe	1600-1600	Northeast Anatolia
Sideritis germanicopolitana* subsp. *germanicopolitana	Perennial	6-8	Pine forests, Rocky limestone slopes	100-2000	Northern and central Anatolia
Sideritis germanicopolitana* subsp. *viridis	Perennial	6-8	Pine forests, Rocky limestone slopes	400-2000	Northeast Anatolia
Sideritis caesarea	Perennial	7-9	Calcareous step	1500-2400	Central Anatolia

Sideritis huber-morathii	Perennial	7-7	Calcareous step	1235-1235	South Anatolia
Sideritis vuralii	Perennial	7-9	calcareous rocky places	1200-1900	South Anatolia
Sideritis libanotica* subsp. *linearis	Perennial	5-9	Limestone rocks and grouse, dry slopes, steppe	1100-2800	Western and southern Anatolia
Sideritis libanotica* subsp. *viol ascens	Perennial	9-9	Rocky slopes	1500-2440	South Anatolia
Sideritis serratifolia	Perennial	7-7	Pinus nigra forests	1280-1850	South Anatolia
Sideritis pisidica	Perennial	7-8	Dry rocky slopes, mushroom bush	0-2100	Southwest Anatolia

(Anonymous: TÜBİVES)

General distribution of endemic *Sideritis* Species

Figure 1.

Sideritis romana* subsp. *romana

Sideritis hololeuca

Sideritis phlomoides

Sideritis erythrantha* var. *erythrantha

Sideritis erythrantha* var. *cedretorum

Figure 2.

- ***Sideritis brevidens***
- ***Sideritis strica***
- ***Sideritis vulcanica***
- ***Sideritis condensata***
- ***Sideritis cilicica***

Figure 3.

- ***Sideritis niveotomentosa***
- ***Sideritis arguta***
- ***Sideritis lycia***
- ***Sideritis leptoclada***
- ***Sideritis brevibracteata***

Figure 4.

- ***Sideritis albiflora***
- ***Sideritis rubriflora***

Sideritis argyrea
Sideritis bilgerana
Sideritis hispida

Figure 5.

Sideritis dichotoma
Sideritis trojana
Sideritis phrygia
Sideritis amasiaca
Sideritis galatica

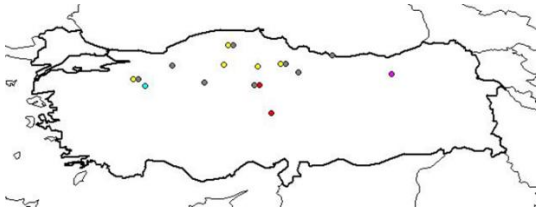

Figure 6.

Sideritis gulendamiae
Sideritis armeniaca
Sideritis germanicopolitana **subsp.** ***germanicopolitana***
Sideritis germanicopolitana **subsp.** ***viridis***
Sideritis caesarea

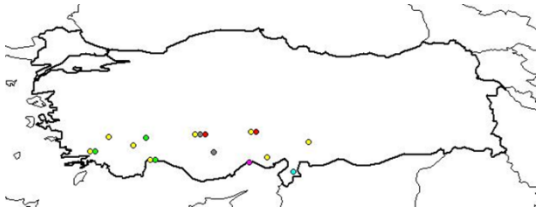

Figure 7.

- ***Sideritis huber-morathii***
- ***Sideritis vuralii***
- ***Sideritis libanotica* subsp. *linearis***
- ***Sideritis libanotica* subsp. *violascens***
- ***Sideritis serratifolia***
- ***Sideritis pisidica***

Traditional use

Sideritis is known by many different names in Anatolia, such as "dağ çayı" (mountain tea), "yayla çayı" (plateau tea), "sarıkız çayı" (scarlet tea), "kuyruk çayı" (tail tea), and "adaçayı" (sage tea). In Turkey, *Sideritis* speciesare widely consumed as herbal tea, especially in the Mediterranean and Aegean regious. Additionally, they are also commonly used for culinary and medicinal puposes. In Turkish folk medicine, certain *Sideritis* species are believed to have appetite-stimulating, anti-inflammatory, tonic, carminative, muscle-relaxing, diuretic, digestion-facilitating, pain-relieving, and cold-alleviating properties (Aytaç and Aksoy, 2000; Kırımer et al.,2000).

Plants constitute the raw materials for natural remedies used in traditional and modern treatment methods since ancient times. The traditional use of mountain tea, recognized by the public for its healing properties, involves sourcing the plant through wild harvesting from nature. In the case of *Sideritis* species, the flowering branches of the plant are commonly collected and consumed as herbal tea.

Mountain tea, in addition to being collected and consumed by the local community, is also widely sold in both domestic and international markets in various forms. Some companies collect and dry the plant, which is then sold

directly or processed into tea bags made from ground dried herbs (Dinçer et al., 2008).

Sideritis species contain pleasant-smelling oils and widely used aromatic compounds, making them useful in the treatment of colds. Additionally, these plants' pleasant aromatic scents, essential oils, and extracts from aromatic plants have strong antioxidant activity in the lipid sublayer (Akaya et al., 2002; Yeşilada et al., 1989).

Plant-derived bioactive compounds, known for their beneficial and versatile effects, are increasingly used in the prevention and treatment of various metabolic diseases. In the case of Sideritis species, research and reports have shown a growing interest due to their di- and triterpenes, which contribute to their anti-inflammatory, antiviral, hepatotoxic, cytotoxic/antitumor activities, as well as their flavonoids, which possess antioxidant, antimicrobial, cytotoxic, and other activities (Yordanova et al., 2000; Ezer et al., 1991).

Recent studies have shown that extracts from certain *Sideritis* species possess antifeedant (substances that inhibit normal feeding behavior, appetite suppressants) properties (Bondi et al., 2000), antistress effects (Öztürk et al., 1996), analgesic properties (Aydın et al., 1996), antioxidant activity (Tunalier et al., 2002), antibacterial effects (Ezer et al., 1994; Ezer and Abbasoğlu, 1996), and anti-inflammatory activity (Yeşilada and Ezer, 1989).

The investigation of the biological activities of *S. congesta* and *S. arguta* has revealed significant cytotoxic activity against ovarian and colon cancer, as well as potential antifeedant activity (Ertaş, 2005). *S. trojana* and *S. athoa* extracts have shown antimutagenic activity (Ballı, 2012), and diterpenic compounds isolated from *S. athoa* have demonstrated antimicrobial activity against Bacillus subtilus (Gören, 1997). High phenolic compounds found in the methanol extract of *S. libanotica* subsp. linearis have been found to possess antioxidant activity (Şahin, 2010). *S. ozturkii* and *S. caesarea* extracts have been suggested for use as natural antimicrobial and antioxidant agents in food preservation and human health (Sağdıç et al., 2007).

The most commonly used form of *Sideritis* species: Herbal tea

Medicinal and aromatic plants have been the most accessible and practical source of healing for humanity for centuries. Throughout human history, numerous plants have been used in forms such as poultices and teas, which provide easy application and quick effectiveness.

Infusion, especially in the preparation of herbal teas, is a simple extraction method. When fresh or dried plant material is steeped in boiled water for a certain period, some of the bioactive compounds are transferred to the water. If the material is simmered in water instead of steeping, it is called a decoction. Decoction is more suitable for extracting bioactive compounds from tough materials such as fruits, roots, and barks, which are stable at high temperatures.

Plant infusions are appreciated for their natural and refreshing flavors, as well as their other sensory properties (Koch et al., 2012). Due to the naturally occurring antioxidant compounds they contain, they are also recognized as a healthy choice (Monbaliu et al., 2010).

The quality of herbal teas is important for their potential benefits. The phytochemicals in plants can vary significantly depending on various factors. Failure to provide the expected benefits or potential harm to health can occur due to these variations. We can classify the factors that directly or indirectly affect the components of plants as follows:

ı. Geographical factors: climate, rainfall, humidity, and the number of sunny days can cause variations in the components of plants.

ıı. Agricultural factors: soil structure, irrigation, fertilization, and the timing of plant harvesting significantly influence the bioactive compounds of plants.

ııı. Environmental factors: residues of agricultural pesticides, contamination risks in irrigation water, and the presence of heavy metals and radiation residues due to proximity to main roads and industrial facilities can contaminate plants.

ıv. Processing method: it is important to use the most suitable drying method for plants and to package them in a way that preserves their appropriate components.

v. Storage conditions: how plants are processed before packaging is as important as the conditions in which they are stored. The humidity and temperature of the storage environment, as well as the duration of storage, are crucial factors in the effectiveness of the plant.

It is important to adhere to good manufacturing practices and hygienic production practices during the collection, drying, storage, sales, and packaging stages of herbal teas due to these reasons.

The most preferred form of consumption for *Sideritis* species since ancient times is in the form of herbal tea. It is enjoyed and consumed due to its aromatic taste provided by the presence of monoterpenes in its chemical composition.

Conservation and Cultivation

Turkey has a wide range of plant species that naturally grow due to its climatic conditions, holding an important place in the global economy. Approximately 500 plant species are relevant to medicinal and aromatic plants, and they are mainly produced in Turkey through wild collection. Looking at the global perspective, the industrial and complementary medicinal use of medicinal and aromatic plants has become widespread.

The production of medicinal and aromatic plants can be carried out through wild collection or cultivation as a crop. Wild plants, due to their unfavorable and competitive growing conditions, tend to maintain their phytochemical synthesis in an active state. As a result, wild-collected plants generally have higher levels of bioactive compounds and greater diversity compared to those grown under cultivation conditions. However, there are several advantages to cultivating these plants. While the content and quality are essential in medicinal and aromatic plants, sustainability and standardization are also indispensable industrial requirements for large-scale utilization.

The widespread use of *Sideritis* genus as herbal tea for various ailments among the public has led to numerous pharmacological research studies on this plant, revealing many important properties. The cultivation of *Sideritis* and the implementation of good agricultural practices have become crucial for its industrial utilization and ensuring the sustainability of its raw materials. These cultivation efforts and practices not only enable the production of high-quality and high-yielding crops on a unit area but also contribute to the production of standardized products.

When the natural and cultivated forms of the endemic species *Sideritis stricta* Boiss. & Heldr. in Turkey are compared, no significant anatomical differences have been observed. However, it has been observed that wild-collected plants have higher essential oil yield and higher levels of certain nutritional elements (potassium, calcium, manganese, and boron) compared to cultivated ones. The similarity in the proportion of the main component of the essential oil suggests that under appropriate conditions, similar quality products can be obtained from cultivated plants as those collected from the wild (Dülgeroğlu, C., 2013).

The mountain tea species, which are widely used by the public, are facing the threat of extinction. Therefore, it is necessary to identify, protect, and carry out cultivation studies for economically important *Sideritis* species.

There is a limited amount of research on the propagation and cultivation of Sideritis species. While pharmacological research on *Sideritis* species is increasing in Turkey, there is a lack of agronomic studies.

The climate, rainfall, humidity, soil structure, and harvesting time of their respective regions affect the chemical compositions of *Sideritis* plants. Even within the same species, there will inevitably be quantitative differences in the chemical profiles of plant samples collected from different regions or at different times within the same region, although qualitative differences may not be significant.

Mountain tea is a typical Mediterranean plant that thrives in hot and sunny, wind-sheltered, and sloping terrains. It is highly tolerant to heat and drought, but sensitive to extreme cold and frost. Mountain tea particularly

thrives in calcareous, alkaline soils that are well-drained, sandy-loamy, or loamy-sandy in texture. It grows well and exhibits good development in such soil conditions.

Mountain tea can be propagated both generatively and vegetatively. It can be propagated by directly sowing the seeds in the field, producing seedlings from seeds sown in trays, or by taking cuttings.

One of the measures taken to prevent the risk of extinction caused by the collection of medicinal plants from the wild is the propagation through stem cuttings. This method has been investigated for *Sideritis trojana* Bornm species. It is believed that determining the optimal rooting method and cultivating the plant in its native region can prevent its extinction. Three different rooting media (sand, coconut coir, perlite) and four different doses of IBA hormone (0-1000-2000-4000 ppm) were studied for the propagation of *Sideritis trojana* through stem cuttings. The results showed that the highest rooting rate (57%) was achieved in the sand medium with 1000 ppm IBA application (Türkmen, 2019).

In the cultivation of mountain tea, weed control and irrigation in the early years are crucial for yield and quality. Mountain tea plants begin to emerge from the soil surface approximately 2-3 weeks after sowing under suitable soil and air temperature conditions. When the plant reaches a height of 4-5 cm, thinning should be performed to prevent yield loss due to shading and overcrowding. Hoeing and weed control ensure soil aeration and moisture retention. Weed control should continue until mountain tea seedlings reach a level where other weeds cannot grow. Various cultural measures should be taken for this purpose. Manual weeding is important in weed control. Additionally, mechanical methods can be used for weed management. Planting norms are important considerations for cultural weed control, yield, and harvest. In this regard, the effects of plant density on yield were studied for *Sideritis perfoliata* L. species. Seedlings were obtained from seeds collected from the wild in Izmir. Two different inter-row distances (45 cm and 70 cm) and three different intra-row distances (10 cm, 20 cm, and 30 cm) were applied. The planting density significantly influenced the total green herbage yield. As a result of the study, a row spacing of 70 cm was recommended for mountain

tea cultivation due to its suitability for mechanization (Sarı et al., 2005). Mountain tea is highly resistant to drought. It is recommended to irrigate the plant until it establishes in the soil during the vegetation period. Irrigation can be done through furrow, flood, drip, or sprinkler methods. The disadvantages of sprinkler, flood, and furrow irrigation in mountain tea cultivation include the ease of disease spread, the formation of magnifying lens effect on wet leaf surfaces causing sunburn, and a decrease in herb quality. Drip irrigation, on the other hand, reduces the occurrence of diseases as water is directly supplied to the soil rather than the plant leaves, minimizes evaporation losses compared to other methods, and ensures uniform water distribution. Irrigation applied during spring and summer seasons, especially in hot and dry periods, increases the yield of herbal material. In general, during cultivation, soil analysis is conducted and fertilization programs can be established based on the nutrient requirements or to enhance yield. Potassium sulfate fertilizer can be applied to increase resistance to winter cold. Phosphorus fertilizer can be applied to promote flowering and enhance aroma intensity. Among the important macro-nutrients, nitrogen is essential for plant growth. Endemic species such as *Sideritis stricta* Boiss. & Heldr. and *Sideritis congesta* Davis & Huber-Morath have been cultivated under Konya's ecological conditions. Studies have been conducted on certain yield and quality characteristics based on three different fertilizer doses (0, 5, 10 kg/ha) and drying methods. The results indicate that cultivating *Sideritis* species (*Sideritis stricta* and *Sideritis congesta*) for flower yield of herbal material is suitable under ecological conditions similar to Konya, particularly with the application of 10 kg/ha nitrogen fertilizer (Bilginoğlu, 2015).

The volatile oil content in mountain tea is known to be influenced by factors such as soil structure, texture, climate, irrigation, planting density, and harvest time, along with fertilization. In a study conducted on cultivated mountain tea species, namely endemic *Sideritis libanotica* Labill. ssp. *linears* (Bentham) Borm. and *Sideritis bilgerana* P.H. Davis, their volatile oil yields were determined as 0.20% and 0.15%, respectively. The highest volatile oil components were reported as 25.92% hexadecanoic acid and 21.49% δ-cadinene for *Sideritis libanotica*, and 19.82% β-pinene and 14.60% α-pinene for Sideritis bilgerana (Kan et al., 2018).

To achieve high yield and quality in the cultivation of medicinal and aromatic plants, it is important to cultivate them in suitable ecological conditions and determine appropriate maintenance practices and harvest timing. As a perennial plant, mountain tea can be utilized from the same plantation for three to four years.

In mountain tea, a perennial plant that can be harvested multiple times during the vegetation period, the timing of harvest, drying methods, and cutting height have an impact on yield and quality. The optimal harvest time for mountain tea is during full flowering. The cutting height is done above the soil level, typically around 10-15 cm, and the flowering branches are harvested (Bilginoğlu, 2015). Medicinal and aromatic plants can contain high levels of moisture after harvest. Drying, which refers to the process of reducing the moisture content in the plant to the final desired level with minimal energy and without causing any deterioration in plant quality, ensures safe storage (Polatcı and Tarhan, 2009).

In order to obtain high-quality herbal drugs, medicinal and aromatic plants should be dried using appropriate techniques. Drying of medicinal and aromatic plants can be performed in two ways: natural drying and artificial drying. Natural drying is carried out in shade on drying racks placed on a clean surface. In Turkey, due to favorable climate conditions, drying is commonly done in shade and under sunlight. The harvested product should not be left directly under the sun for a long time to preserve its unique color and aroma. It is important to have proper air circulation in the drying area. Artificial drying, on the other hand, involves sending hot and dry air to the product in drying chambers or ovens to facilitate drying. To avoid any loss in product quality, the temperature should not exceed 40˚C. For mountain tea, the preferred drying method to preserve the original color and aroma of the product is shade drying. Studies conducted on *Sideritis congesta* and *Sideritis stricta* have determined that shade drying is the most suitable drying method, with volatile oil yields of 0.3 ml/da for *S. congesta* and 0.1 ml/da for *S. stricta* (Bilginoğlu, 2015).

Medicinal plants are the richest biological source for traditional medicine systems, modern drugs, folk remedies, pharmaceutical intermediates, nutraceuticals, dietary supplements, and synthetic drugs, owing to their

chemical compositions. Aromatic plants, on the other hand, serve as a source of fragrance and aroma in aromatherapies, cosmetics, health beverages, and more. In developed countries, medicinal and aromatic plants are often traded in bulk from many developing countries to generate higher added value and become subjects of global trade. Various technologies and methods are employed in industrial processes to harness the value of medicinal and aromatic plant resources, leading to the production of herbal preparations.

Polyphenolic compounds, due to their significant biological activities, need to be identified in order to determine their presence in plants. In previous studies on *Sideritis* species, various chemical components such as volatile oil constituents, terpenoids, flavonoids, and phenolic compounds have been identified.

Numerous studies have been conducted on the chemical components of *Sideritis*. These studies generally focus on the oil constituents, flavonoids, and diterpenes of the *Sideritis* taxon. In addition to these chemical compounds, the *Sideritis* genus also contains sterols, phenylpropanoid glycosides, lignans, iridoid glycosides, coumarins, alkanes, triterpenoid saponins, and essential fatty acids (Akcoş, 1994).

Conclusion

When it comes to quality in medicinal and aromatic plants, there are certain aspects that need to be considered. These include the botanical name of the plant, country or region of origin, harvesting time, sensory tests, foreign matter tests, macroscopic tests, as well as chemical and chromatographic analyses. Additionally, tests for rodent and insect infestation, moisture content, ash content, pesticides, radioactivity, and microbial contamination should be conducted. The microbiological quality of plants is influenced by the region where they are grown/cultivated, drying conditions, post-processing environmental, climatic, and anthropogenic factors, as well as transportation, storage, and packaging conditions. Detecting microscopic contamination in plants is important to determine the microbial load caused by pathogenic organisms, bacteria, mycotoxins, and microbial toxins present in the plant material. Therefore, the microbial content in herbal products with various

applications can be considered as an indicator of good and hygienic production, in other words, a marker of quality and safety (Bayram et al., 2010; Faydaoğlu and Sürücüoğlu, 2011). Ensuring compliance with a series of controls mentioned above for *Sideritis* species, establishing quality criteria, and conducting related studies will contribute to their introduction to the global market and reaching a larger population in the field of medicinal and aromatic plant trade in the coming years.

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ISBN: 978-625-367-194-5