

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**THE EFFECTS OF NANOPARTICLES ON IN VITRO
MICROPROPAGATION OF AVOCADO (PERSEA AMERICANA MILL.)**

KÜBRA ULUTAŞ BAYAR

A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

ADVISOR: PROF. DR. YELDA ÖZDEN ÇİFTÇİ

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GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ**

**NANOPARTİKÜLLERİN AVOKADONUN (PERSEA
AMERICANA MILL.) İN VİTRO MİKROÇOĞALTIMI
ÜZERİNDEKİ ETKİLERİ**

KÜBRA ULUTAŞ BAYAR

**YÜKSEK LİSANS TEZİ
MOLEKÜLER BİYOLOJİ VE GENETİK
ANABİLİM DALI**

DANIŞMAN: PROF. DR. YELDA ÖZDEN ÇİFTÇİ

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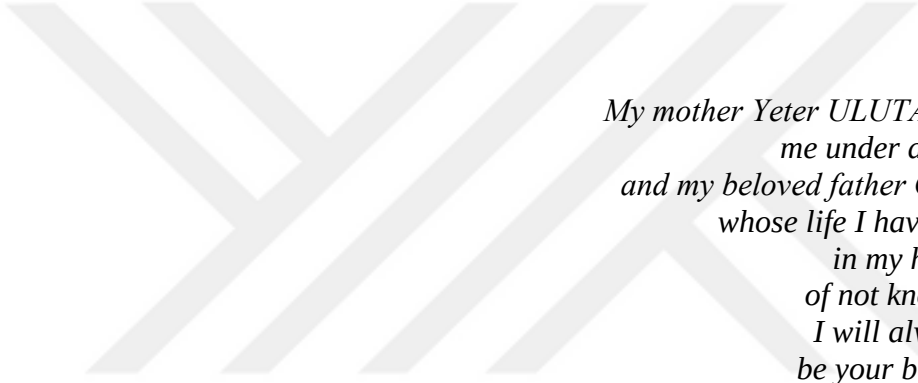
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*My mother Yeter ULUTAŞ has supported
me under all circumstances
and my beloved father Cezair ULUTAŞ,
whose life I have always carried
in my heart the sadness
of not knowing him more,
I will always be proud to
be your beloved daughter.*

ABSTRACT

Avocado (*Persea americana* Mill.) is a tropical, subtropical fruit and evergreen plant belonging to the Lauraceae family. Avocado fruit is known for its high nutritional value and healthy oil thus its production is increasing rapidly. Thus, *in vitro* propagation methods could be used to enhance its production. Moreover, the utilization of nanoparticles (NP) in plant *in vitro* propagation have also gained momentum in recent years. One of the nanoparticles that could be used in these studies could be zero valent iron nanoparticle (nZVI) since it has low phytotoxicity. This thesis nodal buds were cut from seedlings that is grown, *P. americana* Mill. cv. Zutano was used in greenhouse, sterilized and transferred to *in vitro* conditions with supplementation of the medium with different concentrations of 6-benzyl adenine (BA). Upon determination of optimum BA concentration, the influence of various concentrations of nZVI nanoparticles (12.5; 25; 50 mg/L) were also assessed on shoot tips and nodal buds to promote proliferation. After 30 days of *in vitro* culture, the percentage of proliferation, the average number of microshoots per explant, and the average microshoot length were calculated. In addition, rooting of microshoots were also tried to by incorporation of different concentrations (0.5, 1 and 2 mg/L) of naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA). However, no root formation was obtained from avocado explants tested in this thesis under culture conditions. As a result, it was determined that lower concentrations of nZVI (12.5 and 25 mg/L) resulted a increase in microshoot proliferation in shoot tips.

Keywords: *Persea americana* Mill., Zero Valent Iron Nanoparticles, Micropropagation, Plant Tissue Culture.

ÖZET

Avokado (*Persea americana* Mill.), *Lauraceae* familyasına ait tropikal, subtropikal bir meyve ve yaprak dökmeyen bir bitkidir. Avokado meyvesi yüksek besin değeri sebebiyle çeşitli şekillerde tüketilir. Sağlıklı içeriği bulunan yağı ise, kozmetik, farmakoloji gibi alanlarda sıkça kullanılmasıyla bilinir. Bu nedenle üretimi hızla artan üretim talebini karşılamak için üretimini artırmak için *in vitro* çoğaltma yöntemleri kullanılabilir. Ayrıca, bitkilerin *in vitro* çoğaltımında nanopartiküllerin (NP) kullanımı da son yıllarda ivme kazanmıştır. Bu çalışmalarda kullanılabilecek nanopartiküllerden biri, düşük fitotoksositeye sahip olduğu için sıfır değerlikli demir nanopartikülü (nZVI) olabilir. Avokado ve nanopartiküller üzerinden yapılan sınırlı çalışmalar sonucunda, farklı türde nanopartiküllerin uygulanması gündemdedir. Yüksek dozda sıfır değerlikli demir nanopartikülünün kullanımın bitkiler üzerinde toksik etki yaptığı çalışmalarla ispatlandığında, düşük dozda sıfır değerlikli demir nanopartikül konsantrasyonları tercih edilmiştir. Bu nedenle, serada yetiştirilen *P. americana* Mill. cv. Zutano bitkisinin fidelerinden nodal tomurcuklar kesilmiş, sterilize edilmiş ve ortamın farklı konsantrasyonlarda 6-benzil adenin (BA) içeren besiyerilerine *in vitro* koşullara aktarılmıştır. Optimum BA konsantrasyonunun belirlenmesinin ardından, sıfır değerlikli demir nanopartiküllerinin çeşitli konsantrasyonlarının (12.5; 25; 50 mg/L) etkisi de çoğalmayı teşvik etmek için sürgün uçları ve nodal tomurcuklar üzerinde değerlendirilmiştir. 30 günlük *in vitro* kültürden sonra, çoğalma yüzdesi, eksplant başına ortalama mikro sürgün sayısı ve ortalama mikro sürgün uzunluğu hesaplanmıştır. Ayrıca, naftalin asetik asit (NAA) ve indol-3-bütirik asidin (IBA) farklı konsantrasyonları (0.5, 1 ve 2 mg/L) katılarak mikro sürgünlerin köklenmesi de denenmiştir. Ancak, bu tezde kültür koşulları altında test edilen avokado eksplantlarından kök oluşumu elde edilmemiştir. Sonuç olarak, sıfır değerlikli demir nanopartikülünün daha düşük konsantrasyonlarının (12,5 ve 25 mg/L) sürgün uçlarında mikro sürgün çoğalmasında artışa neden olduğu belirlenmiştir.

Anahtar Kelimeler: *Persea americana* Mill., Sıfır Değerlikli Demir Nanopartikül, Mikroçoğaltım, Bitki Doku Kültürü.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percent
°C	: Celsius
μl	: Microliter
Å	: Angstrom
BA	: 6-Benzyladenine
C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ · 2H ₂ O	: EDTA, Disodium, Dihydrate
C ₁₂ H ₁₇ ClN ₄ OS · HCl	: Thiamine, Hydrochloride
C ₂ H ₅ NO ₂	: Glycine
C ₂ H ₆ O	: Ethanol
C ₆ H ₁₂ O ₆	: Myo-Inositol
C ₆ H ₅ NO ₂	: Niacin/Nicotinic Acid
C ₈ H ₁₁ NO ₃ · HCl	: Pyridoxine, Hydrochloride
CaCl ₂	: Calcium Chloride, Anhydrous
CNT	: Carbon Nanotubes
CoCl ₂ · 6H ₂ O	: Cobalt Chloride, Hexahydrate
CuSO ₄ · 5H ₂ O	: Cupric Sulfate, Pentahydrate
DMSO	: Dimethyl sulfoxide
ENP	: Engineered Nanoparticles
FAO	: Food and Agriculture Organization6
Fe	: Iron
FeO-NPs	: Iron Oxide Nanoparticles
FeSO ₄ · 7H ₂ O	: Ferrous Sulfate, Heptahydrate
g	: Gram
H ₃ BO ₃	: Boric Acid
HCl	: Hydrogen Chloride
IBA	: Indole-3-Butyric Acid
KH ₂ PO ₄	: Potassium Phosphate, Monobasic, Anhydrous
KI	: Potassium Iodide
KNO ₃	: Potassium Nitrate
M±SE	: Mean ±Standard Error
MgSO ₄	: Magnesium Sulfate, Anhydrous
mL	: Milliliter
MnSO ₄ · H ₂ O	: Manganese Sulfate, Monohydrate
MS	: Murashige Skoog
Na ₂ MoO ₄ · 2H ₂ O	: Molybdic Acid Sodium Salt, Dihydrate
NAA	: Naphthalene Acetic Acid
NanoCaP	: Calcium Phosphate Nanoparticles
NaOH	: Sodium Hydroxide
NH ₄ NO ₃	: Ammonium Nitrate
nZVI	: Zero-Valent Iron Nanoparticle
pH	: Power of Hydrogen
PVP	: Poly N-Vinylpyrrolidone
SFC	: Shoot Forming Capacity
SPSS	: Statistical Package for the Social Sciences
TUIK	: Türkiye İstatistik Kurumu/ Turkish Statistical Institute
v/v	: Volume per Volume
ZnSO ₄ · 7H ₂ O	: Zinc Sulfate, Heptahydrate

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1. INTRODUCTION

Avocado, with the scientific name *Persea americana* Mill, is a fruit that has grown in popularity worldwide in recent years. Avocado consumption is increasing rapidly on a global scale due to its high nutritional value and health benefits. It has a wide spectrum of vitamins (A, B, C, D, E and K) and minerals, as well as a diverse content of essential nutrients, carbohydrates, proteins and fats. It has become an important agricultural crop. The consumption of avocado is increasing, particularly in many Western European countries with high economic income levels.

The most reliable method of plant propagation through shoot tips and nod buds is the most effective in terms of maintaining the genetic stability of elite selections. In woody plants such as avocado, the morphogenetic capacity of adult material is generally lower than that of juvenile material. Consequently, micropropagation is typically developed using seedling explants, and the resulting data can be applied to direct the propagation of material of adult origin. The process of avocado micropropagation may be a potentially useful technique for the mass propagation of selected rootstocks or the regeneration of adult material, which could subsequently be multiplied more efficiently through conventional methods.

The interaction of nanoparticles with plants results in a range of morphological and physiological alterations, which are contingent upon the specific properties of the nanoparticles in question. The utilization of zero-valent metals in environmental applications was initially documented in 1972 [Pachi et al., 2020]. In recent years, zero-valent iron nanoparticles have been employed extensively in laboratory settings because they are cheap and non-toxic and have been reported to have the capacity to increase plant growth. The efficacy of nanoparticles is contingent upon several factors, chemical composition, including their reactivity, surface coating, size, and, most crucially, the doses at which they are effective. The effect of nanoparticles on plants depends on their composition, size, concentration, physical and chemical properties, and plant species. Furthermore, the efficacy of nanoparticles is contingent upon their concentration, exhibiting variability across different plant species. This review examines the impact of nanoparticles on plant growth, stem length and stem number.

1.1. Purpose of The Study

Various difficulties are encountered in the traditional propagation of avocado from seed or cutting method. Therefore, plant tissue culture, in which sterile micropropagation of isolated cells or tissues under appropriate *in vitro* conditions, has recently gained importance for the mass production of this species. In recent years, interest in the potential applications of iron nanoparticles in plant biotechnology and agriculture has been increasing. The objective of this thesis is to examine the *in vitro* production process and propagation of avocado by applying tissue culture techniques together with to reveal out the influence of zero-valent iron (ZVI) nanoparticles on micropropagation. To determine the effect of nZVI on *in vitro* proliferation of shoot cultures, shoot tips and nod buds were sterilized and incubated with various concentrations of 6-benzyl adenine as cytokinin and then explants were excised from *in vitro* grown seedlings and transferred to different concentrations of nZVI nanoparticles. Explants were selected from appropriately concentrated nZVI culture for rooting experiments. Two different auxin-derived hormones, Naphthalene acetic acid (NAA) and Indole-3-butyric acid (IBA), were incubated in root cultures at different concentrations.

2. GENERAL INFORMATION

2.1. Avocado (*Persea americana* Mill.)

The avocado (*Persea americana* Mill.) is a fruit that is scientifically classified as member of the *Lauraceae* family. It is native to Mexico and Central America, genus *Persea*, and comprises two subgenera: The two subgenera are *Eriodaphne* and *Persea*. The avocado, cultivated for agricultural production, belongs to two species of the genus *Persea*. It is divided into three botanical varieties: *Persea nubigena* Miller var. *Guatemalensis* (Guatemalan species) *Persea americana* Miller var. *American* (West Indian species) and *Persea americana* Miller var. *Drymifolia* (Mexican species) [Duarte et al., 2016]. The tree of the avocado fruit has a woody trunk. While avocados are classified as evergreen, some varieties undergo a period of leaf loss prior to flowering. Leaves can range in length from 7 to 41 cm and exhibit a diversity of shapes. They are typically hairy and reddish in color during their juvenile phase, becoming smooth and dark green upon reaching maturity. The fruit is characterised by a single large seed encased in an oily pulp (Figure 2.1). The avocado is composed of 73% fruit, 16% seed, and 11% pulp, although the proportion of fruit can vary [Goldag and Dogan, 2024].



Figure 2.1: *Persea americana* Mill. a)Avocado tree. b)Avocado tree leaves and fruits. c)Avocado inside and seed view.

Avocado is a fruit with high nutritional value and a healthy oil, has offers many potential benefits for human health, experienced a surge in popularity worldwide in recent years, becoming a significant agricultural product. In contrast to most fruits, which are either acidic or sweet, avocados have a smooth and buttery texture with a rich flavour. The oil extracted from avocados can be used for cooking and preparing

saucers, marinades and salads. In addition to its applications in the oil, cosmetic, soap, and shampoo industries, avocados are widely consumed as a fresh fruit globally. Avocado oil can also be used in the production of skin care products including cleansing creams, sunscreen lotions and moisturisers, as well as make-up bases and hair conditioners [Subba et al., 2023]. It could also be utilized for the treatment of diseases [Chindo et al., 2012]. For instance, the fruit of the common medicinal plant, *P. americana*, which is often consumed as food, has been documented to be efficacious in the treatment of gastric distress, bronchitis, diabetes, hypertension, and other conditions [Muanya et al., 2008]. Moreover, different parts of *P. americana* have been reported to be used for the treatment of various ailments. For instance, avocado bark is employed in the treatment of cough, fever, diabetes, asthma, as well as diseases of the kidney and liver. The avocado leaf is used in the treatment of hypertension, ulcer, diarrhea, and cough, while the avocado root is used in the treatment of skin convulsion, hormonal disorders in women, blemishes, and epilepsy [Oluwole et al., 2011].

The avocado is a tropical fruit that contains a high concentration of bioactive compounds [Tremocoldi et al., 2018]. Its consumption is increasing rapidly on a global scale due to the perceived health benefits associated with it [Akan, 2021]. It has been reported that the nutritional composition of avocado pulp exhibits a moisture content ranging from 67-78%, a lipid content ranging from 12-24%, a carbohydrate content ranging from 0.8-4.8%, and a protein content ranging from 1.0-3.0% [Araújo et al. 2018]. The avocado is a rich source of essential minerals and vitamins, as well as potassium, phosphorus, calcium, iron, zinc and sodium. It is also a significant source of essential amino acids, including phenylalanine, valine, threonine, lysine, leucine, methionine and isoleucine. Additionally, it contains unsaturated fatty acids, including oleic, linoleic and linolenic acids. These nutritional properties contribute to the avocado's overall health benefits [Tremocoldi et al., 2018], [Goldag and Dogan, 2024]. Ingestion of avocado fruit in quantities of 1-2 pieces per day has no adverse toxic effect. The demand for avocados and avocado-containing products in the daily diet is increasing due to their high energy content and their positive effects on abdominal pain, diarrhea, diabetes and cardiovascular diseases. Additionally, they contain vitamins, minerals and phytochemicals. It is recommended that products containing monounsaturated fatty acids be included in the diet to prevent cardiovascular disease. The results of a study indicate that an increased consumption of dietary fiber is

associated with a reduced risk of cardiovascular disease [Weschenfelder et al., 2015]. The avocado is a fruit that has a favorable influence on several cardiovascular risk factors. These include inflammatory processes, blood pressure, high cholesterol, blood glucose levels, and metabolic syndrome. Additionally, they encompass insulin concentrations, body weight, and body fat composition. [Demircan et al., 2021]. *P. americana* Mill. was introduced from California in the early 1970s through the Food and Agriculture Organization (FAO) with the objective of popularizing the commercial cultivation of avocados in our country. The breeding of four important cultivars (Hass, Bacon, Fuerte and Zutano) facilitated the introduction of *P. americana* Mill. to our country. This fruit is also referred to as the "American pear" by some individuals in Turkey. It has been posited that certain regions of the Mediterranean coastal zone of Turkey are particularly conducive to avocado cultivation [Demircan et al. 2021]. In 2023, Turkey produced 38,462 tons of avocados. Figure 2.2 presents the avocado production amounts in Turkey over the past five years in tons. As indicated by data from the Turkish Statistical Institute [TUIK, 2024], the distribution of production amounts by province is presented in Figure 2.2. have been grown in the provinces of Antalya, Adana and Mersin (Figure 2.2) [Web 1, 2024].

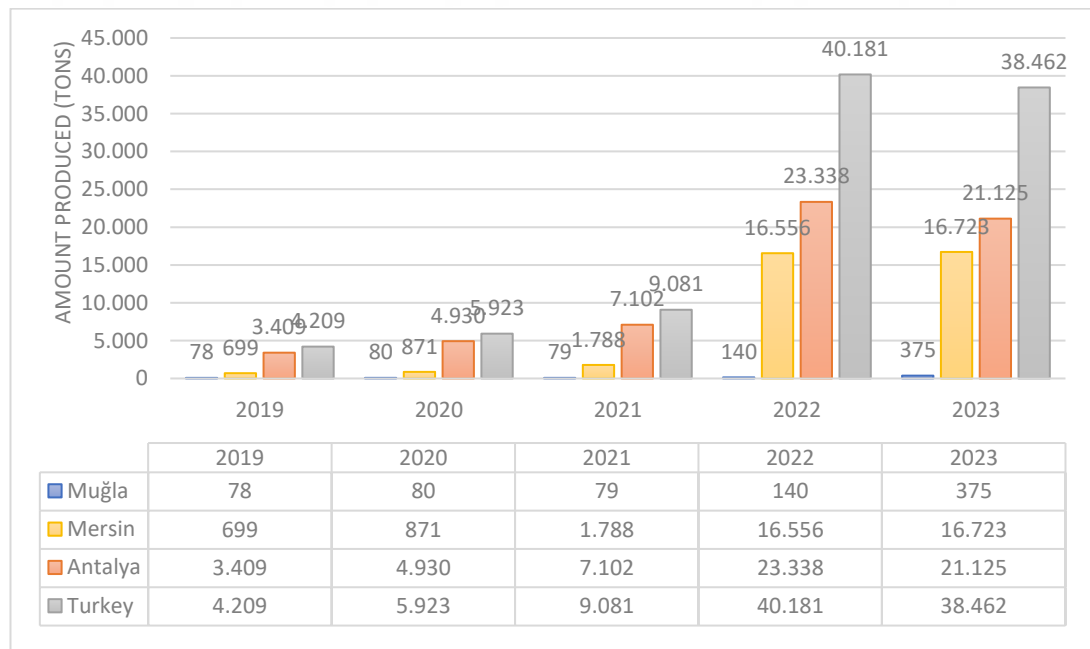


Figure 2.2: Avocado production in the last 5 years by provinces in Turkey [TUIK, 2024].

Among the different avocado cultivars, cv Zutano is selected as a plant material in this thesis. It has shiny, yellow-greenish skin which is thin, and the seed is medium sized. The fruit flesh displays a range of oil content, from 15 to 18%, with dry matter content varying between 23 to 25%. The tree grows to a considerable height, with a strong, upright structure. The 'Zutano' variety has well-developed cold tolerance. It is a variety with consistent and high productivity. In the Serik-Antalya region, the yield of the 'Zutano' variety was found to be between 280 and 290 pieces and 75 to 80 kg per tree (Table 2.1). The market value and suitability for transport of this variety are moderate.

Table 2.1: Fruit and tree characteristics of 'Zutano' cultivar.

Fruit and Tree Characteristics	
Optimum Harvest Period	November-December
Stay In on The Tree	Medium (2-3 months)
Cold Damage Threshold	-3.0 °C
Storage Condition	2 months +4°C
Tree Habitat	Medium
Economic Productive	Age 8-10 years
Average Fruit Weight (g)	200-400
Fruit Peel colour	Light green and shiny
Fruit Peel Structure	Smooth
Shell Thickness	Thin
Peelability	Relatively Difficult
Seed Shape	Conical
Fruit Shape	Ovoid or pear
Flesh Colour	Light Yellow-Cream
Taste and Flavour	Medium flavour
Yield (kg/tree)	(10-12 years old) 75-80
Yield (pcs/tree)	(10-12 years old) 280-290
Industrial Use	Food, cosmetics and pharmaceutical industry

2.2. Plant Tissue Culture

Plant tissue culture is a plant breeding method that was developed in the 1950s. Traditional breeding techniques were unable to meet the required product demand, which led to the emergence of tissue culture as a significant development in breeding applications. This technique allows us to produce multiple copies of a plant in a short period of time. The process makes use of the totipotency property of the plant cell, which means that any cell from any part of the plant can be used. Tissue culture is employed for the cultivation of thousands of genetically identical plants derived from a single parent plant, designated as soma clones. This process is known as micropropagation. The method offers an advantage that can be used to produce a completely new plant methods, as it can be used to develop disease-free plants from diseased plants by using their meristems (shoot tips and nod buds) as explants [Custodio et al., 2023]. Plant tissue culture is a process that involves the cultivation of whole organs, tissues, plants or cells under controlled aseptic conditions in a laboratory to facilitate the growth of cells and the production of genetically identical organisms. These culture systems provide all the water, nutrients and energy necessary for the growth of the plant or explants to establish a basal environment. The provision of optimal light and temperature settings facilitates the creation of conducive incubation conditions. The manipulation of plant development can be achieved through the addition of natural or synthetic phytohormones, also known as plant growth regulators (PGRs), to the plant's environment at specific stages of growth or maturation. The selection of the PGRs type and concentration is contingent on the specific plant species, organ or tissue under consideration, and the objective of the experiment. [Phillips et al., 2019].

2.3. Nanoparticles

Nanotechnology represents an innovative and transformative field of science. The present-day applications of nanotechnology make use of advances in biotechnology, materials science, physics, and chemistry to create novel materials with distinctive properties resulting from their nanometer-scale structures. These altered properties have led to the development of numerous new and effective products and applications.

Industries within the agriculture, food, and medicine sectors have been investing significantly in nanotechnology research [Kim D et al., 2017].

The interaction of nanoparticles with plants results in a range of morphological and physiological alterations, which are contingent upon the specific properties of the nanoparticles in question. The research team posited that the impact of engineered nanoparticles (ENPs) on plant growth and development may be either positive or negative, depending on the specific characteristics of the ENPs in question. These characteristics include composition, concentration, size, and physical and chemical properties, as well as the plant species in question. The efficacy of NPs is determined by several factors, including their chemical composition, surface covering, size, reactivity, and most importantly, the dose at which they are effective [Siddiqui et al., 2015].

Nanoscale zerovalent iron (nZVI) is the most utilized nanomaterial for the remediation of environmental contamination. The effects of nZVI on terrestrial organisms have recently become the subject of research, with findings indicating that plant growth is enhanced by nZVI treatment at various concentrations. It is therefore essential to investigate the detailed physiological and biochemical responses of plants to nZVI treatment to ascertain its suitability for agricultural applications. [Yoon et al., 2019].

nZVI has become a widely utilized remediation technique due to its exceptional efficacy in the treatment of contaminated groundwater [Karn et al., 2009]. The application of nZVI has recently been explored as a remediation strategy for soils contaminated with pesticides and heavy metals, with promising results in terms of soil quality improvement [Jiang et al., 2018]. It may be the case that an optimal concentration of nZVI is required to stimulate plant growth. It must be acknowledged that the effects of nZVI on plants are species dependent. Furthermore, the underlying mechanisms that lead to an increase in biomass or cause toxicity in plants remain unclear. Therefore, before nZVI can be applied in agriculture, it is essential to understand the physiological and biochemical responses of plants to nZVI exposure.

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Plant Material

P. americana Mill. cv. Zutano was used in this thesis. The seedlings were obtained from Adana and transferred to the greenhouse of Gebze Technical University, Department of Molecular Biology and Genetics (Figure 3.1). Figure 3.1 shows the seedlings of *P. americana* Mill. The growth of the seedlings is under greenhouse conditions of 25 °C, 16 hours of light and 8 hours of dark photoperiod.



Figure 3.1: *Persea americana* Mill. plant cv. Zutano in the GTU greenhouse.

3.1.2. Nanoparticle

In this study, zero-valent iron nanoparticles (nZVI) were employed as nanoparticles. The nZVI were obtained as dry matter from Mr. Prof. Dr. Anatoli Dimoglo (Duzce University, Department of Environmental Engineering) and his team. The ZVI-NPs were produced by reduction of iron salts via a chemical reduction procedure, without the use of ultrasonic treatment. As a stabilizing agent, low molecular weight poly N-vinylpyrrolidone (PVP) of 18 was employed. The magnetic core size is 12.0 and 12.4 Å, while the particle size is 200 nm.

3.1.3. Chemicals

The consumables used in the thesis study are shown in Table 3.1.

Table 3.1: Chemicals used in the *in vitro* proliferation stage.

<i>Persea americana</i> Mill. Media Contents		
Chemical Name	Manufacturer Company	Catalogue Number
Murashige and Skoog (MS)	Caisson	MSP09-50LT
Plant Agar	Duchefa	9002-18-0
Sucrose	Caisson	SO11-1KG
6- Benzyl Adenine (BA)	Sigma	B-3408
DMSO (dimethyl sulfoxide)	Alfa Aesar	Q17D720
Commerical Bleach	Domestos	-
Safa Captan 50 WP	Safa	5460
Ethanol (C ₂ H ₆ O)	Duzey Lab	64-17-5
L-Ascorbic Acid	Sigma	A-2174
Naphthalene acetic acid (NAA)	Sigma	20K3255

3.1.4. Equipments and Devices

The equipments used in the thesis study is shown in Table 3.2.

Table 3.2: Equipments used in the thesis study.

Equipment Name	Equipment Model	Manufacturer Company
pH Metre	pH 209	Hanna Instrument
Laminar intelligent cabin	Cleanair	PMU Company
Precision Scale	TP3030	Denver Instrument
Etuve	DIN 12880	Binder
Steriliser	Steri 350	Swiss Made
Plant Growth Room	RivaCold	Genpa Mühendislik
Magnetic Stirrer	MR3001	Heidolph
Autoclave	HV-110L	Hirayama
+4 Refrigerator	RG 1380	İndesit
Deep freezer (-20 °C)	GS26DN13NE	Siemens

3.2. METHODS

3.2.1. Murashige and Skoog (MS) Media Preparation

The calculated amounts of 4.43 g/L MS and 30 g/L sucrose, which were determined as optimal values in the literature studies [Osorio et al., 2018], were added to the glass beaker. The remaining volume was made up to 1000 ml with distilled water. Subsequently, HCl and NaOH were added, and the pH was adjusted to 5.70 ± 0.1 using a pH meter. A solution of 7 g/L plant agar was added, and the medium was sterilized by autoclaving at 121 °C for approximately 20 minutes. The medium was then poured into glass tubes in equal volumes under sterile conditions in a horizontal flow laminar flow cabinet and allowed to solidify. Following solidification, the medium was stored in the plant growth room. Table 3.3 shows the composition and quantity of the MS [Murashige & Skoog, 1962] medium. Furthermore, different concentrations of the cytokinin 6-benzyl adenine (BA) were employed, namely 0.5 mg/L, 1 mg/L, 1.5 mg/L and 2 mg/L.

Table 3.3: Murashige & Skoog Media Components

Components	mg/L
Ammonium Nitrate (NH_4NO_3)	1650.00
Boric Acid (H_3BO_3)	6.20
Calcium Chloride, Anhydrous (CaCl_2)	332.20
Cobalt Chloride, Hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$)	0.025
Cupric Sulfate, Pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.025
EDTA, Disodium, Dihydrate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$)	37.26
Ferrous Sulfate, Heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	27.80
Glycine ($\text{C}_2\text{H}_5\text{NO}_2$)	2.00
Magnesium Sulfate, Anhydrous (MgSO_4)	180.70
Manganese Sulfate, Monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	16.900
Molybdc Acid Sodium Salt, Dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	0.250
Myo-Inositol ($\text{C}_6\text{H}_{12}\text{O}_6$)	100.00
Niacin/Nicotinic Acid ($\text{C}_6\text{H}_5\text{NO}_2$)	0.500
Potassium Iodide (KI)	0.830
Potassium Nitrate (KNO_3)	1900.00
Potassium Phosphate, Monobasic, Anhydrous (KH_2PO_4)	170.00
Pyridoxine, Hydrochloride ($\text{C}_8\text{H}_{11}\text{NO}_3 \cdot \text{HCl}$)	0.500
Thiamine, Hydrochloride ($\text{C}_{12}\text{H}_{17}\text{ClN}_4\text{OS} \cdot \text{HCl}$)	0.100
Zinc Sulfate, Heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	8.600

3.2.2. Sterilization of Avocado Shoots and Determination of Optimum Cytokinin Concentration

The shoot explants were subjected to a 30-minute wash under tap water. Subsequently, the nodal buds were excised and subjected to a 10-minute treatment with a solution comprising 70% (v/v) ethanol and 1% fungicidal agent under laminar flow cabinet conditions. Subsequently, the buds were treated with 4% (v/v) sodium hypochlorite (commercial bleach) for 25 minutes, after which they were rinsed three times with sterile distilled water. The sterilized explants were cut into 1-2 cm nodal buds in an aseptic manner. Then, to prevent the formation of phenolic compounds in the cut bud areas, they were treated with 600 mg/mL ascorbic acid for 1 h and nodes were allowed to dry on sterile filter paper in a laminar cabinet. Finally, the dried sterile explants (nodes and shoot tips) were inoculated into glass tubes of MS medium containing different concentrations (0.5 mg/L, 1 mg/L, 1.5 mg/L and 2 mg/L) of 6 benzyl adenine (BA). The explants were incubated for 30 days at a temperature of $22\pm 2^{\circ}\text{C}$ and light intensity of 4000 lux with a photoperiod of 16 hour light/8 hour of darkness, under plant growth room conditions (Figure 3.2).



Figure 3.2: The excised explants that were transferred to BA containing MS media.

3.2.3. Preparation of Media Containing Zero Valent Iron Nanoparticles (nZVI) and Assessment of Its Influence on Micropropagation

For the preparation of media containing different concentrations of nZVI, firstly, Fe(0) NP calculated as 50 mg/L Fe(0) NP was weighed and dissolved in dimethyl sulfoxide (DMSO). The prepared stock solution was first kept in a sonicator for 20 minutes at room temperature. The aggregation of the material in the suspension was partially prevented by sonication. Then, nZVI was added to the medium under aseptic conditions in the amounts indicated in Table 3.4 to MS medium containing 1 mg/L BA, which was found to be optimal in our previous trials. After the medium was stored in a light-proof refrigerator, it was stored at 4°C.

Table 3.4: Concentrations of nZVI used.

nZVI Concentration	12.5 mg/L		25 mg/L		50 mg/L		Control	
Media	40mL	40mL	40mL	40mL	40mL	40mL	40mL	40mL
Explant Type	Shoot Tip	Nod Bud	Shoot Tip	Nod Bud	Shoot Tip	Nod Bud	Shoot Tip	Nod Bud
nZVI NP Quantity	40 µl	40 µl	80 µl	80 µl	160 µl	160 µl	-	-

In line with the optimization studies performed for the determination of optimum BA concentration, explants were transferred to MS medium containing 1 mg/ml BA, which was found to be the optimal value, to each magenta containing 12.5 mg/L, 25 mg/L and 50 mg/L zero valent iron nanoparticles incorporated MS medium. The samples were incubated in plant growth chamber for 30 days with the conditions indicated above.

3.3. Statistical Analysis

In vitro culture of the sterilized avocado shoot tips and nodal buds were done in glass tubes (one explant/tube). A total of 20 shoot tips and nodal buds were utilized with at least two replicates. The influence of nZVI on avocado micropropagation was assessed

by using at least 20 explants and repeated twice. After 30 days of incubation, the proliferation rate (%), the average number of microshoots proliferated per explants, and the average length of the microshoots per explant were calculated and statistically analyzed. Shoot forming capacity (SFC) of the explants was calculated with the below formula.

$$\text{SFC} = \text{Proliferation percentage} \times \text{Average number of microshoots proliferated per explant} / 100$$
 [Lambardi et al., 1993].

A statistical analysis of the data was conducted to ascertain the percentages of proliferation. The selected non-parametric statistical test was the Chi-square (X^2) test for homogeneity of differences. The number of microshoots and their length, as well as the number of proliferations per explant, were subjected to analysis of variance (ANOVA), followed by the least significant difference (LSD) test at $P \leq 0.05$, with the aim of comparing the means.

4. RESULTS

4.1. Sterilization and Proliferation of Avocado Explants

In this study, nodal buds were used to establish *in vitro* culture in MS medium containing different concentrations (0.5 mg/L, 1 mg/L, 1.5 mg/L and 2 mg/L) of BA. At the end of 30 days, according to the results of statistical analyses; the highest proliferation percentage was recorded 1 mg/L BA containing MS medium while the lowest proliferation rates were calculated with the highest BA concentration (2.0 mg/L, Table 4.1). Accordingly, the highest SFC index together with the average longest microshoots were obtained with 1.0 mg/L BA containing medium. Although the maximum number of microshoots proliferated per explant was calculated in the highest tested nZVI concentration, the obtained microshoots were relatively shorter in comparison with the 1.0 and 1.5 mg/L supplemented media.

Table 4.1: The proliferation of avocado nodal buds in BA containing medium.

BA Concentration	Proliferation ^b (%)	Shoot/ explant ^c (Mean±SE) ^d	SFC ^e	Shoot Length ^c (Mean±SE) ^d (mm)
0.5 mg/L	70.00 ^c	1.56±0.16 ^a	1.09	0.9±0.19 ^c
1.0 mg/L	85.00 ^a	1.62±0.16 ^a	1.38	16.3±0.18 ^a
1.5 mg/L	80.00 ^b	1.23±0.08 ^b	0.98	15.9±0.17 ^a
2.0 mg/L	55.00 ^d	1.67±0.21 ^a	0.92	7.6±0.12 ^b

^a Each trial was performed using a minimum of 20 explants and repeated twice.

^b The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the Chi-Square (χ^2) test.

^c The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the LSD test.

^d Mean $\pm$ standard error.

^e Shoot forming capacity (SFC) is calculated as the percentage of proliferin explants multiplied by the average number of shoots proliferated per explant, divided by 100. This method is based on the approach described by Lambardi et al. (1993).

4.2. Effects of Zero Valent Iron Nanoparticles

4.2.1. Shoot Tips

The result of 30 days incubation of avocado shoot tips in nZVI containing proliferation medium is shown in Figure 4.2. According to the results of statistical analyses, 100% proliferation was recorded in culture media containing 12.5 and 25 mg/L nZVI. Accordingly, the longest microshoots with the highest SFC index were obtained in the medium containing 50 mg/L nZVI, while the shortest microshoots with the lowest SFC index were observed in the medium containing 12.5 mg/L nZVI.

Table 4.2: The proliferation values of avocado shoot tips in nZVI containing medium.

nZVI Concentrations	Proliferation ^b (%)	Shoot/ explant ^c (Mean±SE) ^d	SFC ^e	Shoot Length ^c (Mean±SE) ^d (mm)	Callus Formation (%)
Control	91.66 ^b	1.0±00 ^a	0.92	7.5±0.62 ^a	36.36%
12.5 mg/L	100 ^a	1.0±00 ^a	1.00	6.7±0.37 ^b	33.33%
25 mg/L	100 ^a	1.0±00 ^a	1.00	7.4±0.53 ^a	16.67%
50 mg/L	91.66 ^b	1.0±00 ^a	0.92	7.5±0.45 ^a	18.19%

^a Each trial was performed using a minimum of 20 explants and repeated twice.

^b The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the Chi-Square (X^2) test.

^c The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the LSD test.

^d Mean ± standard error.

^e Shoot forming capacity (SFC) is calculated as the percentage of proliferin explants multiplied by the average number of shoots proliferated per explant, divided by 100. This method is based on the approach described by Lambardi et al. (1993).

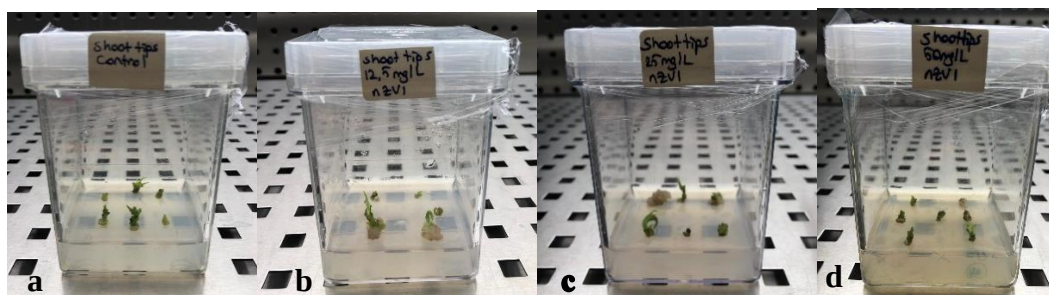


Figure 4.1: After 30 days of incubation of the shoot tips a) Control. b) 12.5 mg/L 25 mg/L nZVI. d) 50 mg/L nZVI.

nZVI seems to have a toxic effect on shoot tip proliferation as the concentration increases, as it can be evidenced from proliferation percentages. On the contrary, it was observed that callus formation decreased as nZVI concentration increased.

4.2.2 Nodal Buds

The result of 30 days incubation of avocado nodal buds in proliferation medium containing nZVI is shown in Figure 4.3. According to the results of statistical analyses, the highest proliferation percentage was recorded in MS medium containing 12.5 mg/L nZVI, while the lowest proliferation rates were calculated in the medium concentration containing 25 mg/L nZVI (Table 4.3) among the nZVI incorporated media. Accordingly, the lowest SFC index was obtained in the medium containing 50 mg/L nZVI, while no difference was observed between the control group and other concentrations (Table 4.3). In addition to this analysis, it was observed that avocado nodal buds formed callus in nZVI culture medium. Similarly to shoot tips, the rate of callus formation was decreased with the concentration of nZVI in the media increased, (Table 4.3).

Table 4.3: The proliferation values of avocado nodal buds in nZVI containing medium.

nZVI Concentrations	Proliferation ^b (%)	Shoot/ explant ^c (Mean±SE) ^d	SFC ^e	Shoot Length ^c (Mean±SE) ^d (mm)	Callus Formation (%)
Control	100 ^a	1.0±00 ^a	1.00	6.7±0.67 ^a	50.00%
12.5 mg/L	90 ^b	1.0±00 ^a	0.90	6.5±0.28 ^a	34.78%
25 mg/L	75 ^c	1.0±00 ^a	0.75	6.5±0.42 ^a	30.00%
50 mg/L	85 ^b	1.0±00 ^a	0.85	6.2±0.33 ^a	10.00%

^a Each trial was performed using a minimum of 20 explants and repeated twice.

^b The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the Chi-Square (X^2) test.

^c The different letters in each column indicate statistically significant differences ($P \leq 0.05$) in comparison to the LSD test.

^d Mean $\pm$ standard error.

^e Shoot forming capacity (SFC) is calculated as the percentage of proliferin explants multiplied by the average number of shoots proliferated per explant, divided by 100. This method is based on the approach described by Lambardi et al. (1993).



Figure 4.2: After 30 days of incubation the nod tips a) Control. b) 12.5 mg/L nZVI. c) 25 mg/L nZVI. d) 50 mg/L nZVI.

4.2.3 Rooting Microshoots

Avocado microshoots that is at least 10 mm in 25 mg/L nZVI culture medium were used for rooting. These microshoots were transferred to MS medium containing indole-3-butyric acid (IBA) or 1-naphthalene acetic acid (NAA) at three different concentrations (0.5 mg/L, 1.0 mg/L and 2.0 mg/L). Control treatments without each auxin (IBA and NAA) in the medium were also included. The pH of the medium was

adjusted to 5.70 before autoclaving. Magenta boxes were used at this stage. Each auxin concentration was performed using 18 explants. The experiment with IBA auxin is shown in Figure 4.3.

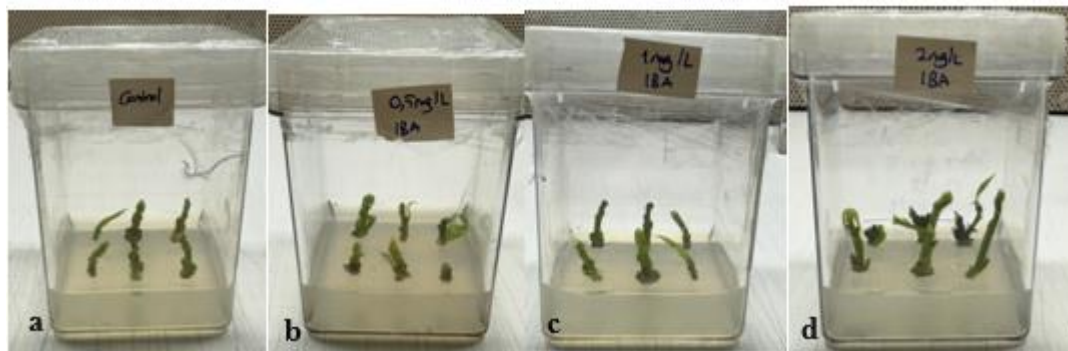


Figure 4.3: After 30 days of incubation the IBA for rooting. a) Control. b) 0.5 mg/L IBA. c) 1 mg/L IBA. d) 2 mg/L IBA.

The other rooting experiment with NAA is shown in Figure 4.4.

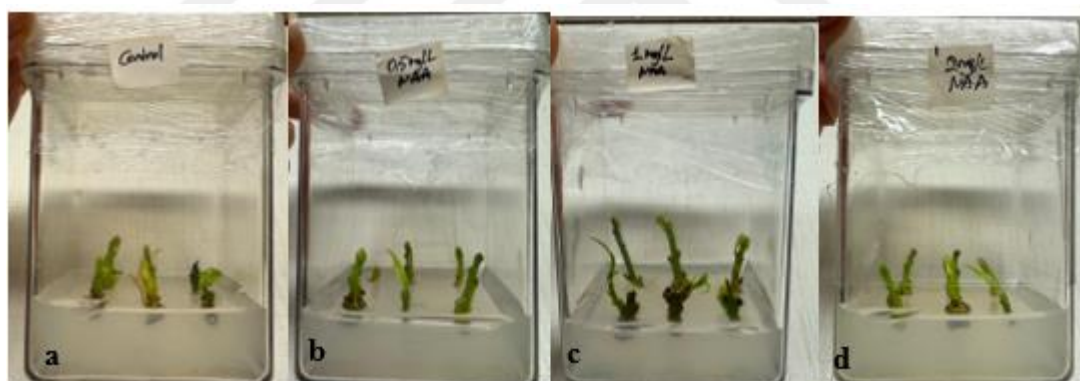


Figure 4.4: After 30 days of incubation the NAA. a) Control. b) 0.5 mg/L NAA. c) 1 mg/L NAA. d) 2 mg/L NAA.

After 30 days of incubation, no root formation was observed from avocado microshoots while basal callusing was evident in both tested auxins (Figure 4.3 and 4.4).

5. DISCUSSION

Studies conducted with different plants and nanoparticles show that NPs offer innovative contributions to fundamental processes such as plant growth, development and stress tolerance. Nanofertilizers, in particular, optimize plant nutrition by providing nutrients in a targeted and long-term manner thanks to their controlled release properties. NPs promote root and shoot growth, increase plant biomass and improve photosynthetic efficiency. NPs, which support photosynthetic processes by increasing stomatal conductance and water use efficiency, also provide protection against diseases by preventing the development of pathogens on the plant surface with their antimicrobial properties. They also contribute to the reduction of oxidative stress by increasing the resistance of plants to environmental stress factors (such as salt, drought, heavy metals). Their controlled release and targeted effects reduce negative impacts on the environment by optimizing the use of fertilizers and pesticides and make significant contributions to agricultural sustainability.

The main purpose of using NPs in plants is to promote growth and increase tolerance to environmental stress factors and increase yield in cases where traditional methods are inadequate. For example, nanoparticles can penetrate the plant cell wall and increase root and shoot growth, optimize water and nutrient uptake and support antioxidant defense mechanisms. Thus, they both improve plant metabolism and provide an innovative solution for sustainable agriculture. Studies on some model plants and NPs are as follows.

In avocado nanoparticle applications, the effects of copper nanoparticles (CuNPs) on growth, biomass increase and photosynthetic performance in avocado plants were evaluated by root irrigation, foliar spray and stem injection methods [López-Luna et al., 2023]. In all methods, cell viability remained high (91–96%), and no signs of toxicity or organelle damage were observed. Cu accumulation was mostly in the leaves (foliar spray) and stem (stem injection). The foliar spray method was determined to be the method that increased stem length (25%) and leaf formation (85%) the most, and stem injection was the most effective in terms of biomass increase. In general, the foliar spray method provided the most efficient uptake of CuNPs and the

environmental risks remained at a low level. These findings reveal the potential of CuNPs as a safe and effective nanofertilizer [López-Luna et al., 2023].

Another study on nanoparticle applications to avocado investigated the effects of calcium phosphate nanoparticles (NanoCaP) in reducing stress due to climate variability in avocado (*Persea americana* Mill., cv. Hass) production areas in Colombia. NanoCaP was tested with foliar and soil applications at concentrations of 15, 30 and 45 mg/L; it was determined that foliar application of 30 mg/L improved growth parameters such as photosynthesis, stomatal conductance, water use efficiency and biomass by 10-30%. The results indicate that NanoCaP is a potential nanofertilizer for reducing stress due to climate variability and increasing yield in avocado production [Ramirez-Gil et al., 2023].

Although the above-mentioned nanoparticles were used in avocado, up to now, there is no study on utilization of nZVI on *in vitro* cultures of avocado. Hence, the aim of this thesis was to investigate the effects of *in vitro* micropropagation of plant explants using different concentrations of nZVI (12.5-25-50 mg/L) on *P. americana* Mill. plant proliferation. In this context, the results obtained from the effect of nZVI on the shoot tips and nodal explants of avocado plants after 30 days were assessed. When the results of nZVI treatment with avocado plants were examined, a statistically positive effect was observed on proliferation in shoot tip explants as the nZVI concentration increased. It was observed that the proliferation percentages of nodal explants were lower than the control. The highest proliferation rate in nodal explants was observed in the group containing 12.5 mg/L nZVI, but it decreased as the concentration increased. Although there was no statistically significant difference, a negative effect was observed as the concentration increased. In shoot tips and nodal explants, it was observed that multiple shoot formation was not achieved. In shoot tip explants applied with nZVI, no statistically significant difference was observed in shoot length. This may be due to the growth and development potential of shoot tips, and no positive or negative effects were observed in nZVI applied groups. On the contrary, a statistically negative effect was observed in the group containing 50 mg/L nZVI in nodal explants than in control groups. As a result, when all data related to *P. americana* Mill. were examined, it was determined that nZVI did not have a significant positive effect on nodal bud proliferation while it has positive influence on shoot tip proliferation at lower concentrations.

In recent years, there have been reports that nZVI has the capacity to enhance plant growth under laboratory conditions. For this reason, various doses of nZVI, including 12.5 mg/L, 25 mg/L and 50 mg/L, were employed to observe the effect on the proliferation of *P. americana* Mill., which is an important and nutrient-rich plant. After 30 days, nZVI was identified in *P. americana* Mill. The impact of plant growth and micr shoot length was then examined. The changes in the proliferation of the avocado plant, which is the subject of this thesis, were examined by exposing the explants to different concentrations (12.5 mg/L, 25 mg/L, 50 mg/L) of nZVI *in vitro*. In this context, the results obtained because of examining the effect of nZVI on the shoot tips and nodal bud explants of the avocado plant after the 30 days are interpreted. In accordance, the application of low concentrations (≤ 500 mg/L) of nZVI has been observed to enhance the biomass of a range of plant species. Conversely, the application of high concentrations of nZVI (>1000 mg/L) resulted in the inhibition of the growth of plant species. For instance, nZVI particles show concentration-dependent effects on different plants. In applications to *Typha latifolia* (cattail), it was observed that nZVI promoted growth at low concentrations (<50 mg/L) but shortened plant height at high concentrations (>200 mg/L) [Ma et al., 2013]. Similarly, in *Populus deltoides* $\times$ *Populus nigra* (hybrid poplar), roots and leaves remained healthy at low concentrations, while transpiration rate decreased at high concentrations [Ma et al., 2013]. In rice plants, growth-promoting effects were observed for nZVI at concentrations below 160 mg/kg, while iron deficiency with chlorosis symptoms occurred at concentrations above 500 mg/kg [Wang et al., 2016]. In peanut plants, no growth inhibition was observed nZVI at concentrations below 160 $\mu\text{mol/L}$, and in some cases, growth was promoted. However, toxic effects and nanoparticle accumulation were detected in plants at concentrations above 320 $\mu\text{mol/L}$ [Li et al., 2015]. In flax plants, no significant effect was observed at concentrations below 250 mg/L, while no seed germination was reported at concentrations above 2000 mg/L [El-Temsah and Joner, 2012]. Similarly, while nZVI concentrations below 250 mg/L did not affect seed germination in barley plants, inhibition of germination was observed at nZVI concentrations above 5000 mg/L [El-Temsah and Joner, 2012]. In applications on *Lolium perenne* (Meadow grass), nZVI concentrations below 250 mg/L had no significant effect on the plant, while seed germination was completely inhibited at nZVI concentrations above 1000 mg/L [El-Temsah and Joner, 2012]. Besides, the development of phosphate-sorbed nZVI has been explored as a means of enhancing

spinach crop growth, acting as both a phosphate fertilizer and an iron fortifier. [Almeelbi et al., 2014]. Studies on *Arabidopsis thaliana* have indicated the impact of varying nanoparticles and concentrations on plant development and physiological processes. nZVI nanoparticles, when applied at a concentration of 0.5 g/L, have been observed to promote root elongation [Kim et al., 2014]. These findings clearly demonstrate the sensitivity of *Arabidopsis thaliana* to nanoparticles and the potential of these materials in agricultural applications.

There are also studies that examined the effects of nZVI on heavy metal bioremediation in soil [Gil-Díaz et al., 2018]. They analyze the responses of plants to contaminants and how nZVI can enhance phytoremediation in this process. Given that nanoremediation is a promising strategy with potential application in contaminated sites, further studies are needed to analyze the effect on plant growth and at the cellular scale with different plant species and different types and doses of nZVI on contaminated soils.

In the tissue cultures performed in this thesis, it was determined that less callus was formed in the experimental groups than the control group. In another study, the effects of iron oxide nanoparticles (FeO-NPs) produced by green synthesis were evaluated in terms of callus formation, plant physiology and microbial contamination control in rice (*Oryza sativa* L.). In the study, it was observed that callus induction and regeneration were significantly accelerated by replacing Fe-EDTA, which is used as the traditional iron source, with FeO-NPs. The addition of FeO-NPs at a concentration of 15 ppm to MS medium significantly increased callus formation rates and diameters in some rice cultivars. In addition, callus contamination rates were greatly reduced and control was achieved [Ullah et al., 2024]. In other words, the potential of iron nanoparticles to induce callus was supported by other studies.

As a result of the rooting experiment, microshoots grown at different concentrations of two different hormones (IBA and NAA) did not show root formation in the culture medium. In plant tissue culture, several factors may contribute to the unsuccessful rooting of avocado microshoots. These include the use of an incorrect hormone combination, the presence of a rooting-inhibiting structure of the nZVI nanoparticle employed, and the lack of optimisation of the plant growth hormone combination.

This thesis study has identified the *P. americana* Mill. plant as a research priority for *in vitro* NP studies. These plants are not sufficiently studied in literature, yet they are economically important. This study will support further studies on the production of these plants. Furthermore, this study demonstrates that by determining the positive effect of nZVI on avocado plants, it is possible to produce healthier and more productive products of this plant. The use of nanoparticles in plant tissue culture is still an evolving approach. It needs more research and studies to apply NPs at optimum doses and understand the effects. Moreover, the precise impact of nanoparticles on living systems remains largely inconclusive and inconsistent. This study will significantly contribute to our understanding of nanoparticles and facilitate the development of more efficient regulations for their use.



REFERENCES

- Akan S., (2021), "Phytochemicals in avocado peel and their potential uses. Food and Health.", 7(2), 138-149.
- Almeelbi T., Bezbaruah A., (2014), "Nanoparticle-sorbed phosphate: Iron and phosphate bioavailability studies with *Spinacia oleracea* and *Selenastrum capricornutum*", ACS Sustain. Chem. Eng., 2, 1625–1632.
- Bayram S. (2012), "Avokado Yetiştiriciliği", Tarım ve Orman Bakanlığı, BATEM Web Sitesi. http://www.batem.gov.tr/urunler/meyvelerimi_z/avokado/avokado.htm (Data Accessed: 24.06.2024).
- Ben-Ya'aco A., Michelson, E., (1995), "Avocado rootstocks", Horticultural reviews, 381-429.
- Ben-Ya'acov A., (1985), "Selection of avocado rootstocks", South African Avocado Grower's Association Yearbook, 8, 21–23.
- Castro M., Cautin R., Fassio C., Darrouy N., (2003), "Introduction, selection and propagation program for avocado rootstocks and cultivars (Abstracts. A–35)", World Avocado Congress V. 2003, Chile, 120–121.
- Castro M., Oyanedel E., Cautin R., (1995), "In Vitro shoot proliferation in avocado (*Persea americana* Mill.) induced by CPPU", Scientia Horticulturae, 165: 44–50.
- Chindo A. B., Anuka A. J., Gamaniel S. K., (2012), "Toxicity screenings of *Ficus platyphylla* stem bark in rats", Pharmacologia. 3(10), 499–505.
- Custódio L., Charles G., Magné C., Barba-Espín G., Piqueras A., Hernández J. A., Ben Hamed K., Castañeda-Loaiza V., Fernandes E., Rodrigues M. J., (2022), "Application of In Vitro Plant Tissue Culture Techniques to Halophyte Species: A Review", Plants (Basel, Switzerland), 12(1), 126.
- Delfani M., Baradarn Firouzabadi M., Farrokhi N., Makarian H., (2014), "Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers", Communications in soil science and plant analysis, 45(4), 530-540.
- Demircan B., Velioğlu Y. S., (2021), "Avocado: Composition and Effects on Health Avokado: Bileşimi ve Sağlık Üzerine Etkileri", Akademik Gıda, vol.19, no.3, 309-324.
- Duarte P. F., Chaves M. A., Borges C. D., Mendonça C. R. B., (2016), "Avocado: characteristics, health benefits and uses", Ciência rural, 46, 747-75.
- Ehikioya C. O., Osagie A. M., Oimage S. O., Oimage K., Azeke M. A., (2023), "Carbohydrate digestive enzyme inhibition, hepatoprotective, antioxidant and antidiabetic benefits of *Persea americana*", Scientific Reports, 13(1), 284.

El-Temsah YS., Joner EJJ., (2012), "Ecotoxicological effects on earthworms of fresh and aged nano-sized zero-valent iron (nZVI) in soil", *Chemosphere* 89(1):76–82.

El-Temsah YS., Joner EJJ., (2012), "Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil", *Environ Toxicol* 27(1):42–49.

Flores M., Saravia C., Vergara C. E., Avila F., Valdés H., Ortiz-Viedma J., (2019), "Avocado oil: Characteristics, properties, and applications", *Molecules*, 24(11), 2172.

Gil-Díaz M., Lobo M. C., (2018), "Phytotoxicity of nanoscale zerovalent iron (nZVI) in remediation strategies", *Phytotoxicity of Nanoparticles*. Springer, 301-333.

Göldağ R., Doğan M., (2024), "Avokado (*Persea americana* Mill.)'nun Besinsel içeriği, Antioksidan Özelliği ve Potansiyel Sağlık Faydaları", *Karamanoğlu Mehmetbey Üniversitesi Mühendislik ve Doğa Bilimleri Dergisi*. 1398303.

Guha T., Ravikumar K., Mukherjee A., Mukherjee A., Kundu R., (2018), "Nanopriming with zero valent iron (nZVI) enhances germination and growth in aromatic rice cultivar (*Oryza sativa* cv. *Gobindabhog L.*)", *Plant Physiol. Biochem.*, 127, 403–413.

Huang D., Qin X., Peng Z., Liu Y., Gong X., Zeng G., Huang C., Cheng M., Xue W., Wang X., (2018), "Nanoscale zero-valent iron assisted phytoremediation of Pb in sediment: Impacts on metal accumulation and antioxidative system of *Lolium perenne*", *Ecotoxicol. Environ. Saf.*, 153, 229–237.

Jiang D., Zeng G., Huang D., Chen M., Zhang C., Huang C., Wan J., (2018), "Remediation of contaminated soils by enhanced nanoscale zero valent iron", *Environ. Res.* 163, 217–227.

Karn B., Kuiken T., Otto M., (2009), "Nanotechnology and in situ remediation: A review of the benefits and potential risks", *Environ. Health Perspect.*, 117, 1813–1831.

Khodakovskaya M. V., De Silva K., Biris A. S., Dervishi E., Villagarcia H., (2012), "Carbon nanotubes induce growth enhancement of tobacco cells", *ACS nano*, 6(3), 2128-2135.

Kim D. H., Gopal J., Sivanesan I., (2017), "Nanomaterials in plant tissue culture: the disclosed and undisclosed", *RSC advances*, 7(58), 36492-36505.

Kim J.-H., Lee Y., Kim E.-J., Gu S., Sohn E.J., Seo Y.S., An H.J., Chang Y.-S., (2014), "Exposure of iron nanoparticles to *Arabidopsis thaliana* enhances root elongation by triggering cell wall loosening", *Environ. Sci. Technol.* 48, 3477–3485.

Lambardi M., Sharma KK., Thorpe TA., (1993), "Optimization of *in vitro* bud induction and plantlet formation from mature embryos of Aleppo pine (*Pinus halepensis* Mill.)", *In vitro Cell Dev Biol* 29:189-199.

Li X., Yang Y., Gao B., Zhang M., (2015), “Stimulation of peanut seedling development and growth by zero-valent iron nanoparticles at low concentrations”, PLoS ONE, 10, e0122884.

López-Luna J., Nopal-Hormiga Y., López-Sánchez L., Mtz-Enriquez A. I., Pariona N., (2023), “Effect of methods application of copper nanoparticles in the growth of avocado plants”, The Science of the total environment, 880, 163341.

Ma X., Gurung A., Deng Y., (2013), “Phytotoxicity and uptake of nanoscale zero-valent iron (nZVI) by two plant species”, Sci. Total Environ. 2013, 443, 844–849.

Martinez-Pacheco M. M., Suarez-Rodriguez L. M., Hernández-García A., Salgado-Garciglia R., Vidales Fernández I., Ángel Palomares M. E., Lopez-Gomez R., (2010), “*In vitro* propagation of mexican race avocado (*Persea americana* Mill. var. *drymifolia*)”, International Symposium on Micro 923 (pp. 47-52).

Muanya C. A., Odukoya O. A., (2008), “Lipid peroxidation as index of activity in aphrodisiac herbs”, J. Plant Sci. 3, 92–98.

Oluwole F. S., Onasanwo S. A., Olaleye S. B., (2011), “Effects of aqueous and methanolic extracts of *Persea Americana* Leaf (Avocado Pear) on gastric acid secretion in male albino rats”, Eur. J. Sci. Res. 61(4), 474–481.

Ozudogru E. A., Ozden-Tokatli Y., Akcin A. (2005), “Effect of silver nitrate on multiple shoot formation of Virginia-type peanut through shoot tip culture”, *In vitro Cellular & Developmental Biology-Plant*, 41, 151-156.

Pachi V.K., Mikropoulou E.V., Gkiouvetidis P., Siafakas K., Argyropoulou A., Angelis A., Mitakou, S., Halabalaki M., (2020), “Traditional uses, phytochemistry and pharmacology of Chios mastic gum (*Pistacia lentiscus* var. Chia, Anacardiaceae): A review”, J. Ethnopharmacol, 254, 112485.

Park H. J., Kim S. H., Kim H. J., Choi S. H., (2006), “A new composition of nanosized silica-silver for control of various plant diseases”, Plant Pathology Journal, 22(3), 295-302.

Phillips G. C., Garda M., (2019), “Plant tissue culture media and practices: an overview”, *In vitro Cellular & Developmental Biology Plant*, 55, 242-257.

Ramirez-Gil J. G., Lopera A. A., Garcia C., (2023), “Calcium phosphate nanoparticles improve growth parameters and mitigate stress associated with climatic variability in avocado fruit”, Heliyon, 9(8), e18658.

Rico C. M., Majumdar S., Duarte-Gardea M., Peralta-Videa J. R., Gardea-Torresdey J. L., (2011), “Interaction of nanoparticles with edible plants and their possible implications in the food chain”, Journal of agricultural and food chemistry, 59(8), 3485–3498.

Ruttkay-Nedecky B., Krystofova O., Nejdil L., Adam V., (2017), “Nanoparticles based on essential metals and their phytotoxicity”, Journal of nanobiotechnology, 15, 1-19.

Siddiqui M. H., Al-Whaibi M. H., Firoz M., Al-Khaishany M. Y., (2015), "Role of nanoparticles in plants", Nanotechnology and plant sciences: nanoparticles and their impact on plants, 19-35.

Sidorenko A., Gutul T., Dvornikov D., Şeker M. G., Arit T., Gutul E., Vaseashta A. , (2022), "Synthesis of nZVI/PVP nanoparticles for bioremediation applications", Bioremediation Journal, 26(2), 162-170.

Skoog F., (1957), "Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*", Symposia of the Society for Experimental Biology, 11, 118-131.

Subba, S., Gurung, S., Mahato, S. K., Thapa, B., & Chhetri, B. (2023), "Introduction of Avocado (*Persia americana*) Fruits in Eastern Himalaya of India: A Review", International Journal of Economic Plants, 127–131.

Tang S.C.N., Lo I.M.C., (2013), "Magnetic nanoparticles: Essential factors for sustainable environmental applications", Water Research, 47: 2613–2632.

Teja A.S., Koh P.Y., (2009), "Synthesis, properties, and applications of magnetic iron oxide nanoparticles", Progress in Crystal Growth and Characterization of Materials, 55: 22–45.

Torney F., Trewyn B. G., Lin V. S. Y., Wang K., (2007), "Mesoporous silica nanoparticles deliver DNA and chemicals into plants", Nature nanotechnology, 2(5), 295-300.

Tremocoldi M. A., Rosalen P. L., Franchin M., Massarioli A. P., Denny C., Daiuto É. R., Paschoal J. A., Melo P. S., Alencar S. M., (2018), "Exploration of avocado by-products as natural sources of bioactive compounds", PLoS ONE 13(2): e0192577.

TUIK, (2024), "İllere ve yıllara göre Avokado Üretim Miktarı (Ton)", Türkiye İstatistik Kurumu <https://www.tuik.gov.tr> (Data Accessed: 23/06/2024).

Ullah J., Gul A., Khan I., Shehzad J., Kausar R., Ahmed M. S., Batool S., Hasan M., Ghorbanpour M., Mustafa G., (2024), "Green synthesized iron oxide nanoparticles as a potential regulator of callus growth, plant physiology, antioxidative and microbial contamination in *Oryza sativa* L.", BMC Plant Biol 24, 939.

Wang J., Fang Z., Cheng W., Yan X., Tsang P.E., Zhao D., (2016), "Higher concentrations of nanoscale zero-valent iron (nZVI) in soil induced rice chlorosis due to inhibited active iron transportation", Environ. Pollut., 210, 338–345.

Wang P., Lombi E., Zhao F. J., Kopittke P. M. (2016), "Nanotechnology: a new opportunity in plant sciences", Trends in plant science, 21(8), 699-712.

Web 1, (2024), <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr>, (Data Accessed: 24/06/2024).

Weschenfelder C., dos Santos J. L., de Souza P. A. L., de Campos V. P., Marcadenti A., (2015), “Avocado and Cardiovascular Health. Open Journal of Endocrine and Metabolic Diseases”, 77–83.

Yoon H., Kang Y-G., Chang Y-S., Kim J-H., (2019), “Effects of Zerovalent Iron Nanoparticles on Photosynthesis and Biochemical Adaptation of Soil-Grown *Arabidopsis thaliana*”, Nanomaterials. 9(11):1543.



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PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Bayar K. U., Çiftçi, Y. Ö., (2024), “*In Vitro* Propagation of Avocado Nodal Buds With Zero Valent Iron Nanoparticles (nZVI)”, Poster presentation at 1st International Congress on Biotech Solutions for Sustainability Kocaeli, Turkey, 7-10 May 2024.

Bayar K. U., Çiftçi, Y. Ö., (2024), “The Effects of Nanoparticules on *In Vitro* Micropropagation of Avocado (*Persea americana* Mill.)”, Oral presentation at 8th GTU Graduate Research Symposium, Kocaeli, Turkey, 30-31 May 2024.

