T. R.
YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

MICROPROPAGATION OF PETUNIA (*Petunia hybrida*)

M.Sc. THESIS

PREPARED BY: Rebaz Rasul HABAS
SUPERVISOR: Prof. Dr. Musa TÜRKER

VAN-2017

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**MICROPROPAGATION OF PETUNIA** (***Petunia hybrida***)" prepares by Rebaz Rasul Habas under supervision of Prof. Dr. Musa TÜRKER in the department of Molecular Biology and Genetics has been accepted as a M.Sc. Thesis according to Legislations of Graduate Higher Education on….... /....... /.......... With unanimity / majority of votes members of jury.

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THESIS STATEMENT

All information presented in the thesis was obtained according to the ethical behaviors and academic rules frame. And also, all kinds of statement and source of information that does not belong to me in this work prepared in accordance with the rules of theses, were cited to the source of information absolutely.

Signature

Rebaz Rasul HABAS

ÖZET

PETUNYA (*Petunia hybrida*) BİTKİSİNİN DOKU KÜLTÜRÜNDE MİKROÜRETİMİ

HABAS, Rebaz Rasul
Yüksek Lisans Tezi
Moleküler Biyoloji ve Genetik Anabilim Dalı
Danışmanı: Prof. Dr. Musa TÜRKER
February/2017/Sayfa: 54

Bu çalışmada Solanaceae familyası süs bitkisi üyelerinden petunya (*Petunia hybrida*) E.Vilm bitkisinin çimlendirilmiş tohumları kullanılarak doku kültüründe mikroüretimi için etkili bir rejenerasyon protokolu geliştirlmeye çalışılmıştır. Tohumlar Istanbul tohumculuk şirketinden satın alınmıştır.

Tohumlar dış ortamda ve steril kültür ortamında çimlendirilerek özellikle en etkin rejenerasyon materyali olarak gövde ucu ve nod eksplantları kullanılmıştır. Eksplantlar %75-90 oranında rejenere olmuşlardır.

Eksplantlar bazal Murashige ve Skoog (MS) ortamında Benzil Amino Pürüin (BAP), Naftalen Asetik Asit (NAA) ve İndol-3-Asetik Asit (İAA), İndol-3-Bütirik Asit (İBA), Giberellik Asit (GA_3) Bitki Büyüme Düzenleyicilerinin (BBD) değişik konsantrasyon ve kombinasyonları ile desteklenerek rejenere edilmişlerdir. En uzun gövde boyuna sahip bitkiler 1 ml/l BAP + 1 mg/l NAA ile desteklenmiş MS ortamından, eksplant başına en yüksek sayıda bitki 0.6 mg/l BAP + 0.5 mg/l IBA ile desteklenmiş MS ortamından elede edilmiştir.

Eksplantlar üzerinde gelişen gövdeler izole edilerek değişik konsantrasyonlarda GA_3 ile desteklenmiş MS ortamında gövde gelişimleri sağlanmıştır. 5-6 cm boyuna ulaşan gövdeler farklı konsantrasyonlarda İBA ve NAA ile desteklenmiş MS ortamında köklendirilmiştir. Doku kültüründe rejenere dilen bitkiler toprak içeren saksılara aktarılarak dış ortama % 70 oranında uyumları sağlanmıştır.

Anahtar kelimeler: Mikroüretim, *Petunia hybrida*

ABSTRACT

MICROPROPAGATION OF PETUNIA (*Petunia hybrida*)

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M.Sc. Thesis; Molecular Biology and Genetics Department
Supervisor: Prof. Dr. Musa TÜRKER
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An efficient plant regeneration protocol has been developed from *in vitro* germinating seeds of *Petunia hybrida* E.Vilm, an ornamentally important plant in the family Solanaceae different part of plant including shoot tip and node were used as explants taken from germinated seedling *Petunia hybrida.*

Shoot tip and node explants of *Petunia hybrida* were cultured on MS basal medium supplemented with different concentrations of Benzyl amino purine (BAP), 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA).The highest shoot length was obtained from MS medium supplemented with 1 BAP mg/l + 1 NAA mg/l, The highest shoot number (3 shoots/explant) were obtained from MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA.

The isolated shoots were transferred to MS basal medium supplemented with different concentrations of GA_3 ranging from 0.05, 0.2 0.5 and 1 mg/l for shoot elongation. The highest shoot length was recorded from the MS medium supplemented with 0.2 mg/l GA3 +0.2 mg/l BAP to the culture medium is (5.75 cm). Rooting of regenerated shoots were achieved on MS medium supplemented with 0.1-1 mg/1 IBA and NAA. The regenerated shoots with well-developed root system were successfully acclimatized and established in pots containing sterilized peat moss and grown under laboratory. Conditions with 70% survival rates.

Keywords: Micropropagation, *Petunia hybrida*

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2017

Rebaz Rasul HABAS

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with description.

Symbols	Description
cm	centimeter
°C	centigrade
Mg/l	milligram / litter
W/v	Weight/volume

Abbreviation	Description
BAP	Benzyl amino purine
IBA	Indole-3-butyric acid
NAA	Naphthalene acetic acid
IAA	Indole acetic acid
GA_3	Gibberellic acid
NaOCl	Sodium hypochlorite
hr	Hour
HCl	Hydrogen chloride
NaOH	Sodium Hydroxide
pH	Hydrogen potential
UV	Ultraviolet
MS	Murashige and Skoog medium
WT	White medium
B5	Gamborg medium
PGR	Plant Growth Regulator

1. INTRODUCTION

Ornamental plants are grown for decorative purposes in gardens and landscape design projects, as house plants, for cut flowers and specimen display. The cultivation of the plants is called floriculture, forming a major branch of horticulture.

Commonly, ornamental plants are grown for the display of aesthetic features including flowers, leaves, scent, overall foliage texture, fruit, stem and bark, and aesthetic form. In some cases, unusual features may be considered to be of interest, such as petunia and the prominent thorns of *Rosa sericea* and cacti. In all cases, the care of plants are for the enjoyment of gardeners, visitors, and the public institutions.

The economic importance of ornamental plants all over the world has introduced a promising horizon for their mass production and world trade in the floriculture industry was estimated at approximately $44 billion with the Netherlands, Japan and the United States of America (USA) leading global production. The commercial production of ornamental plants is growing and the related market is developing fast in the last decades.

Regenerating ornamental plant in the field or *in vivo* directly causes some problems, such as disease and environmental affect, labor cost and large area of earth etc.

Ornamental plants can be regenerated by tissue culture approach. Because, plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of the known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

-The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits, and to quickly produce mature plants, the production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds, and the regeneration of whole plants from plant cells that have been genetically modified.

The production of plants in sterile containers that allow them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens,the production of plants from seeds that otherwise have very low chances of germinating and growing,

i.e.: orchids and *Nepenthes*.to clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Plant tissue culture has come up with an approaching tool and signifies the backbone of plant biotechnology. The better quality planting material is a basic need of growers for boosting productivity. Tissue culture is the propagation of a plant by using a plant part or single cell or a group of cells under aseptic conditions.

Plant tissue culture, also called micropropagation, is a practice used to propagate plants under sterile conditions or in a controlled environment, often to produce clones of a plant. micropropagation is now a well-implanted technology. Like many other technologies, micropropagation was exploited as a research tool and focused on endeavors to culture and study the development of small, isolated section of plant tissues or isolated cells.

The technique was showed as the universal mass organism plant propagation system for the future and the term ‘micropropagation’ was introduced to identify the more accurately to the processes. Many commercial laboratories were established around the world for the mass clonal propagation of horticultural plants (Idowu et al., 2009).

Micropropagation is an alternative method of vegetative propagation, which is well suited for the multiplication of elite clones. It is accomplished by several means, multiplication of shoots from different explants such as shoot tips or axillary buds.

Micropropagation generally involves four distinct stages: initiation of cultures, shoot multiplication, rooting of *in vitro* grown shoots, and acclimatization (Rout et al., 2006).

Each year millions of ornamental plants are produced by *in vitro* culture. Regeneration of ornamental plants *in vitro* has been achieved in many species on culture medium containing auxins and cytokinins, and from various explant sources including tuber segments, shoot tips, stems (node and internode), leaf tissue, peduncles and floral parts.

Around the mid of twentieth century, the notion that plants could be regenerated or multiplied from either callus or organ culture was widely accepted and practical utilization in the plant propagation industry. *Petunia hybrida* belongs to the plant family *Solanaceae:* and is an important ornamental and medicinal plant, several species of *Petunia* sp are ornamentals grown-up in gardens. It is measured to be the first bedding plant; in addition, *Petunia* is the most common among the new developed cultivars (Thenmozhi and Sivaraj, 2011).

Petunia hybrida having colorful flowers and a long florescence; it shows high ornamental value and now is widely cultivated throughout the world. Furthermore, it has become an important model plant.

1.1. Seasons /Availability

The plants are herbaceous and the flowers are funnel-shaped, with petals joined together. The fruit is a dry capsule with two compartments and many tiny seeds,

1.2. Current facts

Petunias can tolerate relatively harsh conditions and hot climates, they need at least five hours of sunlight every day. They grow well in low humidity, moist soil. In drier regions, the plants should be watered daily. Maximum growth occurs in late spring. Petunia short life cycle, clear genetic background, smaller genome and simple cell and tissue operation technique.

1.3. Applications

Petunia hybrida is an important floriculture ornamental crop of high commercial interest. *P.hybrida* is an economically important ornamental plant species. Petunias are among the top 10 bedding plants produced in the United States. In 2008, the 15 largest floriculture-producing states collectively sold 34.5 million flats, pots and hanging baskets at a total wholesale value of $120 million.

Petunia is a mild-acting medicine possessing anti-microbial and shows the mildest antioxidation activity. *Petunias* are ornamentals grown in gardens for their large, showy multicolored flowers and it is also an important cut flower crop. Breeding of petunias began nearly 30 years after the plant’s discovery. Many species other than *P. atkinsiana* are also gaining popularity in the home garden. A wide range of flower colors, sizes, and plant architectures are available in both *P. atkinsiana* and other species.

1.4. Geography / history

Petunia genus is consists of 35 species of flowering plants origination from American, closely related to tobacco, cape gooseberries, tomatoes, deadly nightshades, potatoes, and chili peppers in the same family, *Solanaceae.* The popular flower of the same name derived its epithet from the French, which took the word *petun*, meaning "tobacco," from a Tupi–Guarani language. An annual, most of the varieties seen in gardens are hybrids (*P.* × *atkinsiana*, also known as *P.* × *hybrida*).

Because of economical and ornamental importance of the plant it is necessary to produce *in vitro*. Up to date no more studies available in the literature to regenerate the plant in controlled laboratory location. Therefore an efficient and permanent *in vitro* protocol is necessary for the plant.

2- LITERATURE REVIEW

Petunias are herbaceous perennials, usually grown as annuals that have become one of our most popular garden plants. The petunias that we grow today comprise a large family of hybrids derived from many species including *Petunia axillaris*, *P.violacea,* and *P. inflata*. Petunias are classified into two categories; *Grandiflora* (large-flowered) and *mulitflora* (many-flowered).

The basic petunia flower is funnel shape, but hybridizers have created many variations including singles and doubles with petals that have wavy or fringed margins. Many patterns are available in stripes, speckles and borders in an extensive color palette that includes purple, mauve, lavender, pink, red, white and yellow. Leaves and stems are sticky to the touch and have a distinctive odor.

The plants are herbaceous, generally hairy, and the flowers are funnel-shaped, with petals joined together. The fruit is a dry capsule with two compartments and many tiny seeds.

Petunias can tolerate relatively harsh conditions and hot climates. They need at least five hours of sunlight every day. They grow well in low humidity, moist soil.

Young plants can be grown from seeds. In drier regions, the plants should be watered daily. Maximum growth occurs in late spring. Applying fertilizer monthly or weekly, depending on the variety, will help the plant grow quickly. Petunias can be cultivated in hanging baskets.

In horticulture many terms are used to denote different types of cultivated petunias. These include Grandiflora, Multiflora, Wave (Spreading), Supertunia, Cascadia, and Surfinia.

Petunia is a genus in the family *Solanaceae*, subfamily *Petunioideae.* Well known members of *Solanaceae* in other subfamilies include tobacco (*Nicotianoideae*),

2.1. Taxonomic Classification of *Petunia haybrida*

Kingdom: *Plantae*
Sub kingdom: *Trachiobiota*
Divission: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Solanales*
Family: *Solanaceae*
Genus: *Petunia*
Species: *P. hybrid*
Common name: *Petunia*

The concepts of plant tissue culture are the growth or maintenance of plant cell, tissue, and organs separated from the donor plant on artificial media under aseptic conditions. It includes techniques and methods used for research in many botanical disciplines and has several practical applications (George et al., 2008).

In vitro propagation for ornamental industry has been used as an approach for large-scale plant multiplication of desired varieties. At *in vitro* conditions, plantlets are grown under controlled environment in sterile formulated medium which contain macronutrients, micronutrients, vitamins and plant growth regulators. After the plantlets reach an optimum growth in the culture containers after a certain growth period, they can be transferred to *ex vitro* conditions to allow continuous growth and development of the plantlets (Ahmed et al., 2012).

The theoretical basis for *in vitro* micropropagation was explained the capacity of the separate undifferentiated plant cells to regenerate into an entire plant is termed as totipotency (Cassells and Gahan, 2006). As well as (Sharma and Shahzad, 2013) described totipotency as a phenomenon for plant cells, pieces of leaves stems or roots are capable of performing all the function of development, which are the characteristics of zygote and can often be used to generate a new plant on culture medium given the required nutrients and plant hormones. Furthermore, plant tissue culture is also useful for plant propagators, to achieve mass production of a wide variety of plants especially ornamentals at commercial scales (Flick et al., 1983).

Plant micropropagation also provides around year production independent of season. As it is can be conducted throughout the year, production cycle can be scheduled to meet peak demands (Lineberger, 2002). The explants are taken from a single donor plant, that itself remains uninjured in the tissue harvesting process (Ayenew et al., 2012). Moreover, Plantlets produced by tissue culture are applied for production of pathogen-free plants (Kaviani et al., 2011).

Mulabagal and Tsay, (2004) showed that tissue culture allows the regeneration of large numbers of plants from small pieces of stock plants in a shorter period of time. Culture media, light, and temperature could be more effectively controlled to manipulate the growth, multiplication and regeneration. As well as, plants can be stored *in vitro* in a small space and less labor is required for maintenance of stock plants**.** The process of plant micropropagation aims to produce clones as a clonal propagation.

2.2. *Petunia hybrida*

The South American genus Petunia (*Solanaceae*) consists of about 30 species.The name Petunia is derived from the Brazilian word for tobacco: petun (Sink, 1984). Petunia is one of the most sold bedding plant species. It is probably most economically important in terms of worldwide use as an ornamental plant (Sink, 1984). petunia With respect to plant type, most plants are single-flowered Grandifloras respect to flower color, pink, rose, salmon and red are most popular Furthermore, Petunia plants are widely used a model system for molecule floral development (Quattrochio et al., 1993).

Petunia, which is believed to be the first cultivated bedding plant, is now the most preferred flower crop of the modem bedding plant industry. Petunias can be found in every color of the rainbow in solids, contrasting waves or hedges and star patterns. The flowers may be large or small, ruffled, fringed or double. They bloom from spring until frost. Many petunias, especially white and lavender cultivars have a very sweet fragrance (Sabitha et al., 2009).

2.3. Micropropagation of *Petunia hybrida*

Micropropagation is the modern technique of propagation and developed plants to supply the requirement of the horticultural industries including nursery industries, for pot plant production and homogenous plant size (Maliro and Lamarck, 2004).

To increase the rate of ornamental plants propagation, researchers investigated to find suitable conditions for cultivation, the time suitable for collection of cuttings and the optimal concentration of growth regulators required for the induction of organogenesis (Burritt, 1992). Extensive studies have been conducted on various aspects of the *in vitro* culture of *Petunia hybrida.*

Expanded leaves from both seedlings and regenerated shoots were taken as explants. Explants were placed on MS media supplied with different concentrations and combinations of the plant growth regulators (PGRs) (Abu-Qaoud, 2016).

The production of petunias through breeding is a costly affair and labour oriented. F1 hybrids of petunia are very attractive but there are some limitations in growing such F1 hybrids from seeds, like non-availability of quality hybrid seeds possessing genetic purity.

This necessitates vegetative methods of propagation such as using stem cuttings to maintain the parental lines. However, this method of propagation is not very successful due to problems like low multiplication rate, very slow rooting and susceptibility to tobacco mosaic virus (Sabitha et al., 2009).

In vitro propagation is the only alternative technique which can overcome such problems. It also offers the possibility of multiplying large number of true to type plants in a very short period. The application of tissue culture technique to petunia started in the late 1960's with the work of (Binding, 1971; Sink, 1984).

A moderate rate of multiplication of petunia through tissue culture has been reported earlier through direct organogenesis from shoot apices (Sharma and Mitra, 1976) and through indirect organogenesis from leaf bits and stem segments (Rao et al., 1973) and shoot tips (Dash and Singhsamant, 1990).

2.4. Shoot Initiation stage

Initiation of explants is the first step in micropropagation. This stage refers to the inoculation of the explants without any contamination to be the source of shoot growth on sterile medium to initiate aseptic cultures. Explant initiation in an aseptic condition should be regarded as a critical step in micropropagation (Ahloowalia et al., 2002), many commercial ornamental plants are being propagated by *in vitro* culture on the culture media containing auxins and cytokinins (Laimer and Rücker, 2012).

The effect of different levels of naphthaleneacetic acid (NAA) and benzyl adenine (BA) on shoot multiplication and regeneration of *Petunia hybrida* was studied. Seeds of *Petunia hybrida* were germinated *in vitro* on MS basal medium. The seedlings were used as a source of explants for the multiplication and regeneration experiments (Abu-Qaoud et al., 2010).Growth regulators induce the competence of tissue to respond to further developmental signals.

This present was performed to study the effects of different explants (cotyledons, hypocotyls, shoot tips, and nodal segments) and various concentrations of Benzyl amino purine (BAP) in combination with Indole-3-acetic acid (IAA) on shoot regeneration.

2.4.1 Explants

An explant is the small part of plants (shoot and root tips, leaf tissues, anthers, nodes, meristems and embryos that are used to start *in vitro* cultures, and used for initiating a culture.

Shoot meristem. Multiplication is generally used for producing virus free material and maintaining germplasm via cryopreservation. Several micropropagation protocols have been reported from shoot tip explants. (Kumar et al., 2011).

In vitro multiple shoot regeneration of *Solanum nigrum* L. was accomplished on MS medium utilizing shoot tip and nodal explants. Direct multiple shoots differentiated within 6 weeks when explants were cultured on MS medium containing BAP and KIN (Kavitha et al., 2012).

Multiple shoot regeneration from shoot tip and axillary buds has showed to be a potential tool for mass multiplication of plants *in vitro.* In the present study, shoot proliferation from shoot tip and axillary bud explants and elongation and *in vitro* rooting of the microshoots were significantly influenced by the growth regulators used (Sabitha et al ., 2009).

2.4.2 Surface sterilization of seeds

Sterilization is defined as the process where all the living microorganisms, including bacterial spores, are killed. Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemical sterilants.

Surface sterilization of ornamental plants is difficult as they lack a thick protective surface, and therefore sodium hypochlorite and similar agents can easily damage the delicate tissues. The scope of these techniques has been extended for use in bioprocess technology for production of high value chemicals of immense commercial value in the pharmaceutical and nutraceutical sectors (Munoz et al., 2006).

The sterilants are also toxic to the plant tissues, hence the proper concentration of sterilants, duration of exposing the explants to various sterilants, the sequences of using these sterilants has to be standardized to minimize explants injury and achieve better survival (Badoni and Chauhan, 2010).

The maintenance of sterile conditions are for successful tissue essential culture procedures. Therfore all culture vessels, media, and instruments used in handling tissues, as well as explant itself must be sterilized. The importance is to keep the air, surface and floor free of dust. All operations should be carried out in lamina airflow sterile cabinet (Chawla, 2004).

Sterilization process of explants depends on the plant species and types of explants for establishment of healthy cultures, the explants must sterilized be Contamination free disinfectants widely used are sodium hypochlorite, calcium hypochlorite and ethanol (Ndakidemi at al., 2013).

Explants of ornamental plants with lack a thick protective surface have difficult surface sterilization, and therefore sodium hypochlorite and similar agents can easily damage the delicate tissues (Ahmed et al., 2012).

A wide range of surface disinfectants, such as ethanol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate and antibiotics are used for surface sterilization however, sodium hypochlorite (NaOCl) has been most widely used. NaOCl is highly effective against all kinds of bacteria, fungi, and viruses (Yildiz, 2012; Spaulding et al., 1977).

In vitro seed germination, seedling growth and the viability of the tissue was negatively affected by sodium hypochlorite (NaOCl) at high concentrations (Yildiz and Er, 2002), (Hsiao and Hanes, 1981, Hsiao and Quick, 1984).

Sterilization process under *in vitro* conditions should aim to use the lowest concentration of disinfectant for the shortest time (Yildiz, 2012).

2.5. Multiplication stage

This is the propagation phase in which the explants are placed on a medium with plant growth regulators that enhance multiplication of shoots. At this stage, the number of propagules is multiplied by repeated subculture until the desired number of plants is attained. Sometimes, it is necessary to subculture the *in vitro* derived shoots onto different media for elongation (Ahloowalia et al., 2002).

This stage considered as an important stage in any propagation system, which determine the success or the failure of the propagation. On the other hand, the total number of produced plants for any species depends on this stage (Al-Rifae'e and Al-Shobaki, 2002)

Multiplication stage is the propagation phase in which the explants are cultured on the appropriate media for multiplication of shoots. The primary goal is to achieve propagation without losing the genetic stability. Repeated culture of axillary and adventitious shoots, cutting with nodes, somatic embryos and other organs from Stage I leads to a multiplication of propagules in large numbers.

The propagules produced at this stage can be further used for multiplication by their repeated culture. Sometimes it is necessary to subculture the *in vitro* derived shoots onto different media for elongation. (Ahloowalia et al., 2002).

2.5.1 Effect of Cytokines on Shoot Multiplication

The most important physiological role of cytokinins when added to nutrient media is the promotion of cell division and expansion, buds induction, differentiation, releasing lateral buds from the dominance of terminal buds and inhibition of senescence (Ahmed et al., 2012).

The possibilities of *in vitro* vegetative propagation of almond (*Amygdalus communis* L.) cv. Texas and cv. Nonpareil by shoot-tip culture were investigated. Different levels of IBA (0.0, 0.1 and 0.5 mg/l) and BAP (0.0, 0.5, 1.0, 2.0 and 3.0 mg/l) were tested by observing shoot development and growth during three successive stages, namely initiation, transplantation and multiplication (Gürel and Gülşe, 1998).

Li et al., (2013) improved two optimal media for *in vitro* culture of microspores and germination of mature pollen in *Petunia hybrida*. The effect of different levels of naphthaleneacetic acid (NAA) and benzyl adenine (BA) on shoot multiplication and regeneration of *Petunia hybrida* was studied. Seeds of *Petunia hybrida* were germinated *In vitro* on MS basal medium (Abu-Qaoud et al., 2010).

In vitro multiple shoot regeneration of *Solanum nigrum* L., an Indian medicinal plant was accomplished on MS medium utilizing shoot tip and nodal explants. Direct multiple shoots differentiated within 6 weeks when explants were cultured on MS medium containing BAP (1.0 – 5.0 mg/l) and KIN (1.0 – 5.0 mg/l) individually (Kavitha et al., 2012).

2.5.2 Direct shoot Regeneration

Direct regeneration without undesirable callus formation shortens the time period needed for regeneration and reduces the possibility of the occurrence of somaclonal variability (Košir et al., 2004).

The propagation phase in which the explants are cultured on the appropriate media for multiplication of shoots. The primary goal is to achieve propagation without losing the genetic stability. Repeated culture of axillary and adventitious shoots, cutting with nodes, somatic embryos and other organs from initiation stage leads to multiplication of propagules in large numbers. The propagules produced at this stage can be further used

for multiplication by their repeated culture. Sometimes it is necessary to subculture the *in vitro* derived shoots onto different media for elongation (Prakash and Brinks, 2004).

The objective of multiplication of the shoot stage is to achieve rapid proliferation of shoots which can ultimately give rise to whole plants. The addition of various plant growth regulators and other nutrients to the medium can often control the growth and developments of the cultured tissues. However, specific requirements seem to depend primarily on the species and the physiological age of the original explant. (Warrag, 1990).

2.6. Root formation stage

Auxin is the active ingredient in most rooting mixtures. These products help the vegetative propagation of plants. On a cellular level auxins influence cell elongation, cell division and the formation of adventitious roots. Some auxins are active at extremely low concentrations (Trewavas, 1981).

In vitro rooting on micro-shoots that are produced during multiplication stage are usually transferred to a rooting medium to form complete plantlets (Duhoky, 2012).

Various types of auxins are usually used in rooting micro-shoots and common auxins used in tissue culture the most are IAA, IBA, and NAA. In general, a high auxin and low cytokinins ratio enhances the formation of adventitious roots (Hartmann et al., 1997).

Auxins have clear roles in many developmental processes including promoting cell growth, cell division, induction of the injured tissues to several layers of cells recovering the ability to divide to form callus and promote rooting (Ahmed et al., 2012). Furthermore, there are many differences in metabolism and transport of different auxins, which affect their response or physiological effects on adventitious root formation (Epstein and Miller, 1993).

The effect of different levels of naphthaleneacetic acid (NAA) and benzyl adenine (BA) on shoot multiplication and regeneration of *Petunia hybrida* was studied. Regenerated shoots from leaf explants, the regenerated adventitious shoots were cultured on MS medium without growth regulator. These shoots were then rooted (Abu-Qaoud et al., 2010).

Raja et al., (2015) investigated the influence of shoot tip explant and growth regulators were obtained from the shoot tip culture on MS medium supplemented with BAP (2.5mg/l). The shoots were rooted on MS basal medium supplemented with IBA (3.0 mg/l).

Nisha et al., (2009) employed well-developed microshoots of *Begonia malabarica* generated through axillary-bud proliferation and leaf callus were excised and cultured on MS medium with the addition of different concentrations of auxins for root induction. They found better results on the medium containing IAA or IBA.

2.6.1 Effect of MS Salt Strength

Relatively low salt concentrations in the medium are known to promote rooting of microshoots (skirvin and Chu, 1979). (Hasegawa, 1980) noted that many roses have been rooted well in diluted medium, and half or quarter strength MS salt concentrations often induced rooting. Rooting can take place in full strength culture medium, but is a very common practice to move the shoots to be rooted from high strength media to less concentrated solution. This practice is used for herbaceous plants as well as woody ornamentals, fruit trees and forest species (Murashige and Skoog, 1962). The promoting effect of mineral concentration of the culture medium on rooting can be attained as inorganic ions, which participate in the process of regulating hormonal balance (Amzallag et al., 1992).

Khosh-Khui and Sink, (1982) demonstrated that rooting response was better in half strength MS salt.

Sairkar et al., (2009) reported that the best results for root formation was achieved by using half strength of MS Medium containing activated charcoal.

2.7. Acclimatization stage

At acclimatization stage, the *in vitro* produced plantlets are transferred from heterotrophic phase (sugar-requiring) to autotrophic phase. After forming fully functional root system, they stomatal and cuticular transpiration is completed; the

gradually transplanted plants can survive and grow vigorously after being transferred to soil (Ahmed et al., 2012).

At this stage, the *in vitro* micropropagated plants are weaned and hardened. This is the final stage of the tissue culture operation after which the micropropagated plantlets are ready for transfer to the greenhouse. Steps are taken to grow individual plantlets capable of carrying out photosynthesis (Ahloowalia and Savangikar, 2004).

The hardening of the tissue-cultured plantlets is done gradually from high to low humidity and from low light intensity to high intensity conditions. If grown on solid medium, most of the agar can be removed gently by rinsing with water. Plants can be left in shade for 3 to 6 days where diffused natural light conditions them to the new environment.

The plants are then transferred to an appropriate substrate (sand, peat, compost, etc.), and gradually hardened. Low-cost options include the use of plastic domes or tunnels, which reduces the natural light intensity and maintains high relative humidity during the hardening process. Is the phase where *in vitro* grown plants are transferred to the greenhouse *In vitro* grown plants are produced in high humidity, which can result in less epicuticular wax on the leaves. The lack of epicuticular wax can result in a rapid loss of water for tissue culture plants when placed in a lower humidity environment (Sutter and Langhans, 1979).At acclimatization stage normally consists of an acclimatization step in which the plantlets are acclimated to a lower humidity environment. If stage III plants have been grown on a medium containing agar, the agar is usually washed away from the roots before transplanting to an appropriate rooting medium. After the plants are transplanted, they are usually kept in high humidity environment (e.g. a mist tunnel) for several days and from there they are moved to the greenhouse (Greyvenstein, 2009). Other treatments of *in vitro* plantlets upon transplanting to the outside environment in high phosphate fertilizer to enhance their vigor, fungicides to prevent diseases (Debergh and Read, 1991).

3. MATERIAL AND METHODS

All experiments were carried out in the Molecular Biology and Genetic Department, Faculty of Science University of Yüzüncü Yıl, Van, Turkey. During the period from June 2015 to February 2016.

Figure 3.1. Different steps of plant tissue culture technique in laboratory at the Faculty of Science, University of Yuzuncu Yıl.

3.1. Plant Material

Different explants including shoot tip, nodes were used as explants in this investigation taken from healthy *Petunia hybrid* plantlets germinated from seeds. The seeds were purchased from (Istanbul Tohumculuk, Turkey) Company.

3.2. Sterilization

Sterilization referring to any process that eliminates or kills all forms of life and other biological agents including transmissible agents (such as fungi, bacteria, viruses, spore forms. Sterilization can be achieved with one or more of the following: heat, chemicals, irradiation, high pressure, and filtration.

3.2.1 Seed Sterilization

Each plant seeds may carry covered by bacteria, fungi and reproductive cells. The sterilization time and component are dependent on size and seed structure. Hypochlorite is considered to be the most effective and powerful killer of microorganisms particularly bacteria, even minimum concentrations of it are enough to reduce bacteria. To establish health petunia cultures, different concentrations of NaOCl in time of durations (4, 6, 8 and 10% for 5, 10 minutes) were used for seeds surface sterilization. As a result the best sterilizer at a suitable concentration and duration was determined. The plant seed must be sterilized before placed on culture medium under laminar air flow cabinet.

After sterilization, the seeds were thoroughly rinsed several times with sterile distilled water before being inoculated in the culture medium to remove the traces of the chemicals.

3.2.2. Equipment Sterilization

All culture vessels and glassware including beakers, cylinders, jars, test tubes and petri dishes used in this study were carefully washed with detergent, then two times with distilled water and then all these glassware were autoclaved.

Furthermore, forceps and scalpels were first washed and dried using tissue paper and then autoclaved before use. It is worth mentioning that all the equipment were carefully wrapped with aluminum foil before autoclaving. For sterile water, distilled water was filled up in a bottle and autoclaved.

The cap was let loose during autoclaving and tightened after removal.

Surface of the laminar flow was also swabbed with cotton onto which 70% ethanol alcohol was sprayed. In addition, the working surface of the laminar flow was often sprayed with 70% ethanol and wiped with cotton before using the laminar flow cabinet.

The researcher wear gloves and hands were carefully washed before the process and they were starting continuously sprayed with 70% ethanol during the process. Forceps and scalpels were dipped in absolute ethanol and flamed until red hot, allowed to cool and then used. After opening the caps, the rim of the culture vessels was flamed. Then the explants were cultured inside the culture vessels, their rim was flamed again before closing the cap.

3.3. Preparation of Culture Medium

Plant culture medium was prepared from commercially available premixed powders. The initial medium used for growth in this study was Murashige and Skoog (1962) (Table 3.1) and Gamborg's B5 media (Table 3.3) and White medium (Table 3.2). The media were purchased from Duchefa.

For seed germination PGR free media which contain all nutrients necessary for plant growth and development were used to produce pure explants for *in vitro* culture.

As a pre-mixed medium, 30 gl/l sucrose was added as an energy source and 6 g/l agar was added as a gelling agent (Duchefa). Then the pH was adjusted to 5.7±0.1 with 1N NaOH or HCl. The whole components were dissolved on hotplate magnetic stirrer and the medium was dispensed into culture flasks at a rate of 25 ml and caped with polyethylene or aluminum foils and autoclaved at 121°C and 1.5 atmosphere for 20 min. The flasks were cooled and agar solidified under room temperature to be ready for culture.

Table 3.1 Murashige and Skoog Basal Medium Compassion.

Ingredients	milligrams/liter
Ammonium nitrate	1,650.0
Boric acid	6.20
Calcium chloride (anhydrous)	332.20
Cobalt chloride hexahydrate	0.0250
Cupric sulfate pentahydrate	0.0250
Disodium EDTA dehydrate	37.260
Ferrous sulfate heptahydrate	27.80
Glycine	2.0
Magnesium sulfate (anhydrous)	180.70
Manganese sulfate monohydrate	16.90
Myo-Insoitol	100.0
Nicotinic acid	0.50
Potassium iodide	0.830
Potassium nitrate	1,900.0
Potassium phosphate monobasic	170.0
Ioride pyridoxine	0.50
Sodium molybdate dehydrate	0.250
Thiamine hydrochloride	0.10
Zinc sulfate heptahydrate	8.60
TOTAL gm/litre	**4.736**

The culture media usually contain the following constituents:

1. Inorganic nutrients
2. Carbon and energy sources
3. Organic supplements
4. Growth regulators
5. Solidifying agents
6. PH of medium

3.4. Seedling culture

The seeds were rinsed three times for five minutes in sterile distilled water. Sterilized seeds will be planted in 16 Petri dish (1.6 x 9 cm) each containing 25 ml of medium. Seeds were cultured on three different hormone free media types: MS Murashige and Skoog, (1962) basal medium, and Gamborg basal medium (B5 0): and white basal medium (WM 0). Ten seeds were germinated to the plant in each petri dish.

The double distilled water was used for the preparation of culture media after addition of all the media constituents. The pH will adjust to 5.8 using NaOH or HCl. The medium is autoclaved at 121° C at a pressure of 1.5 atmosphere for 25 min. The cultures were incubated in a growth room, maintained at 25°± 2 C, and a 16/8-h photoperiod, was provided by cool white fluorescent lamps (Phillips Canada, Scarborough, Ont.). Several explants were taken from the seedlings 21 days after initial culture.

3.5. Shoot multiplication stage

For multiplication stage. Explants were prepared under aseptic conditions from pre-sterilized material from the best concentration of the sterilant and exposure duration. The outside edges were usually removed, since these were likely to be damaged by the sterilants.

The tissue was cut into pieces using a sharp scissors to give relatively uniform explants of an appropriate size under the confines of a Laminar-air-flow hood. Two explants were cultured on each culture vessel. Shoot tip were cut aseptically approximately in 1 cm and lateral buds in 1 cm long then initiated on MS medium supplemented with 3 levels of BAP (6- Benzyl aminopurine) (0.3, 0.5, 1 mg/l) with 2 levels of NAA (0.5, 1 mg /l) and 2 levels of IBA (Indole-butyric acid) (0.5, 1 mg/l) for shoot induction. The explants were inoculated on the medium and then placed in the primary growth room under 25±2 ºC exposed to 16/8 h photoperiod provided by cool white fluorescent (Philips Canada, Scarborough, Ont.).

The photoperiod was maintained by an automatic timer system. Observations on the percentage of responded explants were recorded after four weeks from culturing.

3.6. Shoot elongation stage

After four weeks of culture, developed shoots in the petri dish were subcultured and long explants were inoculated in culture jars (5 x 10 cm) in MS basal medium supplemented with in different concentration and combination of BAP and GA_3. Plant shoots were maintained for 3 weeks in elongation medium. Culture jars were capped and incubated under the above mentioned culture conditions. Cultured explants were observed regularly and data were recorded. Elongation, leaf number and node length were measured.

3.7. *In Vitro* Root Induction Stage

In vitro raised shoots in 2 cm and above were excised from the culture tube or culture bottle and subcultured into MS medium containing different concentration of IBA and NAA for root induction. 0.2, 1 mg/l NAA and 0.2, 1 mg/l IBA and 1 mg/l NAA were added separately to MS medium and kept 4 weeks to obtain roots.

3.8. Hardening and Acclimatization

Plantlets with well-developed shoot (5-6 cm) and roots (4-5 cm) were removed from the culture medium and washed thoroughly in running tap water. They were transferred to plastic pots containing sterile soil rite under diffuse light (16/8 h photoperiod) conditions. Potted plantlets were covered with transparent plastic bags to ensure high humidity and watered every three days for two weeks. Plastic bags were removed after two weeks in order to acclimatize the plants to field conditions. After four weeks, acclimatized plants were transferred to the pots containing normal garden soil and maintain in greenhouse under normal day length conditions.

Table 3.2 White Medium w/v Vitamins and Sucrose.

Ingredients	**milligrams/litre**
Potassium nitrate	80.00
Calcium nitrate	221.96
Magnesium sulphate	360.00
Sodium phosphate monobasic	18.98
Potassium chloride	65.00
Sodium sulphate	200.00
Manganese sulphate.H_2O	5.04
Boric acid	1.50
Potassium iodide	0.75
Molybdenum trioxide	0.001
Zinc sulphate.7H_2O	2.67
Copper sulphate.5H_2O	0.01
Ferrous sulphate.7H_2O	2.50
myo - Inositol	100.00
Thiamine hydrochloride	0.10
Pyridoxine hydrochloride	0.10
Nicotinic acid (Free acid)	0.50
Glycine (Free base)	3.00
Sucrose	20000.00
TOTAL g/litre	**21.06**

Table 3.3 Gamborg B5 Medium w/ CaCl2, Vitamins and Sucrose.

Ingredients	**milligrams/litre**
Potassium nitrate	2500.00
Ammonium sulphate	134.00
Calcium chloride.2H_2O	150.00
Magnesium sulphate	122.09
Sodium phosphate monobasic	130.42
Manganese sulphate.H_2O	10.00
Boric acid	3.00
Potassium iodide	0.75
Molybdic acid (sodium salt).2H_2O	0.25
Zinc sulphate.7H_2O	2.00
Copper sulphate.5H_2O	0.025
Cobalt chloride.6H_2O	0.025
Ferrous sulphate.7H_2O	27.80
EDTA disodium salt.2H_2O	37.30
myo - Inositol	100.00
Thiamine hydrochloride	10.00
Pyridoxine hydrochloride	1.00
Nicotinic acid (Free acid)	1.00
Sucrose	20000.00
TOTAL g/litre	**23.23**

4. RESULTS

4.1. Seeds Sterilization and Germination

P.hybrida seeds were surface sterilized to remove bacteria and fungi. Seeds were disinfected with sodium hypochlorite detergent at different concentrations of commercial bleach % (4, 6, 8 and 10) for 10 and 5 minutes.

4.1.1. Effect of different NaOCl concentration in different periods

Table (4.1) Shows that seeds gave significantly higher record for regeneration at 90% when they were treated with 8% of NaOCl for 5 minutes as compared with the other treatments.

Table 4.1. Effect of different NaOCl concentration for 5 minutes on the survival percentage of seeds.

% NaOCl	% seed germination	% contamination
4	0	100
6	92	50
8	90	0
10	20	0

Table (4.2) Shows that seeds gave significantly higher germination record at 75% when they were treated with 8% of NaOCl for 10 minutes as compared with the other treatments. Other ways if the seeds were applied to 10% of NaOCl the survival rate of seeds were recorded 0.0%, after rinsed three times by double distilled water, none of the seeds were germinated because of the high concentration rate of NaOCl. The seeds were cultured on three different type of media MS, B5 and Wt.

Table 4.2 Effect of different NaOCl concentration for 10 minutes on the survival percentage of seeds.

% NaOCl	% seed germination	% contamination
4	10	80
6	85	30
8	75	00
10	00	00

4.1.2. Effect of different media on Seed germination.

Figure (4.1) Shows that the significantly higher germination was recorded in MS medium with 90% following 70% germination in B5 and 50% germination in White medium.

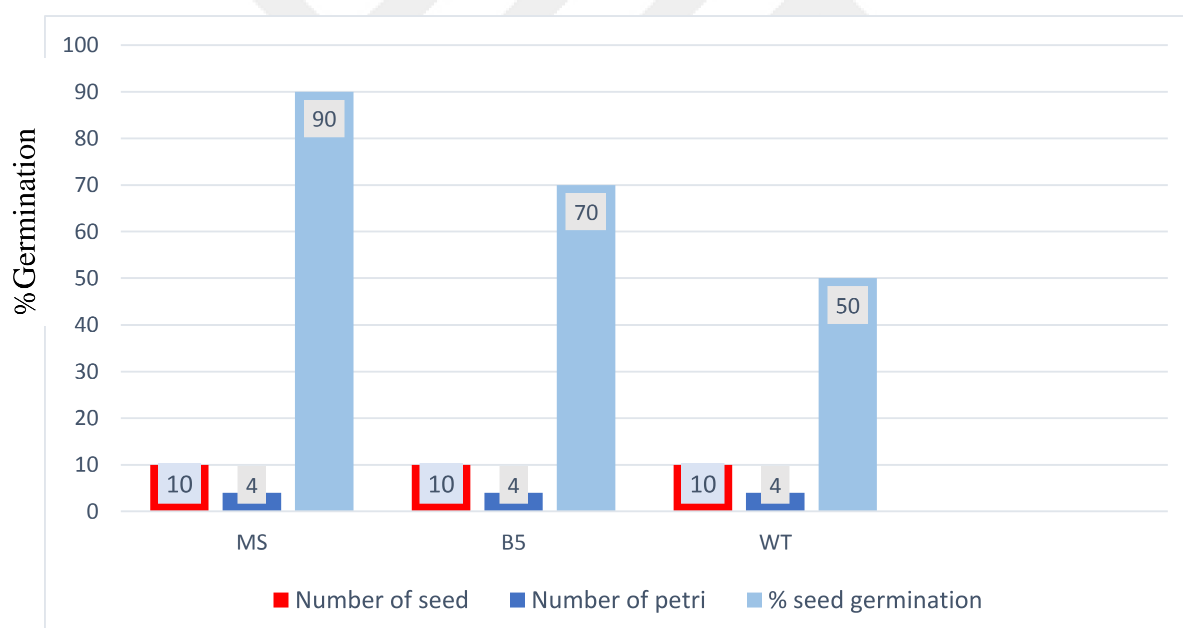

Figure 4.1 Effect of different medium types to seed germination.

4.2. Multiplication Stage

During this stage, multiple shoots were directly induced when shoot tips cultured on MS medium supplemented with different concentration of BAP with NAA and IBA, (Figure 4.1). The highest length of shoots was (2.25 cm) recorded in MS medium supplement with 1 mg/l BAP + 1 mg/l NAA, but the lowest length was recorded (0.25 cm) in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l IBA.

The highest number of shoots (3 shoots/explant) were obtained from MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA, but the least numbers of shoots were recorded (1 shoots/explant) in MS medium supplement with 1 mg/l BAP + 0.5 mg/l NAA, 0.6 mg/l BAP + 0.1 mg/l IBA and 0.3 mg/l BAP + 0.1 mg/l IBA. The highest number of leaves (15 Leaves /explant) was achieved by the addition of 1 mg/l BAP + 1 mg/l NAA. The lowest number of leaves were obtained from (3 leaves/ explant) MS medium supplement with 0.6 mg/l BAP + 0.5 mg/l NAA and 0.6 mg/l BAP + 0.5 mg/l IBA.

The maximum number of roots were recorded (15 roots/explant) in MS medium supplement with 0.3 mg/l BAP + 0.1 mg/l IBA. But at the same time the lowest number of roots in this treatment were obtained (2.5 roots/explant), the maximum length of roots was recorded in MS medium supplement with 1 mg/l BAP + 1 mg/l NAA is (4.67 cm) but minimum length of roots is (0.4cm) in MS medium supplement with 0.3 mg/l BAP + 0.1 mg/l IBA.

Multiple shoot growth was observed directly from nodal explants on MS medium fortified with different concentrations (0.3, 0.6 and 1 mg/l) of BAP combination with different concentration (0.5 and 1 mg/l) of oxines NAA, IBA. The highest length of shoots was produced on node explant (1.5 cm) incubated in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l NAA while the lowest length of shoots was (0 cm) in MS medium supplement with 0.3 mg/l BAP + 0.5 mg/l IBA. Maximum number of shoots were observed in MS medium supplemented with 1 mg/l BAP + 1 mg/l NAA (4 shoots/ explant). But the minimum number of shoots was detected in 0.3 mg/l BAP + 0.5 mg/l IBA.

The highest number of leaves were recorded from nodal explants which cultured on MS medium supplemented with 1 mg/l BAP + 1 mg/l NAA showed about (13.5 leaves/explant). But the lowest number of leaves (0 leaves/explant) were recorded from

node explants in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l IBA. The maximum number of roots per explant was obtained on MS combined with 0.6 mg/l BAP + 0.5 mg/l IBA (6.5 roots/explants), the minimum number of roots (0 roots/explant) was detected in MS medium supplemented with 0.3 mg/L BAP + 0.5 mg/l NAA, 0.6 mg/l BAP + 1 mg/l NAA, 1 mg/l BAP + 1 mg/l NAA, 1 mg/l BAP + 0.5 mg/l IBA and 0.3 mg/l BAP + 0.5 mg/l IBA.

The best result of root length (3.50 cm) was recorded when the shoots were transferred to medium containing 0.6 mg/l BAP +0.1 mg/l IBA, simultaneously, decreased number of root (0 cm) was provided from incubated nodal explants in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l NAA , 0.6 mg/l BAP + 1 mg/l NAA , 1 mg/l BAP + 1 mg/l NAA , 0.6 mg/l BAP + 0.5 mg/l NAA , 1 mg/l BAP + 0.5 mg/l IBA and 0.3 mg/l BAP + 0.5 mg/l IBA.

Table 4.3. Effect of BAP with NAA, IBA on shoot tip and node explants of *petunia hybrida*.

PGR	Con.	PGR	Con.	Explant	Shoot Length (cm) /Explant	No of Shoot/explant	No of Leaves/explant	No. of Roots/explant	Root Length (cm)/explant
BAP	**0.3**	**NAA**	**0.5**	Shoot tip	1.5	2.5	10.67	12.33	3.00
				Node	1.5	2.5	11.00	0.00	0.00
BAP	**0.6**	**NAA**	**1**	Shoot tip	1.5	2	8.50	6.50	3.00
				Node	0.2	2	1.50	0.00	0.00
BAP	**0.3**	**NAA**	**1**	Shoot tip	0.7	2	5.50	2.50	1.60
				Node	0.5	2.5	6.50	2.50	0.60
BAP	**1**	**NAA**	**0.5**	Shoot tip	0.3	1	4.50	4.00	1.75
				Node	0.4	2.5	7.50	0.50	0.15
BAP	**1**	**NAA**	**1**	Shoot tip	2.25	2.5	15.00	10.50	4.67
				Node	1.15	4	13.50	0.00	0.00
BAP	**0.6**	**NAA**	**0.5**	Shoot tip	0.5	2.5	3.00	3.50	0.60
				Node	0.25	3.5	2.50	5.50	0.00
BAP	**1**	**IBA**	**0.1**	Shoot tip	0.7	1.5	6.00	6.00	2.50
				Node	0.5	2	4.50	6.00	1.25
BAP	**1**	**IBA**	**0.5**	Shoot tip	0.6	2.5	6.00	6.00	0.45
				Node	0.3	2	4.00	0.00	0.00
BAP	**0.6**	**IBA**	**0.1**	Shoot tip	0.75	1	5.00	9.50	2.50
				Node	0.8	2	4.00	4.50	3.50
BAP	**0.6**	**IBA**	**0.5**	Shoot tip	0.3	3	3.00	4.50	0.55
				Node	0.2	0	2.50	6.50	0.25
BAP	**0.3**	**IBA**	**0.5**	Shoot tip	0.25	2	3.50	3.00	0.75
				Node	0	0	0.00	0.00	0.00
BAP	**0.3**	**IBA**	**0.1**	Shoot tip	0.7	1	3.50	15.00	0.40
				Node	0.1	1	1.00	1.00	0.20

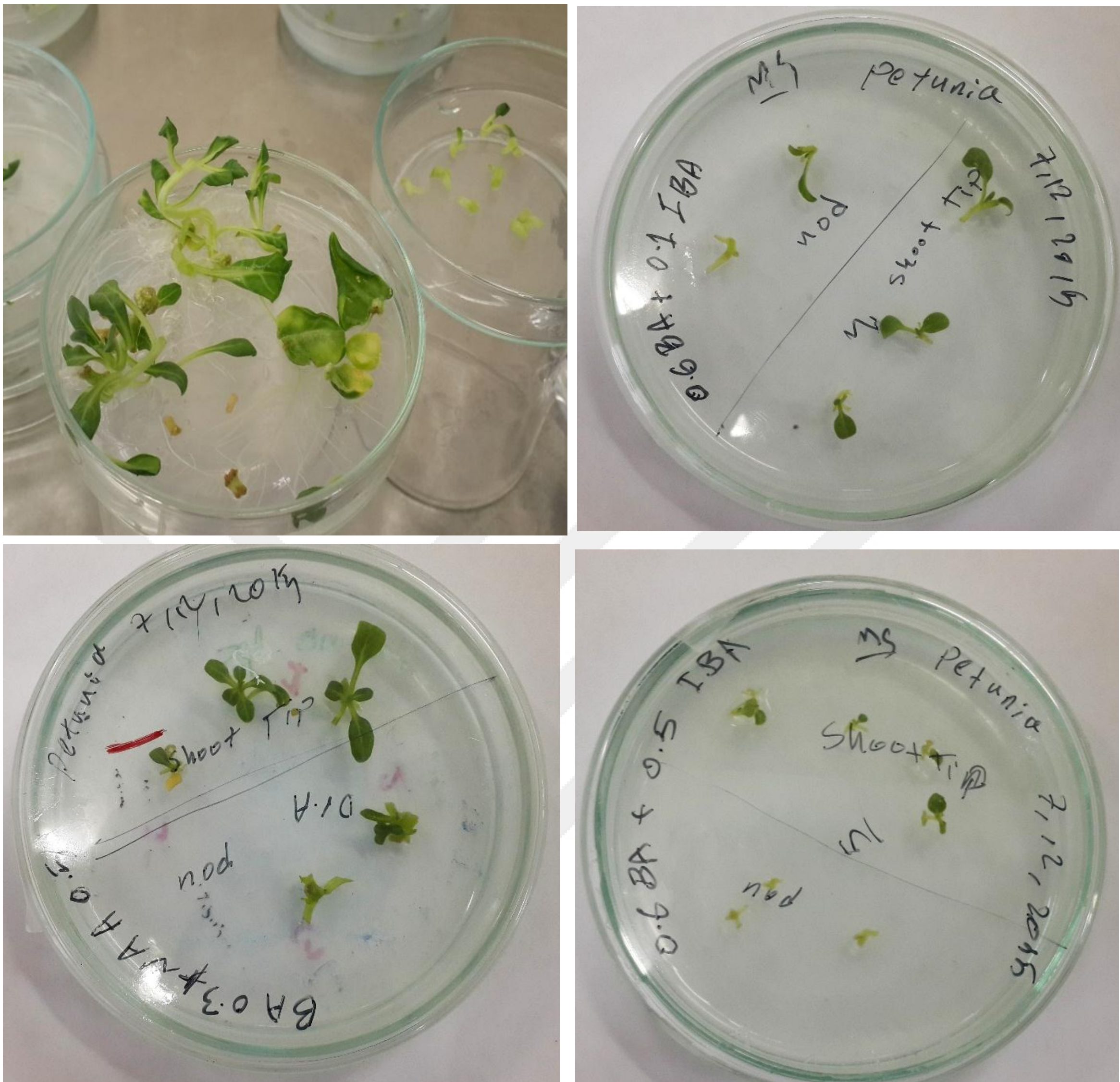

Figure 4.2. The Effect of PGR types and concentration on shoot formation.

4.2.1 Effect of BAP on Shoot Multiplication Stage.

BAP is known the most effective cytokine utilized for shoot multiplication for different plant species. It was tested at different concentration (0.3, 0.6 and 1 mg/l) for shoot multiplication.

The data in Table 4.4 explain the effect of different BAP and NAA concentration on shoot length and number of shoots per explants, number of leaves per explants, root numbers per shoot and root length. The longest shoot was recorded in MS medium supplemented with 1 mg/l BAP + 1 mg/l NAA (2.25 cm). The highest number of shoots was obtained (3 shoots/explant) from shoots tip cultured in MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l NAA. The highest number of leaves per explant was recorded from shoot tip explants in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l NAA (10.5 leaves / explant). The highest number of roots (12 roots/ explant) was obtained from shoot tips cultured in MS medium supplemented with 0.3 mg/l BAP + 0.5 mg/l NAA. The longest roots (4.6 cm) from shoot tip explants were recorded in MS medium supplemented with 1 mg/l BAP +1 mg/l NAA.

Table 4.4. Effect of BA and NAA on shoot multiplication stage from shoot tip explants.

PGR concentration (1mg/l)	**Shoot length (cm)**	**Shoot No:**	**Leaf No:**	**Root No:**	**Root length (cm)**
0.3 BAP + 0.5 NAA	1.5	2.5	10.5	12	3
0.3 BAP + 1 NAA	0.7	2	5.5	2.5	1.6
0.6 BAP + 0.5 NAA	0.5	3	3	3.5	0.6
0.6 BAP + 1 NAA	1.5	2	8.5	6.5	3
1 BAP + 0.5 NAA	0.3	1	4.5	4	1.7
1 BAP + 1 NAA	2.25	2.5	15	10.5	4.6

Table 4.5 showed that different BAP and NAA concentration in MS medium was significantly effective on the number of shoots per explant and the number of leaves

and shoot length and number of roots and root length produced on the shoot from the culture of axillary node explant.

The highest number of leaves (13.5 Leaves /explant) was achieved in MS medium supplemented with 1 mg/l BAP + 1mg/l NAA. The highest number of adventitious shoot regeneration (4 shoots /explant) was produced from axillary node explant in MS medium supplemented with 1 mg/l BAP + 1mg/l NAA. The highest length of shoots was observed in MS medium Supplement with 0.3 mg/l BAP + 0.5 mg/l NAA (1.5cm). The highest number of roots was produced in MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l NAA.

The highest length of roots was recorded (0.6cm) on the shoot tip explant cultured in MS medium supplemented with 0.3 mg/l BAP + 1 mg/l NAA. The other treatments did not produce roots but they produced Callus.

Table 4.5. Effect of BA and NAA on shoot multiplication stage from axillary node explants.

PGR concentration (1mg/l)	**Shoot length (cm)**	**Shoot No:**	**Leaf number**	**Root No:**	**Root length (cm)**
0.3 BAP+0. 5 NAA	1.5	2.5	11	0	0
0.3 BAP +1 NAA	0.5	2.5	6.5	2.5	0.6
0.6 BAP +0.5 NAA	0.25	3.5	2.5	5.5	0.35
0.6 BAP + 1 NAA	0.2	2	1.5	0	0
1 BAP +0.5 NAA	0.4	2.5	7.5	0.5	0.15
1 BAP + 1 NAA	1.15	4	13.5	0	0

BAP was found to be very effective on the shoot multiplication with IBA which also very effective auxin on root formation.

Table 4.6 shows the effect of BAP on shoot regeneration of Petunia shoot tip explants and the effect of IBA on rooting of Petunia microshoots. The addition of 0.6

mg/l BAP + 0.1 mg/l IBA in MS medium was the best concentration on shoot formation, (0.75 cm) per shoot.

The highest number of shoot formation (3 shoots/explant) was recorded from culture in MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA. The highest number of leaves (6 leaves /shoot) were provided from 1 mg/l BAP + 0.1 mg/l IBA and 1 mg/l BAP + 0.5 mg/l IBA supported MS medium. The highest root number was recorded by the addition of 0.3 mg/l BAP + 0.5 mg/l IBA. In that culture medium 15 roots /explant were produced. The longest roots was recorded in the treatments was (2.6 cm) in which MS medium supplement with 0.6 mg/l BAP + 0.1 mg/l IBA.

Table 4.6. Effect of BA and IBA on shoot multiplication stage from shoot tip explants.

PGR concentration (1mg/l)	**Shoot length**	**Shoot No:**	**Leaf number**	**Root No:**	**Root length (cm)**
1 BAP + 0.1 IBA	0.7	1.5	6	6	2.5
1 BAP + 0.5 IBA	0.6	2.5	6	6	0.45
0.6 BAP + 0.1 IBA	0.75	1	5	9.5	2.6
0.6 BAP + 0.5 IBA	0.3	3	3	4.5	0.55
0.3 BAP + 0.1 IBA	0.7	1	3.5	3	0.4
0.3 BAP + 0.5 IBA	0.25	2	3.5	15	0.75

Table 4.7 declares the effect of different BAP and IBA concentration on shooting stage. The highest shoot length was measured (0.8 cm) in MS medium supplement with 0.6 mg/l BAP + 0.1 mg/l IBA. The addition of 0.6 mg/l BAP + 0.5 mg/l IBA in MS medium caused the highest shoot number (4.5 shoot/explant). In axillary node explant the highest number of leaves formation was produced in MS medium supplement with 1 mg/l BAP + 0.1 mg/l IBA. The highest number of root was recorded in MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA (6.5 roots/ explant).The highest length of roots was produced in MS medium supplement with 0.6 mg/l BAP + 0.1 mg/l (3.5cm/shoot).

Table 4.7. Effect of BA and IBA on shoot multiplication stage from axillary node explants.

PGR concentration (1mg/l)	Shoot length (cm)	Shoot number	Leaf number	Root number	Root length (cm)
1 BAP + 0.1 IBA	0.5	2	4.5	6	1.25
1 BAP + 0.5 IBA	0.3	2	4	0	0
0.6 BAP + 0.1 IBA	0.8	2	4	4.5	3.5
0.6 BAP + 0.5 IBA	0.2	4.5	2.5	6.5	0.25
0.3 BAP + 0.1 IBA	0.1	1	1	1	0.2
0.3 BAP + 0.5 IBA	0	0	0	0	0

4.3. Shoot length Stage

Figure 4.2 shows the effect of gibberellic acid (GA_3) on shoot length. Table 4.8 shows the effect of gibberellin on shoot length of Petunia microshoots. The highest length of shoots was recorded from the MS medium supplemented with 0.2 mg/l GA_3 +0.2 mg/l BAP (5.75 cm).The highest number of leaves (23 leaves /shoot) was achieved when GA_3 was added at 0.2 mg/l concentration in MS medium. The addition of GA_3 at 0.2 mg/l concentration gave the best result on axillary node formation (23 node /shoots). The highest internode length formation was produced in MS medium supplement with 0.2 mg/l GA_3 +0.2 mg/l BAP (0.85cm). But the lowest result was taken from MS medium supplemented with 0.05mg/l GA_3 +0.5 mg/l BAP (0.1 cm).

Table 4.8. Effect of GA_3 on shoot length stage.

PGR concentration (1 mg/l)	Shoot length (cm	Leaf number	Axillary node	Enter node length (cm)
0.2 GA_3 + 0.2 BAP	5.75	12	10	0.85
0.2 GA_3 + 0.5 BAP	5.25	16	14	0.55
0.05GA_3 +0.5 BAP	2.2	10	10	0.1
0.05GA_3 + 0.2 BAP	3	13	10	0.2
0.2 GA_3	4.5	23	23	0.3
0.5 GA_3	1.5	7	7	0.2
1 GA_3	4.5	10	9	0.5

Figure 4.3. Effect of different concentration of GA_3 with BAP on shoot length.

4.4. Rooting stage

4.4.1. Effect of IBA and NAA on rooting formation

The results showed that different concentration of IBA and NAA in MS medium had significant effect on root length per explant and the number of roots per explant after four weeks of culture for *petunia hybrida* rooting stage *in vitro* culture (Figure 4.3).

Table 4.9 shows the effect of IBA on root formation of Petunia shoots. The highest number of roots was recorded in MS medium supplemented with 1 mg/l IBA (6.8 roots/shoot), subsequently 0.8 mg/l IBA (5 roots /shoot). The highest root length (3.2 cm) was achieved in MS medium supplemented with 1 mg/l IBA subsequently 0.8 mg/l IBA (3 cm).

Table 4.9. Effect of IBA on rooting formation.

PGR concentration of IBA (1mg/l)	**No of Root/shoot**	**Root length (cm)**
0.1	0.2	0.5
0.3	1.2	1.2
0.5	2.5	2
0.8	5	3
1	6.8	3.2

Table 4.10 declared the effect of NAA on the root formation of Petunias macroshoots. The addition of NAA at 1 mg/l concentration was found to be the best auxin concentration at rooting number (7.5 roots/shoot), subsequently 0.8 mg/l NAA (6 roots/shoot). The lowest result was recorded at 0.1 mg/l NAA (0.5 roots /shoot). The highest length of root formation was observed in MS medium supplemented with 1 mg/l NAA (3 cm), subsequently 0.8 mg/l NAA (2.8 cm). The lowest length of adventitious root formation was in the MS medium supplement with 0.1 mg/l NAA (0.6 cm).

Table 4.10. Effect of NAA on rooting formation.

PGR concentration of NAA (1mg/l)	No of Root/shoot	Root length (cm)
0.1	0.5	0.6
0.3	1.4	1.8
0.5	3	2.2
0.8	6	2.8
1	7.5	3

Table 4.11 shows the effect of IBA with NAA on root formation. 1 mg/l IBA + 1 mg/l NAA resulted in (9 root/shoot) subsequently 1 mg/l IBA + 0.2 mg/l NAA (9 root/shoot). The highest length of roots was produced in MS medium supplemented with 1 mg/l IBA + 1 mg/l NAA (4.2 cm), subsequently with 1 mg/l IBA + 0.2 mg/l NAA (4 cm). But the lowest root length was recorded on 0.2 mg/l IBA + 0.2 mg/l NAA (2 cm).

Table 4.11. Effect of IBA and NAA on rooting stage.

PGR concentration (1mg/l)	No of Root/shoot	Root length (cm)
0.2 IBA +0.2 NAA	0.5	2
0.2 IBA +1 NAA	4.5	3.2
1 IBA +0.2 NAA	6	4
1 IBA +0.2 NAA	9	4.2

Figure 4.4. Effect of different concentration IBA and NAA on rooting formation.

4.5. Hardening and Acclimatization

When the developed plantlets reached to (5-6 cm) and rooted (4-5 cm), they were removed from the culture medium and wash thoroughly in running tap water. Plantlets were transferred to plastic pots containing sterile filled with perlite and peat. (3:1) (Figure 4.5). Plantlets were kept in a chamber with 80–90% relative humidity under a 16/8 h (light/dark) of photoperiod. Potted plantlets were to cover with transparent plastic bags to ensure high humidity and watered every three days for two weeks.

Plastic were removed after two weeks in order to acclimatize the plants to field conditions. After four weeks 70% of plants were adapted. To the ambient condition acclimatized plants were transferred to the pots containing peat moss and maintain under normal day length conditions. The acclimatized plants were than flowered, gave fruit and the seeds from fruit were provided. The whole *in vitro* regeneration stages were completed.

Figure 4.5. Acclimatization plantlets**.**

5. DISCUSSION AND CONCLUSION

The application of tissue culture techniques to Petunia started in the late 1960's (Binding, 1971). The main objective of this work was to study aspects of morphogenesis and organogenesis from organs and tissue. It was found that the normal plants could be regenerated from explants.

The present study includes the works in which the role of explants and plant growth regulators on the regeneration of Petunia was investigated. The role of certain plant growth regulators as major factors *in vitro* morphogenetic processes is reflected in the thesis. We describe the development of explants and their differentiation to whole plants. The primary goal is to clone certain Petunia line *in vitro* for horticultural uses.

Petunia hybrida plants with two types of explants were used in micropropagation as shoot tip and axillary node. Seeds were sterilized in four different concentration of sodium hypo chloride (NaOCl) (4, 6, 8 and 10%), at two different time of period (5 and 10 min.).

The best results was obtained from the application of 10 minutes and 8% of NaOCl concentration. The germination ratio were observed 90% without any contamination. In 10 minutes and 10% of NaOCl resulted in germination ratio in 20%. The main reason for the result was the size of the seeds. The thin layer of small seeds was injured by NaOCl. Thus high concentration of NaOCl caused to death of embryo. But when we change the concentration of NaOCl to 4% we realized that the percentage of seed germination became 0%, and this was due to insufficiently sterilization, and all media were contaminated as it is demonstrated in the Table (4.1).

Due to the property of the Petunia seed, the best outcome of the germination of seeds was observed in 5 minutes, in 8% NaOCl concentration. The germination ratio became 75% and no contamination was noticed. The rate of the germination changeg with the change of NaOCl concentration. Meanwhile, 10% concentration of NaOCl resulted in 0% germination. The reason was the same with the first experiment as it was seen 10 minutes application which was due to the effect of the size of the seed. But it is detected that in 4% of NaOCl, caused 10% of seed germination and contamination of culture was

80%. Moreover, different results were obtained when different time and concentration were used (Hailu et al., 2015; Walla et al., 2016).

In the current study, three types of plant media without PGR were applied to *Petunia hybrida* seed germination. The best results for seed germination was obtained from MS medium with 90%, which was more than the germination ratio in B5 medium and White medium. The results were 70% and 50% respectively. The reason was that the chemical structures, vitamins, macroelements and microelements concentration in each media was different from each other. But a change of color in seedlings was noticed, most of them changed to yellow, so we added (0.005 mg/l Fe) to the media to solve the problem. The result was affective and the color changed to green.

Walla et al., (2016) demonstrated that the multiplication is a rapid increase of organs which can ultimately give rise to plant. This stage repeated at regular intervals to produce large-scale shoot multiplication to be commercially useful to the shoot induction and proliferation depend on plant growth regulators and types of explants**.**

For petunia explants, the exogenous Cytokinin, BAP can control the commitment of explants to produce shoots in micropropagation. For understanding the action of Cytokinin it is required that the actual concentration of active molecules within the tissue be characterized during the developmental process (Fosket, 1980; Van der Krieken et al., 1990).

Explants such as shoot tip and axillary node are one of the reason for the success in this study because of the natural concentration of hormones which are in higher level, more activity and purity than that of another explants. Moreover, the explants contains meristematic cells which cause increase in shoot multiplication rate. In the other hand, regenerated shoots from axillary buds and shoot tips are true types because in their regeneration process (Kharrazi et al., 2011).

In the present study, combining cytokinin and auxin proved a useful effect on shoot organogenesis. The combination of BAP with IBA and NAA resulted to adventitious shoot regeneration, leaves and root formation. Parallel results were reported by Annapurna and Rathore, (2010).

The major effects of BAP and other Cytokinins have been characterized in a limited plant tissues and their activities differ significantly (Laloue, 1977).

The benzyl adenine (BA) glucosides are normally considered to be inactive. The BA ribosides (BAR) and free base may be involved in the regulation of cell division or other developmental processes (Letham and Palni, 1983).

Shoot tip explant was applied for subculturing in MS medium supplement with BAP and combination with NAA and IBA. The highest shoot length was 2.25 cm in MS medium supplement with 1 mg/l BAP +1 mg/l NAA. The increased concentration of BAP caused increasing shoot production. One of the important role of the lower level of auxin in the organogenic stage is nullify the effects of cytokinin on shoot organogenesis and elongation. Vidya et al., (2012) and Mahadev et al., (2014) reported that the combination of auxin and cytokinin support to organogenic differentiation.

Shoot tip explant was applied for subculturing in MS medium supplement with the combination of BAP and NAA. The highest number of shoots (3 shoots/explant) was recorded in MS medium supplement with 0.6 mg/l BAP + 0.5 mg/l NAA. Parallel results was reported by Sharmin et al., (2008).

Axillary node explant was applied for subculturing in MS medium supplement with BAP in combination with NAA. MS medium supplement with 1 mg/l BAP + 1 mg/l NAA gave the maximum number of shoots (4 shoots/explant) and the shoot length (1.15 cm). The synergistic effect of cytokinin and low auxin for multiple shoot induction has been reported in same family *Solanaceae* (Khadiga et al., 2009).

Effect of different concentration of cytokine, BAP and auxins, NAA and IBA on direct shoot regeneration was investigated. The maximum number of shoot induction in shoot tip explants was observed on MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA (3 shoots/explant).

The highest length of shoots was produced on node explant (0.8 cm) incubated in MS medium supplemented with 0.6 mg/l BAP +0.1 mg/l IBA. The results show the higher potential of the collective effect of BAP, NAA for shoot induction than those of BAP, IBA.

The growth of the above ground part was particularly strongly stimulated by 0.2 and 0.05 mg/l of gibberellic acid (GA_3) in combination with BAP (Table 4.8.). Plantlets were developed in MS medium supplemented with 0.2 mg/l GA_3 + 0.2 mg/l BAP (5.75cm). Petunias regenerated on MS medium supplement with 0.2 GA_3 gave the highest number of leaves (23) which is in agreement with the results by Kulpa and Nowak,

(2011). Gibberellins are recognized to have a stimulatory effect on shoot elongation in many species, aside from helping in the further growth and development of preformed organs. This can be ascribed to the increased transport of potassium ions assisted by gibberellin.

The function of rooting stage is to prepare plantlets for transplanting and acclimatization to outside environment (Hartmann and Kester, 1983).

Auxins have several promoting roles in tissue culture and they have the facility to promote root initiation (Wetherell, 1982). Synthetic auxins such as IBA and NAA are frequently used for *in vitro* root initiation and increasing root number and length even present alone or in mixing each other. Regenerated shoots of *petunia hybrid* was established from the previous stage with length 1.5- 4 cm and rooted in MS medium supplement with different concentrations and combinations of IBA and NAA.

Data represented in Table 4.9, 4.10 and 4.11 and Fig 4.4**,** showed that MS medium supplemented with 1 mg/l IBA +0.2 NAA gave the highest number of root formation per explant (9 roots/explant) and also the best root length of root per explant (4 roots/explant) obtained from the similar media after four weeks, while MS medium supplemented with 0.1 mg/l NAA and also 0.1 IBA showed a very few root formation. In our research IBA was more effective than NAA with respect to rooting of the regenerated shoots. The same result has been reported by Husain et al., (1999).

These results in agreements with Otroshy et al., (2011). The MS medium supplemented with 1mg/l NAA was effective for inducing root formation on *Capsicum annuum* L. plants and also IBA was more effective for root induction than NAA even alone or in combination with each other.

In the present study, regeneration of *petunia hybrida* was greatly achieved by using different plant growth regulators supplemented with MS medium. It was concluded that MS medium supplemented with 1mg/l BAP+ 1mg/l NAA was proven to be the best medium for primary establishment of direct organogenesis of *petunia hybrida* in all the treatments based on available shoot tip and nodal explants.

The best result for elongation of microshoots was recorded in MS medium supplemented with 0.2 mg/l GA_3. Plantlets grown under these conditions gave higher number of leaves and auxiliary nodes. Auxins such as NAA and IBA added to MS

medium supplemented with 1 IBA +0.2 NAA appeared to be the most useful for rooting *Petunia hybrida*. Petunias rooted in this way had numerous of roots.

A high survival acclimatization of the *in vitro* grown plantlets was proven the validity of the development into healthy plants after being transferred to chamber.

The present study describes an efficient protocol for *in vitro* regeneration of *Petunia hybrida*. For future perspective, the protocol can be applied for large scale production of the plant for commercial purpose and the regenerated plant may be used for medicinal purpose. The plant was investigated in terms analyzing and application of secondary metabolite and some promising results were reported.

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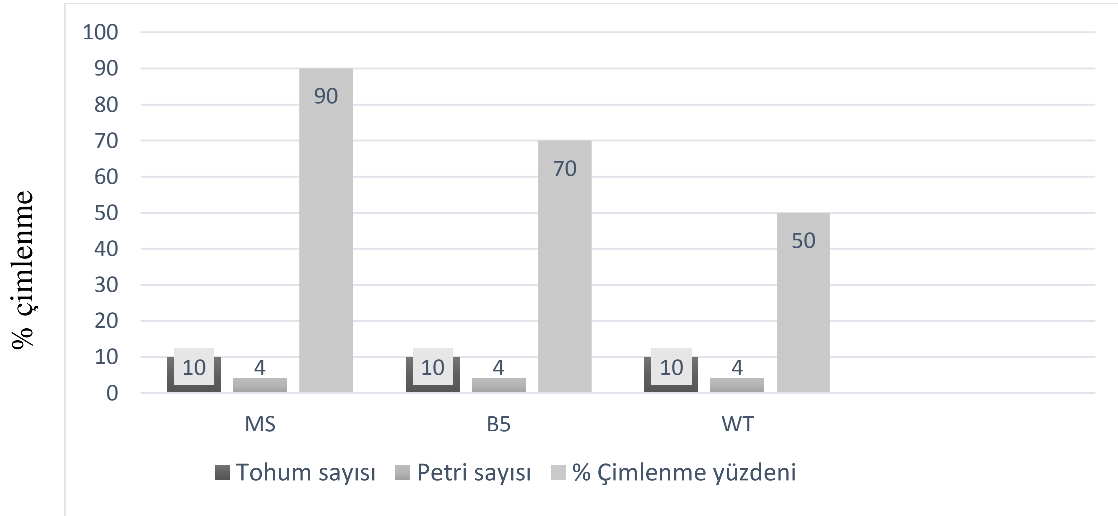
EXTENDED TURKISH SUMMARY

GENİŞLETİLMİŞ TÜRKÇE ÖZETİ

Tezin Adı: Petunya (*Petunia hybrida*) Bitkisinin Doku Kültüründe Mikroüretimi
Yüksek Lisans Öğrencisi: Rebaz Rasul HABAS
Danışman: Prof. Dr. Musa TÜRKER

Süs bitkileri endüstrisi pazarı gün geçtikçe artmakta ve seçkin ve fazla talep edilen bitkilerin doku kültüründe üretimleri yaygın hale gelmektedir. Petunya (*Petunia hybrida*) bitkisi de 35 çiçekli bitki familyası içerisinde Solanaceae familyasına ait oldukça talep edilen bir süs bitkisidir. Petunya bitkisi dünya üzerinde yaygın olarak klasik yöntemlerle çoğaltılmakta ve satılmaktadır. Doku kültüründe de kitlesel üretimi konusunda çalışmalar devem etmektedir. Bitki ABD klasik yöntemlerle, ticari olarak en fazla üretilen 10 bitki türünden bir tanesidir. 2008 yılında ABD deki satış miktarı 120 milyon USD dir. Bitkinin aynı zamanda tıbbi etkisi, antimikrobiyal ve antioksidan aktivitesi de tespit edilmiştir.

Bu çalışmada ekonomik açıdan önemli bir süs bitkisi olan petunya doku kültüründe üretilmiştir. Tohumlar çamaşır suyu ile (NaOCl) sterilize edilerek, Bitki Büyüme Maddeleri (BBD) içermeyen Murashige ve Skoog (MS) çimlendirilmiştir. Tohumlar farklı besi ortamlarında %50- 90 oranında çimlenmiştir (Şekil 4.1).



Şekil 4.1 Farklı besi ortamlarının tohum çimlenmesi üzerine etkileri.

Tablo 4.3 gövde ucu ve nod eksplantlarının farklı konsantrasyon ve kombinasyonlarda BBD ile desteklenmiş MS ortamında rejenerasyon miktarlarını göstermektedir. Bir eksplant üzerinde birden fazla gövre rejenerasyonu gözlenmiştir. En

uzun gövdeler nod eksplantının inkübe edildiği 1 mg/l BAP + 1 mg/l NAA ile desteklenmiş MS ortamında elde edilmiştir. En fazla sayıda gövde (3 sürgün/eksplant) ise 0.6 mg/l BAP + 0.5 mg/l IBA ile desteklenmiş ortandan elde edilmiştir.

Çizelge 4.3. Farklı konsantrasyon ve kombinasyonlardaki BBD'nin petunya bitkisi nod ve gövde ucu eksplantlarunda bitki rejenerasyonuna etkileri.

BBD	**kon.**	**BBD**	**kon.**	**eksplant**	**gövde uzunluğu (cm) eksplant**	**gövde sayısı /eksplant**	**yaprak sayısı / ekplant**	**kök sayısı explant**	**kök uzunluğu (cm) / Ekplant**
BAP	**0.3**	**NAA**	**0.5**	Gövde ucu	1.5	2.5	10.67	12.33	3.00
				Nod	1.5	2.5	11.00	0.00	0.00
BAP	**0.6**	**NAA**	**1**	Gövde ucu	1.5	2	8.50	6.50	3.00
				Nod	0.2	2	1.50	0.00	0.00
BAP	**0.3**	**NAA**	**1**	Gövde ucu	0.7	2	5.50	2.50	1.60
				Nod	0.5	2.5	6.50	2.50	0.60
BAP	**1**	**NAA**	**0.5**	Gövde ucu	0.3	1	4.50	4.00	1.75
				Nod	0.4	2.5	7.50	0.50	0.15
BAP	**1**	**NAA**	**1**	Gövde ucu	2.25	2.5	15.00	10.50	4.67
				Nod	1.15	4	13.50	0.00	0.00
BAP	**0.6**	**NAA**	**0.5**	Gövde ucu	0.5	2.5	3.00	3.50	0.60
				Nod	0.25	3.5	2.50	5.50	0.00
BAP	**1**	**IBA**	**0.1**	Gövde ucu	0.7	1.5	6.00	6.00	2.50
				Nod	0.5	2	4.50	6.00	1.25
BAP	**1**	**IBA**	**0.5**	Gövde ucu	0.6	2.5	6.00	6.00	0.45
				Nod	0.3	2	4.00	0.00	0.00
BAP	**0.6**	**IBA**	**0.1**	Gövde ucu	0.75	1	5.00	9.50	2.50
				Nod	0.8	2	4.00	4.50	3.50
BAP	**0.6**	**IBA**	**0.5**	Gövde ucu	0.3	3	3.00	4.50	0.55
				Nod	0.2	0	2.50	6.50	0.25
BAP	**0.3**	**IBA**	**0.5**	Gövde ucu	0.25	2	3.50	3.00	0.75
				Nod	0	0	0.00	0.00	0.00
BAP	**0.3**	**IBA**	**0.1**	Gövde ucu	0.7	1	3.50	15.00	0.40
				Nod	0.1	1	1.00	1.00	0.20

Eksplantlar üzerinde gelişen çok sayıdaki sürgünler birbirinden ayrıştırılarak bağımsız olarak kavanozlara, farklı konsantrasyondaki GA_3 ile desteklenmiş MS ortamında inkübe edilmiş ve gövdelerin gelişimi sağlanmıştır. En uzun gövdeler 0.2 mg/l GA_3 +0.2 mg/l BAP ile desteklenmiş ortamdan elde edilmiştir. GA_3'ün yaprak sayısı üzerine etkileri de incelenmiş, en yüksek sayıda yaprak 0.2 mg/l GA_3 ile desteklenmiş ortamlardan elde edilmiştir (Tablo 4.8).

Çizelge 4.8 Farklı konsantrasyonlarda BBD'nin gövde gelişimi üzerine etkileri

BBA konsantrasyon (1mg/l)	**Gövde uzunhğu (cm)**	**Yaprak sayısı**	**Aksillar nod**	**Enter nod uzunhğu (cm)**
0.2 GA_3 + 0.2 BAP	5.75	12	10	0.85
0.2 GA_3 + 0.5 BAP	5.25	16	14	0.55
0.05 GA_3 + 0.5 BAP	2.2	10	10	0.1
0.05 GA_3 + 0.2 BAP	3	13	10	0.2
0.2 GA_3	4.5	23	23	0.3
0.5 GA_3	1.5	7	7	0.2
1 GA_3	4.5	10	9	0.5

Gelişen ama kök vermeyen gövdelerin köklendirilmesi için farklı konsantrasyon ve kombinasyonlarda IBA ve NAA kullanılmıştır. 1 mg/l IBA + 1 mg/l NAA, 1 mg/l IBA + 0.2 mg/l NAA ile desteklenmiş ortamlarda en yüksek düzeyde köklenme gözlenmiştir.

Çizelge 4.11. IBA ve NAA'in kök gelişimi üzerine etkileri.

BBD konsantrasyon (1mg/l)	Kök sayısı / gövde	Kök uzunluğu (cm)
0.2 IBA + 0.2 NAA	0.5	2
0.2 IBA + 1NAA	4.5	3.2
1 IBA + 0.2 NAA	6	4
1 IBA + 0.2 NAA	9	4.2

Eksplantlardan sürgün ve kök elde edildikten sonra rejenere olmuş fideler toprak içeren saksılara aktarılarak bir süre %80-90 nem oranına sahip iklim dolaplarında bekletilmiş ve daha sonra dış ortama aktarılmıştır. Dış ortama aktarılan bitkiler %70 oranında hayatta kalmayı başarmıştır.

CURRICULUM VITAE

Rebaz Rasul HABAS was born in Raniya –Sulaimnyah. North Iraq in 1987. He completed primary and secondary school in Hajiawa a small town in Sulaimanyah Governorate. He was graduated in Horticulture Department, Faculty of Agriculture, and University of Sulaimanyah in 2010. He was registered for Master of Science program in molecular Biology and Genetics Department, Yüzüncü Yıl University, Van-Turkey in 2015.

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