T.R.
EGE UNIVERSITY
Graduate School of Applied and Natural Science

MICROPROPAGATION OF PRINCESS TREE (*Paulownia tomentosa* STEUD)

MSc. THESIS

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DECLARATION

I hereby declare that this thesis study entitled as "**Micropropagation of Princess Tree (*Paulownia tomentosa* steud)**" represents my very own work which has been done after registration for the degree of MSc at Ege University and has not been previously included in an exceedingly thesis or dissertation submitted to the present or the other institution for a degree, diploma, or other qualifications. I also declare that each one the knowledge during this document has been obtained and presented in accordance with Ege University academic rules and regulations as well as ethical conduct. Lastly, I declare that, as required by these rules and by these rules and conduct, I have fully cited and referenced all material and results, which does not seem to be original to the current work.

27/06/2022

Getalew Ayizengaw CHANA

ÖZET

PAVLONYA (*Paulowina tomentosa*)'NIN MİKRO ÇOĞALTIMI

CHANA, Getalew Ayizengaw

Yüksek Lisans Tezi, Tohumluk Bilimi ve Teknolojisi Anabilim Dalı
Danışman: Prof. Dr. Eftal DÜZYAMAN
İkinci Danışman: Prof. Dr. Alicia AYERDI GOTOR
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Bu tez çalışması, İzmir İli, Bademli Çiftçi Kooperatifi Bitki Doku Kültürü Laboratuvarında 2022 yılında gerçekleştirilmiştir. Prenses ağacı (*Paulownia tomentosa*) tohumlarından üretilen bitkicikler *mikro* kültürünün başlangıç materyalini oluşturmuşlardır. Tohumlar, 0.5 mgl-1 BAP büyüme düzenleyicileri ile sıvı Murashige ve Skoog (MS) ortamında çimlendirilmiştir. Çoğaltma aşamasında 6-BenzylAminoPurine'nin (BAP) farklı konsantrasyonları (0.0, 0.5, 0.5+0.1 NAA, 1.0+0.1NAA, 2.0+0.1NAA mgl-1) test edilmiştir. BAP konsantrasyonunun (0-1.5 mgl-1) arttırılması bitki başına sürgün ve yaprakların çoğalmasını arttırmıştır. Bununla birlikte, daha yüksek hormon dozları, bitkicik tabanında kallus büyümesine neden olarak olumsuz bir fizyolojik etki ile sonuçlanmıştır. Bu nedenle, sürgün çoğalması için en iyi uygulama (6.67~7 sürgün/eksplant) 1.5 mgl-1BAP ile kaydedilmiştir. Çoğaltılmış sürgünler, farklı konsantrasyonlarda (0.0, 0.5, 1.0 mg/l) İndol bütirik asit (IBA) veya Naftalin asetik asit (NAA) içeren köklendirme MS ortamına aktarılmıştır. IBA, prenses ağacının *in vitro* köklenmesinde NAA'dan daha etkili olmuştur. Test edilen her iki IBA konsantrasyonu (%100) köklenme yüzdesi vermiştir. 0,5 mgl-1 IBA ile desteklenmiş MS ortamı, en iyi kök büyümesini ve gelişimini sağlamıştır. Köklü Bitkicikler perlit içinde başarılı bir şekilde iklimlendirilmiştir: Cocopeat: turba (1:1:1 V/V/V) ortam bileşimleri.

Anahtar Kelimeler: BAP, IBA, o, MS, NAA, Pavlonya, Prenses ağacı

ABSTRACT

MICROPROPAGATION OF PRINCESS TREE (*Paulownia tomentosa* STEUD)

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This study was undertaken in Plant Tissue Culture Laboratory of Bademli Farmer's Cooperative, Izmir Province, Turkey in 2022. For micropropagation of Princess tree (*Paulownia tomentosa)* seeds were used. The seeds were germinated onto liquid Murashige and Skoog (MS) medium strength with 0.5mgl^{-1} BAP growth regulators. Different concentrations (0.0, 0.5, 0.5+0.1 NAA, 1.0+0.1NAA, 2.0+0.1NAA mgl^{-1}) of 6-BenzylAminoPurine (BAP) were tested during multiplication stage. Increasing the concentration of BAP (0-1.5 mgl^{-1}) increases the proliferation of shoot and leaves per plant. However, further increase leads to a negative physiological effect by producing abundant callus growth on the base of the plantlet. Hence, the most effective treatment for shoot multiplication (6.67~7 shoots/explant) was recorded by 1.5 mgl^{-1}BAP. Multiplied shoots were transferred into a rooting MS medium containing different concentrations (0.0, 0.5, 1.0 mg/l) of Indole butyric acid (IBA) or Naphthalene Acetic Acid (NAA). IBA was more effective than NAA for in vitro rooting of Princess tree. Both tested concentrations of IBA gave (100%) root proliferation. MS medium supplemented with 0.5 mgl^{-1} IBA produced the most effective root. Rooted Plantlets were successfully acclimatized using perlite: Cocopeat: peat (1:1:1 V/V/V) media compositions.

Key words: BAP, IBA, In vitro, MS, NAA, Paulownia, Princess tree.

PREFACE

Plant Tissue culture is a well-known techniques of growing new plant tissues by transferring them into an artificial environment in which they can continue and function. It's a very fast multiplication techniques that's being used increasingly for propagation of various tree species. For princess tree **(***Paulownia tomentosa* STEUD) it helps to produce disease free and large number plantlets within a short period of time from a small amount of plant tissues (explants). That helps to speed up the production of new varieties into the marketplace. The production techniques also known as in vitro or micro propagation techniques because small amount of plant material has been used to produce several thousand plantlets.

Now adays copping strategies for climate change is a critical factor for the safe dwelling of the human being. Therefore, well planned afforestation programs could be a steppingstone for the conservation of soil, water, and biosphere in general which has indispensable role for the alleviating climate change. With this regards, Izmir Metropolitan Municipality has a firm stand and work with Bademli Cooperative to propagate several thousand Princess tree plantlets that will be ready for afforestation programs. However, the cooperative has not been conducted a study about the shoot and root growth hormone requirements. Therefore, the main aim of this study was to determine the optimum concentration of growth regulator used for better shoot, root growth and development of Princess Tree plantlets under tissue culture.

Plant growth regulators are phytohormones or chemicals that are used to modify plant growth such as increasing branching, altering shoot growth, hasten blooming, removing excess fruit or alter maturity. Therefore, Studying the optimum concentration of growth regulators are very important for the multiplication of vigorous and uniform plantlets. Nevertheless, during in vitro multiplications of plants soma clonal variations are attributed as a major factor. Therefore, the fidelity of clonal cultures needs to be assessed regularly. Otherwise, higher variability could result in. In the present study, it was observed visually by comparing the number of shoots, nodes and roots developed during subsequent cultures. While not many changes were observed morphologically. Hence, molecular assessment can also be done using various markers.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Explanation
2,4, D	Dichloro Phenoxy Acetic Acid
ANOVA	Analysis of Variance
BAP	6-BenzylAminoPurine
Ca	Calcium
Cd	Cadmium
Cu	Copper
DMRT	Duncan Multiple Range Test
ha	hectare
$HgCl_2$	Mercuric chloride
IAA	Indole Acetic Acid
IBA	Indole-3 -Butyric Acid
IMMG	Izmir Metropolitan Municipality Garden
K	Potassium
K_2SO_4	Potassium sulfate
m	meter
m.a.s.l	meter above sea level
MCPA	2 Methyl 4 Chloro Phenoxy Acetic Acid
MENA	Methyl Ester of Naphthalene acetic Acid
Mg	Magnesium
mm	millimeter
MS	Murashige and Skoog medium
Na	Sodium
NAA	Naphthalene Acetic Acid
RH	Relative Humidity
Spp	Species pluralis
TDZ	Thidizuron
tRNA	transfer RNA
Zn	Zinc

1. INTRODUCTION

The most notable perennial, quickly growing, and deciduous tree in the *Scrophulariaceae* family is a peculiar feature of the Princess Tree (Paulownia tomentosa STEUD (Rout et al., 2001). For the purpose of reforestation and mine site reclamation projects, the genus Paulownia has received a premium and substantial attention in various part of the world (Bergmann et al., 1997). Additionally, it has been managed as the major crop in large-scale afforestation initiatives or planted as an intercropping in various farming systems. The genus Paulownia includes several significant species, including *P. kawakami, P. tomentosa, P. fortunei, P. austalis, P. elongata, P. tawianiana, P. fargesi, P. catalpifolia and P. albipholea* (Lila et al., 2016; Sivacioglu et al., 2006). Princess trees are indigenous to China and East Asia, but they are now commercially grown in places like Vietnam, Cambodia, Taiwan, Laos, Korea, Japan, and North America (Sivacioglu et al., 2006).

The Princess Tree timber has a unique feature such as the strength-to-weight ratio of its wood is high, and low shrinkage coefficient, which helps to prevents it from easily warping and fracturing and improves its insulation capabilities (Chunchukov & Yancheva, 2015). A charcoal from princess tree is vital for high fireworks and the preparation of gunpowder (Rout et al., 2001). On top of these, they are playing an indispensable role as a source of secondary metabolites (such as flavonoids) which is high antioxidant activities and better pharmacological roles and used to treat different infectious disease (Bahri & Bettaieb, 2013). On the top of that the leaves are also used for animal feed and soil improvement, flowers for bee farming, and woods for quality timber production (Bahri & Bettaieb, 2013).

Princess trees could be conventionally propagated either by seed or root cuttings (Lila et al., 2016). However, the Propagation of Princess tree using seed is untrustworthy due to impeding by disease and pest problems and the inherent nature of unreliable germination and slow growth compared with cuttings. Modern tissue culture techniques are by far more important and sustainable. In vitro propagation techniques are among the more advanced methods of reproduction and may provide a quick and easy way to produce large numbers of plantlets (Venkateswarlu et al., 2001). Nonetheless, there are also a few inherent impediments of in vitro propagation of plantlets. For Instance, excessive callus proliferation at the base of the explant, deformed axillary shoot, explant exerted out of the jar by producing unmanaged leaves, and development of adventitious shoots on the callus base of explant (Litwińczuk & Bochnia, 2012).

Perhaps problems related to in vitro propagation of the Princess tree could be solved with different strategies. For instance, proper aseptic and working conditions in the lab, determination of proper medium type, and composition of different components especially the growth regulator hormones and sucrose can modify the growth, development, and success of different species multiplication in tissue culture (George et al., 2008). Hence, most importantly concentration of growth hormones which is vital for better shoot and root formation should be given better attention (Lila et al., 2016).

The objective of this study was to determine the optimum concentration of growth regulator used for the better shoot and root growth, development, and mass multiplication of Princess tree plantlets.

2. LITERATURE REVIEW

Botanical Description: Princess tree is upright, evergreen, short growing tree with a rounded and spreading branching habit. The *Paulownia* genus has roughly nine rapidly growing species including a few hybrids native to China and Southeast Asia (Yadav et al., 2013). All species are resilient, rapidly expanding, and capable of effectively to a new environment. East Asia, more specifically China, Korea, and southern Japan, where princess trees have been grown for a century are also considered as a center of origin and diversity (Ede et al., 1997; Yadav et al., 2013). In temperate climates of East Asia, Princess trees were producing a definite broad conical crown and spread branching and the tree will probably have reach 10-25m height, and up to 15meter canopy width in an open area (Czarapata, 2005; Reza Eddin, 2017; Zhu et al., 1986). Its normally a winter deciduous cultivated or Wild plant grown below 1800 m.a.sl., and flowering in spring (Apr-May) and fruiting in summer and autumn (Jul-Nov) (Pao, 1998). The tree reaches to marketable size in 5-8 years (Kiaei, 2012).

Princess tree has several applications. For example, numerous components of the plant, such as leaves, flowers, fruits, and barks have been utilized in traditional medicine to treat a variety of health-related problems (He et al., 2016). However, Princess tree is most utilized for timber production, soil improvement and fodder (Bergmann & Whetten, 1998; Tang et al., 2010). Princess tree plantations are also utilized to counteract climate change and restoration through carbon sequestration and decreasing strain on old age forests due to their quick growing and short rotation characteristics (Bergmann, 2003; Xu Dyeing et al., 2001). Princess tree is also catching the attention of producers and end-user’s due to its benefits for bioenergy, industrial raw material, and its unique nature of good adaptability to marginal soil without competing for main crops grown. Princess tree is a low demand water plant due to its deep-rooted nature (Lu, 2006) and detoxification of the heavy metals (Caparros et al., 2008; Wang et al., 2010). As a result, *Paulownia* species becomes popular around the world, including Spain, Italy, Austria, Turkey, Israel, India, United States of America, Canada, Mexico, and Brazil (Borja et al., 2010). Because of their resilience and ability to survive in nutrient-depleted soils, they are a highly valued item in the furniture business (Ipekci et al., 2001). Princess tree might become a significant commodity for Turkey's wood sector as a demand for higher output grows, and the plantations must guarantee that new materials utilized have the best attributes. This also includes essential characteristics of commercial timber production such as the reliability of timber, trunk yield and ease of large-scale production.

Many growth factors are required for Princess tree species utilized in successful planting efforts (Bergmann & Whetten, 1998). Because of variations in ecological factors at plantation sites, the features of the stock plants used for propagation, and the technique of propagation utilized, the development and shape of the princess tree are very inconsistent (Bergmann, 2003). *Paulownia elongata* is the most often used species for timber production since it exceeds the others in terms of yield potential (Bergmann et al., 1997). Neverthless, other varieties of Princess tree are still sought prominent attention for their best timbering characteristics (Bergmann & Whetten, 1998).

The potential for within species out crossing is naturally high in Princess tree. Hence, hybridization of closely related species is a common phenomenon (Zhu et al., 1986). Therefore, regardless of the methods of propagation used, the plantations of princess tree may contain unknown genetic background or hybrids (Finkeldey, 1992; Zhu et al., 1986). Therefore, maximum care must be sought when choosing the propagation techniques, so that time and resources are not used as counterproductively during production (Bergmann & Whetten, 1998).

Major Diseases: Like many other plants, princess tree can get sick and are susceptible to various pathogens. However, there is little information available about the common disease effected Princess tree. As few authors reported so far are caused by fungi and oomycetes (Bergmann, 2003; Milenković et al., 2018; Ray et al., 2005). The most well-known lethal disease in princess tree is the Paulownia witches' broom, a condition that has been observed in China and nearly all over the world for many years (Hiruki, 1997; Nakamura et al., 1996). The infected princess tree branches will show a very small yellowish leaves followed by branch dieback (Liu et al., 2013). Studies have determined it is caused by phytoplasma belongs to the Aster Yellows group "*Candidatus* Phytoplasma asteri" to be the causal agent of witches' broom, which is potentially transmissible between trees by the stinkbugs *Halyomorha mista* Uhler and *H. halys* Stål(Gao et al., 2008; Liu et al., 2013; Yue et al., 2008). The mode of action could be related to change of gene-expression in response to the phytoplasma (Cao et al., 2021). Some studies have suggested that there may be genetic resistance to the disease in the hybrid cultivar *P. tomentosa* × *P. fortune* (Tao et al., 2005). In addition, several other diseases have been found, such as Phytophthora root and collar rot (Aloi et al., 2021) and rot caused by *Trametes hirsuta* in Serbia (Milenković et al., 2018). Recently reports also indicated the presence of nematodes (*Meloidogyne hapla*) in the roots of *P. tomentosa* in Poland (Skwiercz et al., 2019).

In vitro propagation: Princess tree has been reproducing sexually or asexually; however, for mass production of princess tree asexual reproduction mostly preferred (Jakubowski Marcin, 2022). Asexual reproduction can be conventional propagation methods such as root splitting (Bergmann, 2003) or using advanced technologies in tissue culture(Bahri et al., 2013; Litwińczuk et al., 2012; Mariusz et al., 2019; Mohamad et al., 2022; Rout et al., 2001; Yadav et al., 2013). The seedling production using seed is rarely used because the seed germination is too slow and uneven. Therefore, the most prudent and effective methods of propagation of princess tree are conventional and modern asexual propagations (Damir Drvodelić, 2018). Hence, specialized, and well-equipped laboratories in different part of the world have been established to grow Princess tree using in vitro propagation. For instance, in United States of America Carolina Pacific International, Inc are well known company for mass propagation of Princess tree using advanced technologies (Pezzaniti Michael, 2017).

The in vitro propagation provides healthy, high quality planting stock which might aid for mass production and afforestation of princess tree. As mentioned earlier efficient vegetative in vitro propagation has many advantages than the seedling of Paulownia ssp. (Markovic et al., 2013). This approach allows for the multiplication of basic material without the risk of infection and yields large number of healthy and uniform plants that will be suitable for further multiplication or development depending on the production goals (Rahman et al., 2013) .

Hybrid Princess Tree: The most common cultivated species of princess tree are *P. tomentosa, P. elongata, P. fortunei, P. taiwaniana, P. fargesii, P. galbrata and P. catalpifolia* (Lila et al., 2016; Sivacioglu et al., 2006). In the early introduction of paulownias in different part of the world, pure botanical species were used. United States of America was one of the first countries to introduce princess tree (*P. tomentosa*) on a large scale in 1840's. Because of its fast growth, it was named "the tree of the future". Over the last 150 years, and it has spread widely across various states; however, recently causing a great deal of trouble that has resulted in a heated debate regarding all species of *Paulownia*. *Paulownia tomentosa* has officially been declared an invasive species, therefore, it has been eradicated from many states. More indulgent treatment has been given to other species such as *P. elongata*, which is not as invasive as *P. tomentosa* but is also accepted hesitantly(Jakubowski Marcin, 2022).

In some other countries, certain species of *Paulownia* have been declared dangerous, such as *P. tomentosa*, which has been recognized as an invasive species in Austria(Essl Franz, 2007). The Czech Republic, too, has declare it as invasive, pass the status of an alien species requiring constant monitoring (Pergl et al., 2016). State authorities in Poland have also been cautious about the introduction of paulownia species on a mass scale(Jakubowski et al., 2018). Natural *Paulownia* species are still being grown throughout Asia, and Turkey, but are now increasingly being replaced by hybrids. In some countries, such as Bulgaria, hybrids have only gained importance as a potential product after previous attempts to cultivate pure species failed to grow(Gyuleva, 2010). To produce hybrids, individuals are selected from several popular species that exhibit high productivity and high environmental adaptability, including *P. elongata* × *P. fortune* (García-Morote et al., 2014) and *P. fortune* × *P. tomentosa* (Ayan et al., 2006). Following these some of the best-known hybrids are the clones in vitro 112 (Berdón et al., 2017; Icka et al., 2016; Kadlec et al., 2020) , Cotevisa 2, Sundsu 11 (Antonio et al., 2013) and Shan Tong (Ištok et al., 2020; Luca et al., 2014). There are also naturally occurring hybrids, such as *P. taiwaniana* from a cross between *P. kawakamii* × *P. fortunei* (Wang et al., 2013; Wang et al., 1994). Sometimes, more unusual hybrids “9501” are formed by a double cross between ((*P. fortunei* × *P. elongate*) × (P. fortunei × P. tomentosa)) (Ištok et al., 2020). The princess tree plantations are mainly based on the end purpose. The most used spacing is ranging from 2 × 1.5 m^2 to 4 × 4 m^2. For biomass production, about 2000–3300 plants/ha are planted, while for timber production, far fewer are planted, which is about 550–750 trees/ha (Berdón et al., 2017; Icka et al., 2016). Paulownia hybrids grown for timber production mostly taken 6–10-year cycle, but these cycles can be even shorter for biomass production(Berdón et al., 2017).

Explants: Most of the time in vitro propagation of *Paulownia tomentosa* has been achieved through shoot bud regeneration directly from leaf explants or via the callus induction (Bergmann et al., 1997), through seed culture (Mohamad et al., 2022; Shtereva et al., 2014) and nodal culture has also been reported (Bahri Nada Ben & Bettaieb Taoufik, 2013; Chunchukov & Yancheva, 2015; Mariusz Pozoga et al., 2019; Rahman et al., 2013).

Surface Sterilization: For the successful in vitro propagation, the material used as an explant should be cleaned from debris and other deleterious microorganisms following the lab protocols. Regardless of the types of explants used the explant should be washed with distilled or tab water followed by surface disinfected by Deeping in to 70% ethylene alcohol for seven minutes followed by 0.2 % $HgCl_2$ for three and 10 minutes, respectively (Mohamad et al., 2022; Shtereva et al., 2014). The concentration, frequency and the duration depends on the specific lab protocol, availability, cost, the type, and quality of propagating material.

Medium Composition: Murashige and Skoog medium (MS) (Murashige & Skoog, 1962) has been widely implemented for in vitro propagation of the Princess tree. As a result, among the different growth regulators investigated, MS medium enriched with Benzyl Amino Purine (BAP) is significantly more effective than the other growth hormones in most *Paulownia* species; nevertheless, some species may exhibit variable responses. For example, TDZ in conjunction with IAA elicited the greatest regeneration response from robust leaf explants of *P. tomentosa* (Corredoira et al., 2008). In general, In vitro propagation of princess tree root is as simple as employing micro cutting MS basal medium without inclusion of an auxin growth hormones(Bahri & Bettaieb, 2013). Several Paulownia Species have been shown to produce hyper hydric shoots during in vitro culture (Yadav et al., 2013).

3. MATERIAL AND METHODS

3.1 Plant Material and Explant Source

This study was undertaken in plant tissue culture laboratory of Bademli Farmer's Cooperative, Izmir, Turkey. For this experiment, mature Princess tree (*Paulownia tomentosa*) seed was collected from Izmir Metropolitan Municipality Garden (IMMG). The pericarp was broken, taken out the seed and brought to the laboratory to use as an explant source for this experiment.

3.2 Establishment of Aseptic Intact Seedlings

The seed was washed carefully under running tap water for 25-30 minutes, followed by liquid anti-bacterial detergent for extra 15-20 minutes. After repeated washing with distilled water (three times), the explants were finally treated with 0.1% $HgCl_2$ for half-hour under the laminar air flow cabinet and washed three times with autoclaved double-distilled water.

3.3 Culture media and Culture Condition

After surface sterilization of the seed, it was transferred to the germination chamber and 20 seeds per jar were placed inside on Murashige and Skoog medium (MS) (Murashige & Skoog, 1962) fortified with 0.5mg l^{-1} BAP, myoinositol (100 mgl^{-1}), K_2SO_4 (990 mgl^{-1}), 3g l^{-1} agar and sucrose (3%, w/v).

Shoot regeneration: After germination and raising seedlings in a growth chamber 30-day old aseptic seedlings shoot tips were used as an explant for shoot regeneration. As shown on (Figure 3.1) two shoots per each jar were excised and transferred to MS media containing 3% sucrose solidified with $3gl^{-1}$ agar supplemented with different concentrations of BAP(6-Benzylaminopurine) and NAA (Naphthalene Acetic Acidic) (Table 3.1). 5.6 as a pH of the media was maintained thoroughly. After

4 weeks of culture, the average number of shoots per explants, the length of shoot, and number of leaves per shoot were recorded.

Figure 3.1. Excised shoot used for shoot induction.

Root induction: as shown on (Figure 3.1&Figure 3.3) five in vitro raised shoots (1.5-2.0 cm in length) were cultured on MS basal medium supplemented with different rate of Auxin (IBA and NAA) for two weeks (Table 3 2). After two weeks the mean number and length of roots per explant were recorded.

All seated cultures were incubated under 16/8 h photoperiod in the growth chamber under light intensity of 40 μMm2s^{-1} with cool-white, fluorescent lamps at 22 °C – 25 °C temperature and 65% – 70% humidity for about two weeks (Figure 3.4).

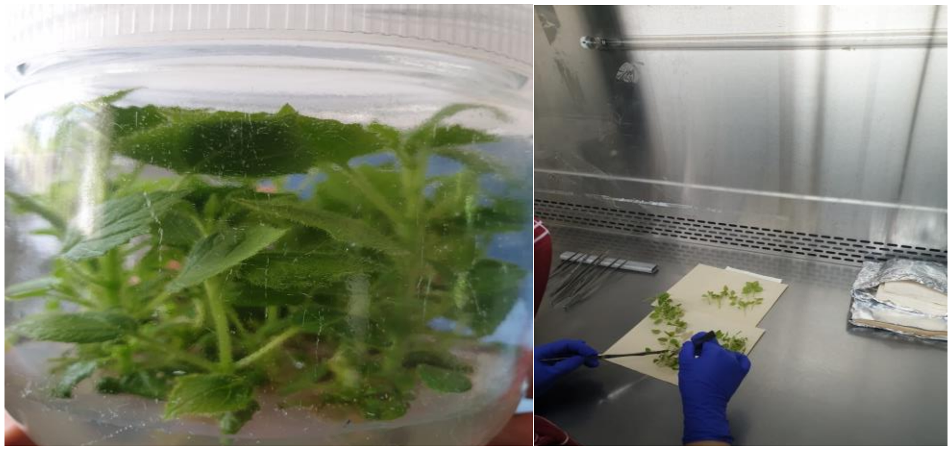


Figure 3.2. In vitro raised young shoots (1.0-1.5cm) excised for root induction.

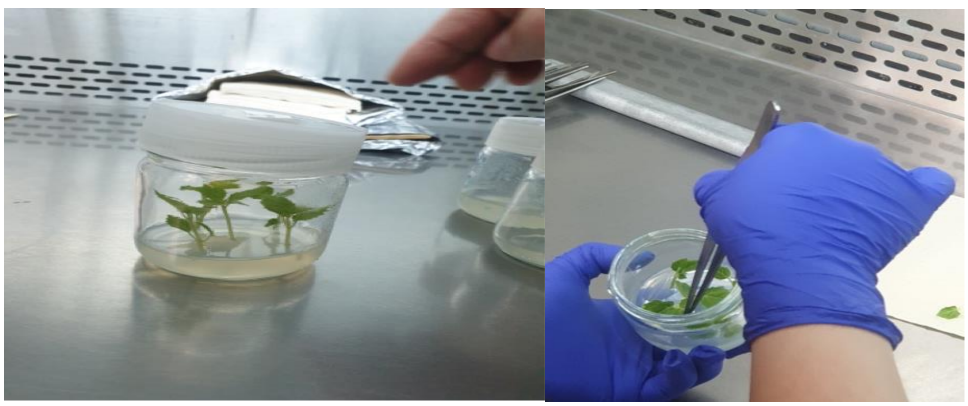


Figure 3.3. Excised shoot treatment with different growth inducing hormones for root induction.

Figure 3.4. Rooting treatments inside growth chamber.

Table 3.1. BAP with NAA combinations for shoot induction

Treatment No	Cytokinin (mgL^{-1})	Auxin (mgL^{-1})
	BAP	NAA
1	0	0
2	0.5	0
3	0.5	0.1
4	1.0	0.1
5	1.5	0.1
6	2.0	0.1

Table 3.2. NAA and IBA Medium rating for root induction

Treatment No	Auxin (mgL^{-1})	
	NAA	IBA
1	0	0
2	0.5	0
3	1.0	0
4	0	0.5
5	0	1.0

3.4 Acclimatization of Plant Under In vivo Condition

Normal, and healthy plantlets were taken out from the culture medium, and the roots were thoroughly washed under running tap water to remove agar. Plantlets were transferred into a plastic pot (5-8cm in diameter) containing a mixture of 1:1:1 ratio of cocopeat, perlite, and peat. Root trainers with micronutrients were then accommodated in the screenhouse for 25-30 days under 30±2°C and 65± 5% RH and then transplanted to the field.

3.5 Experimental Design and Statistical Analysis

This experiment was laid out using Completely randomized design with 3 replications. The obtained data were analyzed using one way Analysis of variance (ANOVA) by means of R statistical software to determine the variation between treatments as explain by Gomez and Gomez (Gomez & Gomez, 1984). Whenever analysis of variance showed significance result between any of the two means at p=0.05 (the probability chosen for the F value), Duncan Multiple Range Test (DMRT) were determined.

4. RESULT AND DISCUSSION

4.1 Effect of BAP + NAA Concentration on Shoot Proliferation and Development

The Analysis of variance (ANOVA) showed that Plant growth regulator (BAP + NAA) presented a very highly significant effect (*$P \leq 0.001$*) on the average number of shoots and leaves produced explant^{-1} of Princess Tree. However, there was no significant effect (*$P \leq 0.05$*) observed on shoot length (Table 4.1).

As shown below on (Table 4.2) there is no significance difference among used hormones on shoot initiation. Technically speaking shoot initiation without the application of growth hormone were possible.

Table 4.1. Mean square values of ANOVA for shoot induction related parameters of paulownia as affected by applications of different rate of growth regulator hormones.

SOV	DF	NSH	SHL	ANL
Treatment	5	8.35^{***}	0.25^{ns}	7.95***
Error	12	0.33	0.12	0.27

SOV (Source of Variation), DF (Degree of Freedom), NSH (Number of Shoot), SHL (Shoot Length), ANL (Average Number of Leaves), ns (non-significance), *** which has equivalent in designation with p<0.001(Very highly significance effect).

As depicted on (Table 4.2) the maximum (6.67~7.0) shoots explant^{-1} was recorded with the application of 1.5 BAP+ 0.1NAA followed by 2.0 BAP+0.1 NAA (5.0 shoot explant^{-1}) and 1.0 BAP+0.1 NAA (4.67~5.0 shoots explant^{-1}). However, the least number of shoots explant ^{1}(2.0) was recorded with no application of growth hormones (control).

The extrinsic and intrinsic application of growth regulators has a determinantal effect on the metabolic and morphologic change of paulownia. As we have seen on the result increasing an application of BAP (0-1.5) increases the number of shoot and leaves of paulownia.

This result indicates that the progressive increase of BAP concentration (from 0 to 1.5 mg l^{-1}) enhancing shoot proliferation. This result also further supported by Salem et al. (2022) who reported that there was a significance effect observed with increasing concentrations of BAP from 0 to 2.0 mgl^{-1}. However, further increase results negative physiological effect by producing abundant callus growth on the base of the plantlet. Similar results were also reported by Fahmy & Gendy (2018) on Paulownia Hybrid tree, Abdi et al. (2013) on *Aloe Vera*, Hang et al. (2013) on *Philodendron Xanadu* and Krishnan et al. (2018) on *Ophiorrhiza mungos.*

These results could be explained by the fact that cytokinin have important physiological function, as they have been shown to stimulate cell division as well as cell elongation and development of lateral bud through activation of RNA synthesis that helps to stimulate protein synthesis and enzyme activity (Sachin & Homrai, 2021; Wu et al., 2021).

Table 4.2. Effect of Different BAP+NAA Concentration on Shoot Proliferation and Growth of Princess tree After four weeks of a multiplication stage.

Plant growth regulators (BAP+NAA mgl^{-1})	Shoot initiation (%)	Number of shoot $explant^{-1}$	Length of shoot (cm)	Number of leaves $shoot^{-1}$
Control	100%	2.00^{d}	1.50^{ns}	4.00^{e}
0.5+0.0	100%	3.33^{c}	1.90^{ns}	5.33^{d}
0.5+0.1	100%	3.00^{cd}	1.00^{ns}	5.67^{cd}
1.0+0.1	100%	4.67^{b}	1.46^{ns}	6.33^{c}
1.5+0.1	100%	6.67^{a}	1.51^{ns}	7.33^{b}
2.0+0.1	100%	5.00^{b}	1.60^{ns}	8.67^{a}
mean		4.1	1.49	6.2
CV		14	23	8.4

BAP (Benzyl Aminopurine), NAA (Naphthaleneacetic Acid), CV (Coefficient of Variation in percentage), ns (non-significant difference), Means in the same letter and columns are not significantly different at 5%).

As shown above on (Table 4.2) the average number of leaves per shoot (6.2) was recorded. Hence, the maximum leaf number (8.67~9) was recorded with the application of maximum amount of growth regulator hormone (2.0 BAP+0.1NAA mgL^{-1}) followed by seven (7.0) leaves per shoot was recorded while the author applied (1.5BAP+0.1NAA mgL^{-1}). However, the least number of leaves per shoot (4.0) was recorded with no application of BAP and NAA). Lila et al. (2016) also reported that the abundant number of leaves per plant were obtained while maximizing the BAP concentration from 0.5 to 2.5 mgl^{-1}. However, a contrasting result was reported by Salem et al.(2022) who reported that there were no significance effect observed on leaves number of princess tree with application of different rates of BAP.

4.2 Effect of Different Concentration of IBA and NAA on Root Proliferation and Development

As shown on (Table 4.3) application of different rating of IBA and NAA has revealed a highly significant effect ($p \leq 0.01$) on number of roots per plantlet and root length of princess tree. Except control (neither IBA nor NAA applications) and 0.5 NAA mgl^{-1}the remaining treatments (0.5 IBA, 1.0 IBA, 1.0 NAA mgl-^{1}) were resulted a 100% root initiation (Table 4.4).

Table 4.3. Mean square values of ANOVA for root induction related parameters as affected by applications of different rate of growth regulator hormones.

SOV	DF	NR	RL
Treatment	4	70.31**	3.39**
Error	10	7.07	0.38

SOV (Source of Variation), DF (Degree of Freedom), NR (Number of Root), RL (Root Length), ns (non-significance), **(p<0.01) or (Highly significant effect, *** which has equivalent designation with p<0.001(very highly significance variation).

Hence, the maximum (18.6~19) and the minimum (5.0) roots per shoot was recorded by applications of 1.0 NAA mgl^{-1} and control, respectively. However, there was no statistical difference observed between 0.5-1.0 mgl^{-1} IBA and 0.5 mgl^{-1}NAA on number of roots per shoot (Table 4.4). And this result showed that IBA is better for rooting than NAA. The results are fully in agreement with that obtained by Rahman et al. (2013) and they also concluded that IBA is far better than NAA for root growth and development.

Table 4.4. Effect of different concentration of IBA and NAA on root proliferation and growth of Princess tree after two weeks of induction.

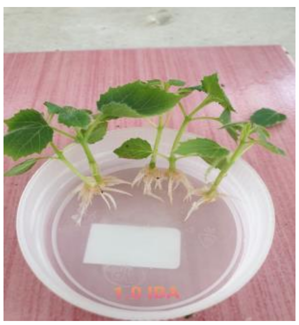
Plant growth regulators (IBA and NAA mgl^{-1})	Root initiation (%)	Number of Roots $Shoot^{-1}$	Length of Root (cm)
Control	93.3%	5.06^{c}	2.73^{ab}
0.5_IBA	100%	10.9^{b}	3.12^{a}
1.0_IBA	100%	12.9^{b}	1.74^{bc}
0.5_NAA	93.3%	12.3^{b}	0.76^{c}
1.0_NAA	100%	18.6^{a}	0.89^{c}
mean		11.9	1.85
CV		22	33

IBA (Indole-3- Butyric Acid), NAA (Naphthaleneacetic Acid), CV (Coefficient of Variation in percentage), Means in the same letter and columns are not significantly different at 5%).

There was a difference on response of Princess tree root length on the type of growth hormone used and concentration. As notice on above (Table 4.4) after two weeks of application the maximum root length (3.12cm) was recorded with the application of 0.5 mgl^{-1} IBA. This was basically not statistically difference observed on root length with no application of growth hormones. However, applying 0.5-1.0 mgl^{-1}NAA for root induction resulted shorter root length than 0.5 mgl^{-1}IBA (Figure 4.1). These results in line with that obtained by (Rahman et al., 2013).

a) Control

b) 0.5 IBA mgl^{-1}



c) 1.0 IBA $mgl^{-1.}$

d) 0.5 NAA $mgl^{-1.}$

e) 1.0 NAA mgl^{-1}

Figure 4.1. Princess tree root response for different application rate of Auxin (IBA & NAA).

Root length and diameter are the most important component of the higher plant such as princess tree that helps to tap water and nutrient to increase biomass and survive. A study carried out by Thevs et al. (2021) clearly showed that in abundant water Princess tree biomass grew by 4.81 kg at 2.36 l/day in average (water productivity 11.9 g/l). Therefore, on marginal soil large tap root plays an indispensable role for maximizing water productivity and tapping soil moisture effectively.

5. CONCLUSION

Princess tree are becoming the most popular tree species in Turkey for its prominent use in paper industry, honey production, afforestation, and land- scape designing. The Aegean Region of Turkey has extreme hot weather conditions during summer and the fast-growing Princess tree (*P. tomentosa*) could be a better solution to ameliorate the altered weather conditions. Micropropagation is known to be the most effective method in Paulownia spp. And an efficient regeneration of *P. tomentosa* Steud. was established using 4 weeks old shoot tip explants that was from in vitro germinated seedlings in laboratory (Bademli cooperative). Within four weeks we were able to regenerate Seven shoots on average recorded 1.5 cm long with the application of 1.5 mgl^{-1} BAP+0.1 mgl^{-1} NAA. Compared with NAA application of IBA were successful on root regeneration. Hence, lesser (0.5 mgl^{-1}) application of IBA far better than 1.0 mgl^{-1} IBA and it produces longer and more fine roots that could probably help the tree for tapping water, nutrient, and essential minerals.

The result of this work is a one-year experiment and could help as a preliminary information. Therefore, more research has to be done on the type of medium requirements of Princess tree, molecular study on soma-clonal variation, *ex vitro* propagation techniques of princess tree and efficient acclimatization protocol development.

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