BOLU ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

IN VITRO GERMINATION AND GROWTH OF SESAME (*Sesamum indicum* L.)

MASTER OF SCIENCE

LEBOGANG CHARLOTTE THEMBA

BOLU, NOVEMBER 2020

BOLU ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF BIOLOGY

IN VITRO GERMINATION AND GROWTH OF SESAME (*Sesamum indicum* L.)

MASTER OF SCIENCE

LEBOGANG CHARLOTTE THEMBA

TEZ DANIŞMANI
Doç. Dr. Songül Gürel

BOLU, NOVEMBER 2020

APPROVAL OF THE THESIS

IN VITRO GERMINATION AND GROWTH OF SESAME (*Sesamum indicum* L.) submitted by LEBOGANG CHARLOTTE THEMBA and defended before the below named jury in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, Graduate School of Natural and Applied Sciences of Bolu Abant Izzet Baysal University in 30.11.2020** by

Examining Committee Members	**Signature**
Supervisor Assoc. Prof. Dr. SONGÜL GÜREL BOLU ABANT IZZET BAYSAL UNIVERSITY	.....
Member Prof. Dr. ARZU TÜRKER BOLU ABANT IZZET BAYSAL UNIVESITY	.....
Member Prof. Dr. ATALAY SÖKMEN KONYA FOOD AND AGRICULTURE UNIVERSITY	.....

Graduation Date: .../12/2020

Prof. Dr. Osman GÖRÜR **……..……**

Director of Graduate School of Natural and Applied Sciences

DEDICATION

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, **Eric and Elizabeth Themba** whose words of encouragement and push for tenacity ring in my ear. My brothers **Jabulani and Tshegofatsi Themba** for their moral support and always being by my side

To my late grandmother, **Asina Mondlani**, who taught me to be like river reed that survive in shallow waters, can withstand small and big water currents and cannot be blown by big winds. This taught me perseverance and to stand firm in my goals and dreams.

To my late grandfather, **Johannes Makhubela**, and grandmother, **Christinah Makhubela**, who raised me and build me to be the person that I am and taught me to always be me.

And, to my entire family for their unconditional and unwavering love and for allowing me to follow my dreams no matter how crazy they looked.

I also dedicate this dissertation to my many friends in Turkey and South Africa who have supported me throughout the process. I will always appreciate all they have done and finally a very especial thanks to my best friends **Nilza Delmara Do Nascimento Silva** and **Mareme Ndeye Ba** for being there for me throughout the entire masters program. Both of you have been my best cheerleaders.

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

LEBOGANG CHARLOTTE THEMBA

_____

ABSTRACT

***IN VITRO* GERMINATION AND GROWTH OF SESAME (*Sesamum indicum* L.)**
MSC THESIS
LEBOGANG CHARLOTTE THEMBA
BOLU ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: ASSOC. PROF. DR. SONGÜL GÜREL)

BOLU, NOVEMBER 2020

ABSTRACT: Sesame (*Sesamum indicum* L.) is one of the oldest oilseed in the world. The aim of this research is to study *in vitro* response ability of two sesame cultivars, Baydar-2001 and Muganlı-57. The experiments are classified in 5 groups; (i) testing sesame growth on control MS media against 0.5 mg/l gibberellic acid (GA_3) hormone, (ii) comparing the plant growth regulators kinetin (KIN) and benzylaminopurine (BAP) at 0.5 mg/l on the effect of sesame regeneration, (ii) studying the effect of different sucrose concentrations (0 g, 5 g, 7.5 g, 10 g and 15 g), on germination, (iv) optimizing the best suitable sterilization protocol using 2% sodium hypochlorite at different time intervals (5, 10 or15 min), and (v) to attempt sesame regeneration using cotyledon and hypocotyl explants excided from salt-pre-treated seedlings using NaCl at different concentrations (0 mM, 50 mM, 100 mM and 150 mM). GA_3 hormone at 0.5 mg/l resulted in a higher germination rate in explants compared to the control medium but the growth rate observed in the control medium was higher. According to the results, KIN at 0.5 mg/l is suitable to be used in sesame regeneration. Even though explants grown in BAP supplemented media had the longest root length at 8.5 cm highest shoot length 2.15 cm and shoot number 8.17 was observed on explants grown in KIN supplemented media, which might indicate that plants do not receive nutrients well with BAP. Of all the sucrose concentration tested, control (sucrose-free) treatment was sufficient for sesame germination and contamination rate to be reduced. Sucrose in sesame is directly proportional to its contamination rate. For sterilization 2% NaOCl concentration for 5 minutes is effective at reducing contamination of *S. indicum* L. Concentration for a longer time interval (10-15 minutes) inhibits seed germination. Data analysis showed that sesame salt tolerance is at zero mM, growth rate slows down at 50 mM and germination and growth at 100 mM -150 mM is inhibited and that pre-salt treatment of seeds inhibits or reduces regeneration ability in cotyledons and hypocotyl explants of the both sesame cultivars. Callus induction was unsuccessful but whole plant regeneration was observed in both cultivars with Baydar-2001 producing the highest cotyledon response rate (75%) and hypocotyl response rate (67%) and Muganli-57 had the lowest**.**

KEYWORDS: *Sesamum indicum* L, Regeneration, Plant growth regulators,

Sucrose, Sodium chloride, Hypocotyl, Cotyledon

ÖZET

SUSAM (*Sesamum indicum* L.) BİTKİSİNDE İN VİTRO ÇİMLENME VE BÜYÜME
YÜKSEK LİSANS TEZİ
LEBOGANG CHARLOTTE THEMBA
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI
(TEZ DANIŞMANI: DOÇ. DR. SONGÜL GÜREL)

BOLU, KASIM - 2020

ÖZET: Susam (*Sesamum indicum* L.) dünyadaki en eski yağlı tohumlu bitkilerden biridir. Bu araştırmanın amacı, iki susam çeşidinin, Baydar-2001 ve Muganlı-57'nin *in vitro* tepki kabiliyetini incelemektir. 5 Farklı deney yapılmıştır; (i) MS ortamında gelişmenin 0,5 mg/l GA_3'de test edilmesi, (ii) 0,5 mg/l kinetin ve 0,5 mg/l benzil amino pürinin rejenerasyona etkisini karşılaştırmak, (ii) farklı sükroz düzeylerinin (0, 5, 7,5, 10 ve 15 g) çimlenme üzerine etkisini incelemek, (iv) farklı zaman aralıklarında (5, 10, 15 dakika) %2 NaOCl'in optimizasyonu ve (v) farklı düzeylerde (0, 50, 100 ve 150 mM) NaCl kullanılarak ön tuz uygulanmış fidelerden alınan kotiledon ve hipokotil eksplantlarının rejenerasyonunu optimize etmek. 0.5 mg/l'deki GA_3, kontrol ortamına kıyasla daha yüksek bir çimlenme oranı vermiştir. Elde edilen sonuçlara göre 0,5 mg/l KIN susam rejenerasyonunda kullanılmaya daha uygundur. BAP takviyeli ortamda büyütülen eksplantların en uzun kök uzunluğuna sahip olmalarına rağmen (8,5 cm), en yüksek sürgün boyu (2,15 cm) ve sürgün sayısı (8,17) KIN takviyeli ortamda yetiştirilen eksplantlarda gözlenmiştir. Bu da bitkilerin BAP ile besinleri iyi almadıklarını gösterebilir. Test edilen tüm sükroz konsantrasyonları dikkate alındığında, kontrol ortamında susam tohumlarının çimlenme ve kontaminasyon oranlarının azaldığı gözlenmiştir. Ortamdaki sükroz, kontaminasyon oranı ile doğru orantılı olmuştur. Sterilizasyon için, 5 dakika süreyle %2 NaOCl konsantrasyonu, *S. indicum* L.'nin tohumlarının kontaminasyonunu azaltmada etkili olmuştur. Daha uzun süreli (10-15 dakika) sterlizasyonda tohum çimlenmesi ve fide büyümesi olumsuz etkilenmiştir. Veri analizleri, susamın büyüme hızının 50 mM NaCl'de yavaşladığını ve 100 mM ile 150 mM'de çimlenme ve büyümenin engellendiğini, özetle tohumların ön tuz muamelesinin gelişme ve büyümeyi engellediğini veya azalttığını göstermiştir. İki susam çeşidinin kotiledon ve hipokotil eksplantlarında rejenerasyon yeteneğine bakıldığında, kallus indüksiyonu başarısız olmuş, ancak tüm bitki rejenerasyonu her iki çeşitte de gözlenmiştir; en yüksek bitki rejenerasyonu Baydar-2001 çeşidinde kotiledon explantlarında (%75), Muganlı-57 çeşidinde ise hipokotil explantlarından (%67) elde edilmiştir.

ANAHTAR KELİMELER: *Sesamum indicum* L, Rejenerasyon, Bitki büyüme düzenleyicileri, Sükroz, Sodyum klorür, Hipokotil, Kotiledon

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS AND SYMBOLS

BAP : 6-Benzylaminopurine

^{o}C : Degrees Celsius

cm : Centimeter

EtOH : Ethanol

et al. : and others

G : grams

GA(s) : Gibberellin(s)

GA_3 : Gibberellic acid 3

G/L : Gram per litre

HCl : Hydrochloric acid

K : Potassium

KG : Kilogram

KIN : Kinetin

M : Molar

Mg : Magnesium

ML : Millilitre

Min : Minutes

mM : Micro molar

MS : Murashige and Skoog (1962)

NaOCl : Sodium hypochlorite (bleach)

NaOH : Sodium hydroxide

pH : Potential of hydrogen

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my supervisor **Assoc. Prof. Dr. Songül Gürel** for believing in me and passing on the torch of knowledge and for her support and guidance throughout the course of my studies. The author would also like to thank **Prof. Dr. Ekrem Gürel** for his suggestions, comments, inspiring me to reach my highest potential and the generosity of his expertise and precious time. I cordially thank to both of them for their countless hours of reflecting, reading, encouraging, and, most of all, patience throughout the entire process. Their excitement and willingness to provide feedback made the completion of this research an enjoyable experience. Finally, I would like to thank all my mentor-teachers that offered assistance during my research.

1. INTRODUCTION

There is an over-increasing need for sesame in Turkey caused by the increase in consumption and high import/export demands. However, as a result of infertile and small marginal areas that its grown in, and lack of improved cultivars used by farmers, *in vitro* studies and the use of improved sesame cultivars is limited putting the plant at agricultural and economical risks. It is crowned the "queen of oil" because its seed contains 50-60 percent oil that has great stability. Sesame is a valuable herbaceous annual plant from the Pediliaceae family whose plant parts can also be used as food, feed and other health issues and its natural antioxidants are reported to have health promoting effect such as lowering cholesterol levels and hypertension in humans (Noguchi et al., 2001; Sankar et al., 2005). The antioxidants sesamolin, sesamin and sesamol are neutritionally and medicinally beneficial and hence the increasing demand for it. Sesame is used globally with top producing countries being Tanzania, India, China, Nigeria, Myanmar and Sudan (**Figure 1**). Regardless of the it being a globally used product/crop and the many advantages it has compared to other seed crops and other agricultural crops, such as economic advantages, low irrigation requirement, adaptation to a variety of soil and climates, none labor intensive, and high oil content, which makes it the ideal crop to replace other crops especially with the effect global warming has on crops, It has been neglected by the science field, which results to the lack of *in vitro* and regeneration study interest regarding sesame compared to other major crops.

1.1 The Sesame Plant

S. indicum L. (sesame) is a diploid species (2n=2x=26) that is annually grown for its seeds. It is from the Pediliaceae family which consist of 36 species in which 22 are found in Africa, 5 in Asia and 7 commonly found in both continents. It is an erect plant that grows up to 1.5 to 2 meters with a deep taproot of approximately 1m and a well distributed secondary root system (**Figure 2**). The stem, leaves and shape of the crop varies according to the seed type and is affected by soil type, rainfall and day length. Sesame produces bell-shaped white to pale-rose flowers that begin to develop at the leaf axils and is a self-pollinated crop, insect pollination is, however, common. It originates from Africa with traces of domestication found in Asia. The

crop is drought tolerant and is cultivated in tropical and subtropical climates but has been grown in temperate climates as a summer crop (Bedigian, 1985, 1998, 2000, 2004; Bedigian and Harlon, 1986; Weiss, 1983), but is not tolerant to water logging and excessive rainfall. However, with climate change comes lots of rainfall combined with infertility of the small areas which leads to soil salinity that in-turn affect sesame yield.

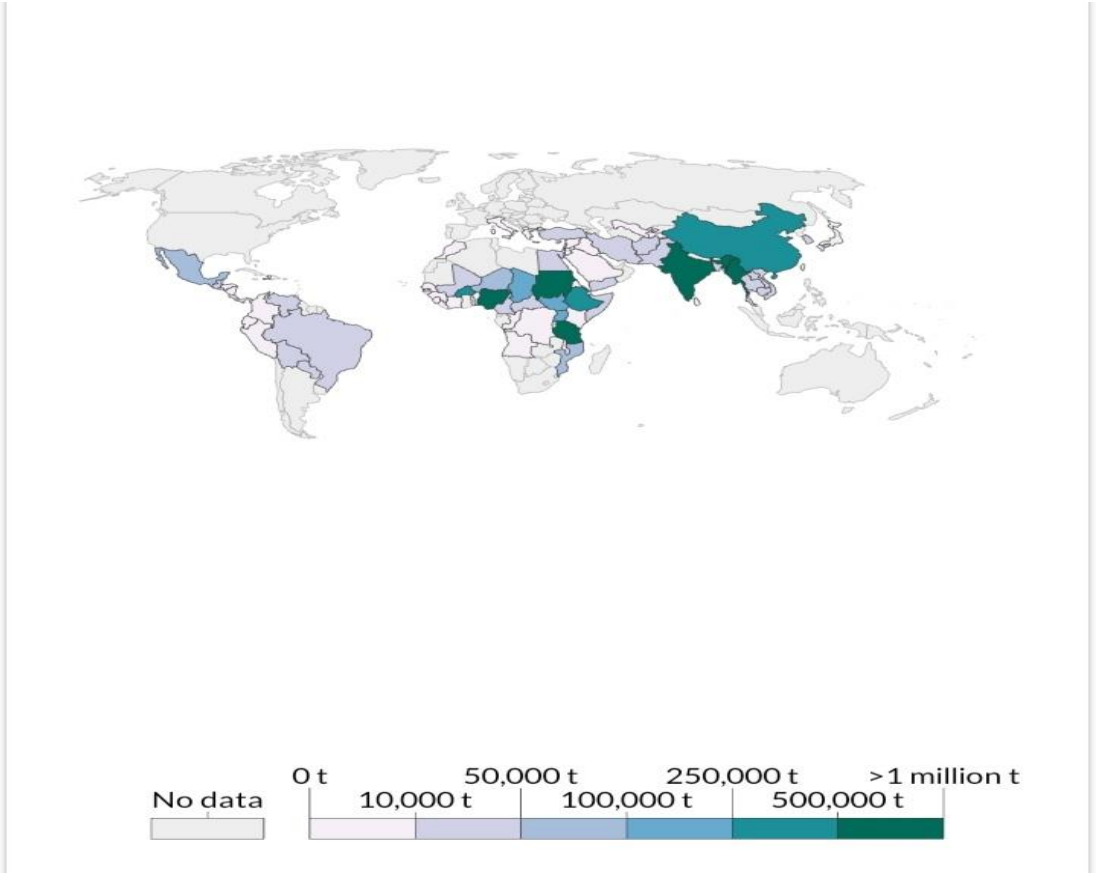


Figure 1. **Sesame seed production in 2018 (measured in tonnes) (UN Food and Agriculture Organization, FAO).**

1.1.1 Sesame in Turkey

Globally known as "the globe sesame" Turkish sesame is used in bakery products, like bagel known as "simit" and confectionery. Sesame is grown in 32 provinces in Turkey, with most of it grown near the southern and western costal region, and in the south east of Turkey with Antalya and Şanlıurfa as the lead sesame production cities (**Table 1**), and the varieties, (**Table 2**) used are grown under a range of ecological conditions (Baydar et al., 1999; Bedigian et al., 1986; Demir,

1962; Hiltebrandt, 1932). The crop is grown as a summer crop and thrives in high temperature. However, the annual sesame production is between 17k-20k and domestic consumption is 130k-150k, which indicates that Turkey is not a self-sufficient country for sesame. Therefore, scientific solutions applied to increase crop yield and battle abiotic stress tolerance are a necessity that will lead to the economical and agricultural benefit of the country.

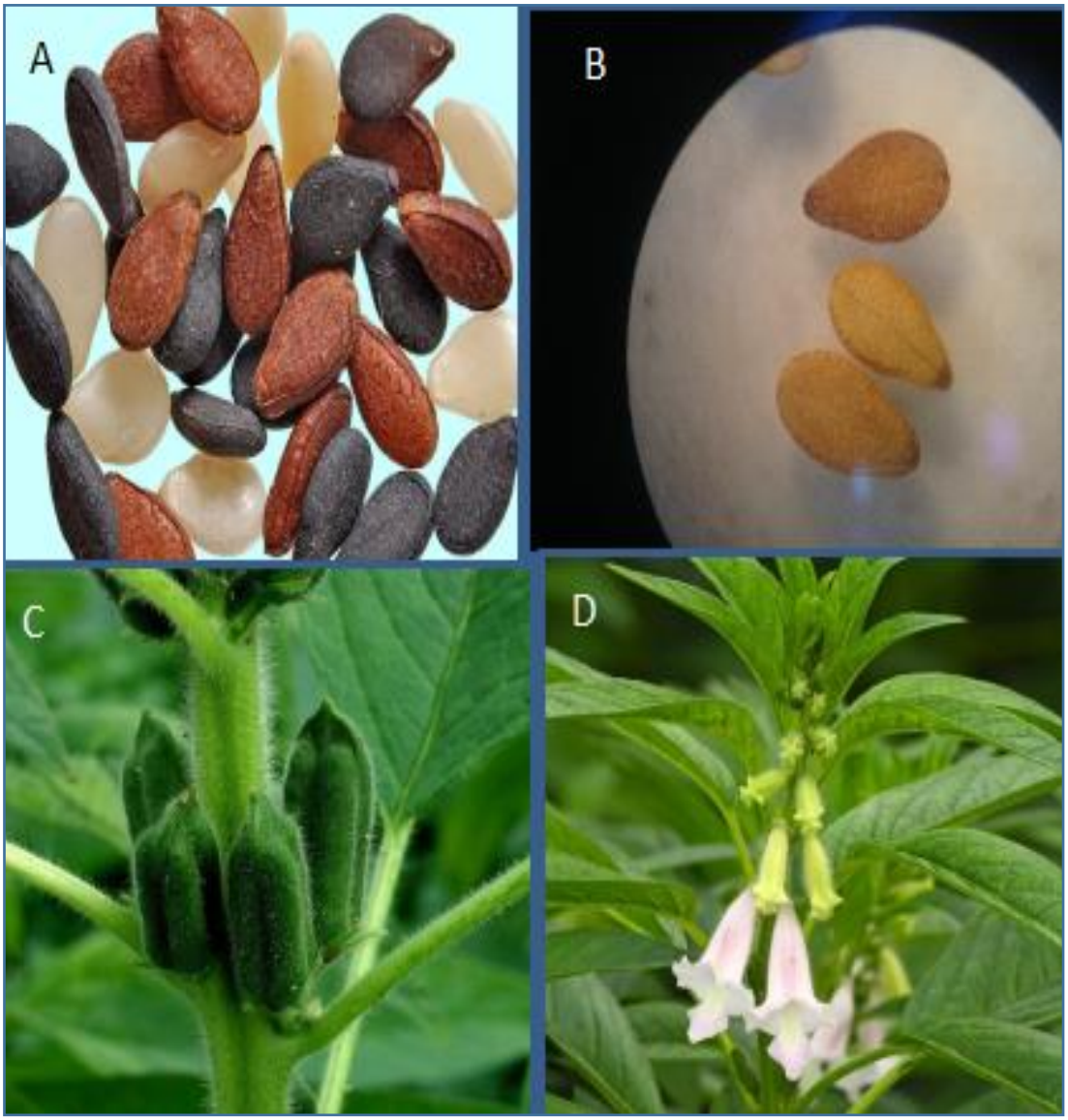


Figure 2. General overlook of *S. indicum* (sesame). a) seeds, b) microscopical view of sesame seeds, c) sesame seed capsule, d) flower (Waynes, 2011).

Table 1. Harvested area, production and yield of sesame in Turkey (DIE, State Institute of Statistics, 2002).

Provinces	Harvested area (ha)	Yield (kg/ha)	Production(T)
Adana	33.3	294	96
Adıyaman	3.084	127	392
Afyon	198	470	93
Antalya	10.814	689	7449
Aydın	83	446	37
Balıkesir	1.008	407	410
Burdur	20	350	7
Bursa	55	491	27
Çanakkale	2.829	491	1389
Denizli	539	468	252
Diyarbakır	642	435	279
Edirne	205	541	111
Elâzığ	105	600	63
Gaziantep	232	384	89
Hakkâri	16	750	12
Hatay	54	222	12
Isparta	21	619	13
Mersin	1.154	782	903
Izmir	123	431	53
Konya	403	370	149
Kütahya	43	488	21
Manisa	1.519	452	686
Kahraman-maras	132	341	45
Mardin	178	180	32
Muğla	6.599	594	3917
Siirt	18	667	12
Sanlıurfa	14.263	297	4243
Uşak	1.89	346	653
Karaman	20	1200	24
Batman	11	455	5
Kilis	461	430	198
Osmaniye	948	344	326

Table 2. Characteristics of Turkish sesame cultivars (Akdeniz University and the Akdeniz Agricultural Research Institute; Uzun et al. 2008; Furat and Uzun 2010).

Cultivar	Description	Year released
Muganlı-57	Plant height is 80–150 cm Maturity at 95–120 days Yellow seed, two carpels, shattering pod 1000-seed weight is 3–5 g, 50–60% oil Average yield of 600–1500 kg/ha	1986 by the West Mediterranean Agricultural Research Institute, Antalya
Özberk-82	Plant height is 90–140 cm Maturity at 95–120 days Yellow seed, two carpels, shattering pod 1000-seed weight is 3–5 g, 45–60% oil Average yield of 600–1500 kg/ha	1986 by the West Mediterranean Agricultural Research Institute, Antalya
Gölmarmara	Plant height is 80–135 cm Maturity at 95–120 days White seed, two carpels, shattering pod 1000–seed weight is 2.5–4 g, 47–61% oil Average yield of 600–1500 kg/ha.	1986 by the West Mediterranean Agricultural Research Institute, Antalya
Kepsut-99	Plant height is 126–157 cm Maturity at 109–115 days White seed, two carpels, shattering pod 1000-seed weight is 3.3–3.52 g, 55.5% oil Average yield of 1300 kg/ha	1999 by the Aegean Agricultural Research Institute, İzmir
Cumhuriyet-99	Plant height is 107–114 cm Maturity at 109–119 days White seed, two carpels, shattering pod 1000-seed weight is 3.13–3.57 g, 55.5% oil Average yield of 1100 kg/ha	1999 by the Aegean Agricultural Research Institute, İzmir
Osmanlı-99	Plant height is 112–120 cm Maturity at 104–111 days White seed, two carpels, shattering pod 1000-seed weight is 3.03–3.45 g, 55.9% oil Average yield of 1150 kg/ha	1999 by the Aegean Agricultural Research Institute, İzmir
Tan-99	Plant height is 112–118 cm Maturity at 106–115 days White seed, two carpels, shattering pod 1000-seed weight is 3.18–3.57 g, 55.3% oil Average yield of 1200 kg/ha	1999 by the Aegean Agricultural Research Institute, İzmir

Orhangazi-99	Plant height is 113–157 cm Maturity at 105–117 days White seed, two carpels, shattering pod 1000-seed weight is 3.48–3.68 g, 56.5% oil Average yield of 1348 kg/ha	1999 by the Aegean Agricultural Research Institute, İzmir
Baydar-2001	Plant height is 120–130 cm Maturity at 90–120 days Pale brown seed, two carpels, shattering pod 1000-seed weight is 2.5–4 g, 59.8% oil Average yield of 1000–1600 kg/ha	2001 by the West Mediterranean Agricultural Research Institute, Antalya

1.2 Salt Stress

Salinity is the most common stress factor in agricultural areas as a result of extensive irrigation and fertilizers application. It occurs when the excess of minerals, [cations: Na (Sodium), K (Potassium), Mg (Magnesium), Ca (Calcium), and anions: NO (Nitrate), HCO (Bicarbonate), SO (Sulfate)], are at high levels in soil and water. This results in osmotic plants stress and reduction in plant growth and production. Saline soil is found at different altitudes and in all climatic regions. Salinity originates from mineral weathering, inorganic fertilizers, soil amendments and irrigation water (Kotuby-Amacher et al., 2000). All soil type(s) contain salt(s), a common necessary component(s) of soil, and many salts such as nitrate and potassium and others are essential plant nutrients (**Table 3**). Calcium (Ca), magnesium (Mg) and sodium (Na) are found in irrigation water, and the precipitation of Ca and Mg leaves Na dominating in the soil (Serrano et al.,1999). Soil dominated by NaCl is a major stress that limits crop production (Deinlein et al., 2014). Sodium and chloride can potentially affect plant enzyme and cause plant swelling, resulting in energy production and other physiological changes (Larcher, 1980). Crops can either be glycophytes, which are most crop plants and do not grow in high salt concentration and are severely inhibited or killed by 100-200 mM NaCl, or halophytes which can survive at 300-400 mM salinity. Crop yields are merely or not affected at low salt concentration (Maggio et al., 2001), but high salinity can affect plant mainly in two manners, high salt concentration disturbs water extraction capacity of root and concentration of salt within the plant, resulting in the inhibition of many physiological and biochemical processes such as nutrient uptake and

assimilation (Hasegawa et al., 2000; Munns, 2002; Munns et al., 1995; Munns and Tester, 2008) , this, in turn, reduces plant growth and development (**Figure 3**).

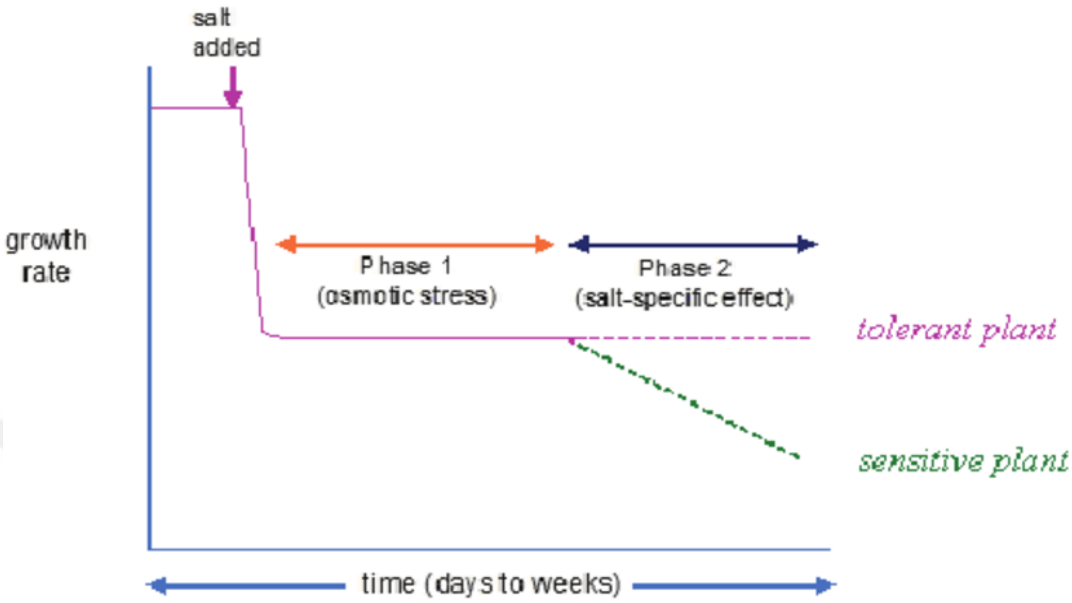


Figure 3. Scheme of the two-phase growth response to salinity (adapted from Munns and Tester, 2008).

Table 3. Form, source, mode of uptake and major function of the 16 plant essential nutrients (Provin and McFarland, https://agrilifeextension.tamu.edu /library/gardening/essential-nutrients-for-plants).

Nutrient Family	Nutrient	Percentage of Plant (dryweight)	Form Taken up by Plant (ion)	Mode of Uptake	Major Functions in Plants
Primary	Carbon	45	Carbon dioxide (CO_2) bicarbonate (HCO_3)	Open somata	Plant structure
	Oxygen	45	Water (H_2O)	Mass flow	Respiration, energy production, plant structure
	Hydrogen	6.0	Water (H_2O)	Mass flow	pH regulation, water retention, synthesis of carbohydrates
	Nitrogen	1.75	Nitrate (NO_3-) Ammonium (NH_4^+)	Mass flow	Protein/amino acid, chlorophyll, cell formation
	Phosphorus	0.25	Dihydrogen phosphate ($[H_2PO_4]^-$, $[PO_2(OH)_2]^-$) phosphate (PO_4^{3-})	Root interception	Cell formation, protein synthesis, fat and carbohydrate metabolism
	Potassium	1.5	potassium(K^+)	Mass flow	Water regulation, enzyme activity
Secondary	Calcium	0.50	Calcium ion (Ca^{2+})	Mass flow	Root permeability, enzyme activity
	Magnesium	0.20	Magnesium (Mg^{2+})	Mass flow	Chlorophyll, fat formation and metabolism
	Sulfur	0.03	Sulfate	Mass flow	Protein, amino acids, vitamin and oil formation
Micro	Chlorine	0.01	Chloride (Cl-)	Root interception	Chlorophyll formation, enzyme activity, cellular development
	Iron	0.01	Iron ion (Fe^{2+}, Fe^{3+})	Root interception	Enzyme development and activity
	Zinc	0.002	Zinc ion (Zn^{2+})	Root interception	Enzyme activity
	Manganese	0.005	Manganese ion	Root	Enzyme activity

			(Mn^{2}+)	interception	and pigmentation
	Boron	0.0001	Boric acid (H_3BO_3) Borate(BO_3^{3}-) tetraborate(B_4O_7)	Root interception	Enzyme activity
	Copper	0.0001	Copper ion (Cu^{2}+)	Mass flow	Enzyme activity
	Molybdenum	0.00001	Molybdenum ion (MoO_4^{2}−)	Mass flow	Enzyme activity and nitrogen function in legumes

1.3 Tissue Culture Studies

Drought and salt stress are one of the few factors that challenge sesame production and cell and tissue culture are conventional techniques that can be used to improve the crop. *In vitro* culture is one of the key tool of plant biotechnology that exploits the totipotency, nature of a plant cell, a concept presented by Haberlandt (1902) and undoubtedly executed for the first time by Steward et al. (1958). The first sesame reported tissue culture was by Lee et al. (1985) and George et al. (1987) and thus far, significant improvement in sesame have been made. However, attempts made to regenerate sesame *in vitro* proved *S. indicum* L. to be a difficult crop to regenerate (Were et al., 2006), which further limits genetic engineering of this crop (**Figure 4**). Explants such as shoot tips, nodes, cotyledons and hypocotyls have been used in the attempts to regenerate sesame in tissue culture. Hypocotyl explants has gained attention in recent years and have proved to be an excellent source for callus induction and subsequent regeneration (George et al., 1987; Jayamary and Jayabbalan, 1997; Rajender Rao et al., 2002 and Seo et al., 2007). Callus achievement is the most basic step in indirect organogenesis where shoot regeneration precedes callus induction. The best *in vitro* shoot regeneration in sesame globally reached 46% in Japan and 66% in India (Shashindhara, 2005). However, Seo et al. (2006) reported a higher regeneration percentage of de-embryonated cotyledon explants.

Figure 4. *In vitro* grown sesame plant.

1.3.1 Plant growth regulators

In biological research, tissue culture refers to a method in which a fragment of tissue (plant or animal) is introduced into a new artificial environment, where they continue to function and grow due to the nutrients it supplies. The success of plant tissue culture as means of plant propagation is greatly influenced by the nature of culture media used. The most commonly used medium in plant tissue culture experiment in laboratories is MS media (Murashige and Skoog, 1962) (**Table 4**).

Table 4. Composition and preparation of stock solution for MS basal medium.

Stock Solutions	Ingredients	Concentration of Ingredient in MS Medium (mg/l)	Stock Solution (mg/l)	Volume to be Taken/ Liter of Medium
Inorganic Macronutrients (20x)				
A	NH_4NO_3 KNO_3 $MgSO_4$ H_2PO_4	1650 1900 370 169	33000 38000 7400 3400	50
B	$CaCl_2.2H_2O$	440	8800	50
Macronutrients (50x)				
C	$MnSO_4.4H_2O$ $ZnSO_4.7H_2O$ H_3BO_3 Kl $Na_2MoO_4.2H_2O$	22.3 8.6 6.0 0.83 0.25	1115 430 310 41.5 12.25	20
D	$CuSO_4.5H2O$ $CoCl_2.6H_2O$	0.025 0.025	1.25	20
Micronutrients (10x)				
E	$Na_2EDTA.2H_2O$ $FeSO_4.7H_2O$	37.3 27.3	373 278	10
Organics				
F	Thyamine HCI Pyridoxine HCI Nicotinic acid Myo-inositol Glycine	0.1 0.5 0.5 100 2	10 50 50 10000 200	10

1.3.1.1 Gibberellins

Gibberellins (GAs) were discovered in a rice disease study conducted by scientist and farmers in Japan 1930s. GAs are composed of a 20-carbon atom skeleton or 19 carbon skeletons, in which all biologically active GAs possess 19 carbon atoms (Tarkowská et al., 2014). GA synthesis occurs via the terpenes route from the geranylgeranyl diphosphate by plants and fungus. An estimate of 140

known GA molecules have been isolated from plant and microorganism (MacMillan, 2001; Kawaide, 2006), and the ones with the highest biological properties and are commercially available are GA_3, GA_4 and GA_7.

GA_3 (gibberellic acid) is a tetracyclic dihydroxy-y-lactone acid containing C1-C2 double bound, C10 y-lactone ring and an OH group in C13 (**Figure 5**). It is a white crystalline powder that is soluble in alcohol, acetone, ethyl acetate and butyl acetate and has a melting point of 233-235 ^{o}C. It decomposes at high temperature, alkaline pH values and aqueous solutions and is stable in dry conditions. GAs are plant hormones that participate in regulation of many growth and developmental process in various plants but because it can inhibit callus growth and auxin-induced adventitious root formation, GA_3 is infrequently used in plant cell cultures (Van Bragt and Pierik, 1971). It is however useful in studies on morphogenesis.

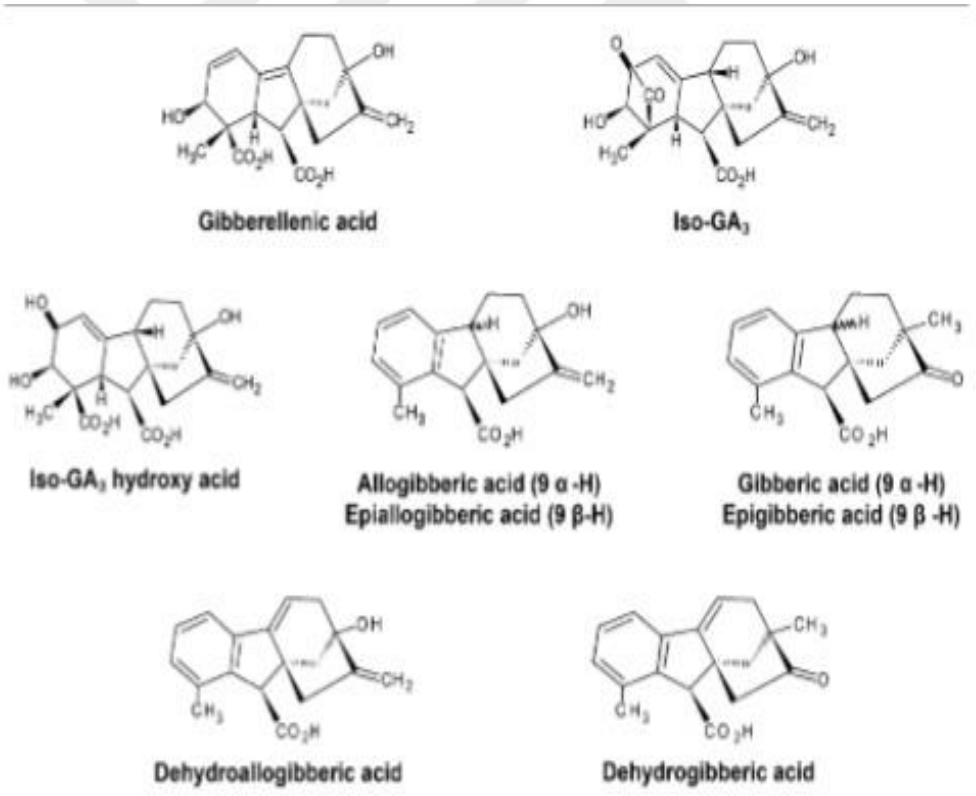


Figure 5. Chemical structures of giberellins.

1.3.1.2 Cytokinins

The first cytokinins were discovered by Haberlandt (1913) in a phloem compound but the first natural occurring cytokinin to be identified was zeatin in immature maize endosperm (Letham, 1973), which is also found abundantly in coconut milk. Cytokinins that occur naturally are adenine derivatives with a distinct substitutions attached to the N6 position of the adenine ring (**Figure 6**) and a typical cytokinin has isoprenoid side chains. Cytokinins can also be found in plants as a riboside or a ribotide (Brzobohaty et al., 1994; Mok and Mok, 2001). They are found in meristematic regions and growing tissue, and are synthesized in the roots and trans-located via the xylem to the shoot. Cytokinin biosynthesis happens through the biochemical modification of adenine (McGaw, 1995; Salisbury and Ross, 1992). There are more than 200 natural and synthetic cytokinin combined and BAP and KIN are synthetic cytokinins. Adenine is the parent compound of natural occurring cytokinins.

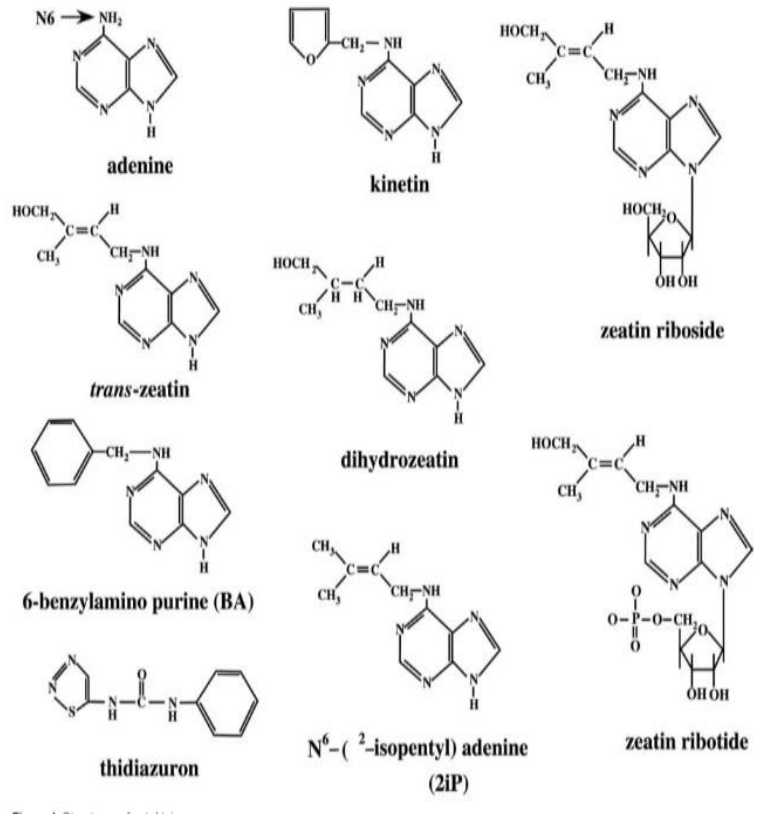


Figure 6. Chemical structures of cytokinins. Adenine is the parent compound of natural occurring cytokinins.

1.3.2 Sucrose

The most commonly used carbon source in plant cell, tissue and organ culture is sucrose. Three percent sucrose containing media has been used since Murashige and Skoog (1962). It acts as a carbon source, sustains photomixotrophic metabolism, maintains osmotic potential and converts water in cells to ensure optimal development. High sucrose concentration in media restricts photosynthetic efficiency (Hazarila et al., 2006) and studies have also shown that plantlets grown under culture conditions cannot/do not fix sufficient carbon dioxide in sucrose absence (Gautheret et al., 1955; Joe et al., 2009).

1.3.3 Aseptic techniques

Cell culture achievement highly depends on keeping cells free from contamination by microorganisms (**Figure 7**). Aseptic techniques are a set of procedures that are designed to provide a barrier between microorganism in the environment and sterile cell cultures to reduce contamination probability. Most plants have microbial contamination within the vascular system, some seeds (Donnarumma et al., 2011), and intercellular spaces in leaf mesophyll, xylem vessels lumen and intercellular spaces of callus (Miyazaki et al., 2011).

Disinfectant agents such as ethanol (EtOH), sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$), mercuric chloride ($HgCl_2$) and others are used to sterilize explants. Sodium hypochlorite is the most commonly used chemical for sterilization due to its strong oxidizing property and is high effectiveness against microorganisms. Proper sterilization using NaOCl depends on the concentration, application period and temperature as it has hazardous effects on tissue health.

In a study on sterilization on seed germination and growth in *Lathyrus chrysanthus* Boiss, 3.75% NaOCl at 15 min produces the best results (Telci et al., 2011) and contamination occurred with decreased concentration and time interval lesser than 15 minutes. In the study we focus on NaOCl concentration and time interval factors on germination and growth of sesame.

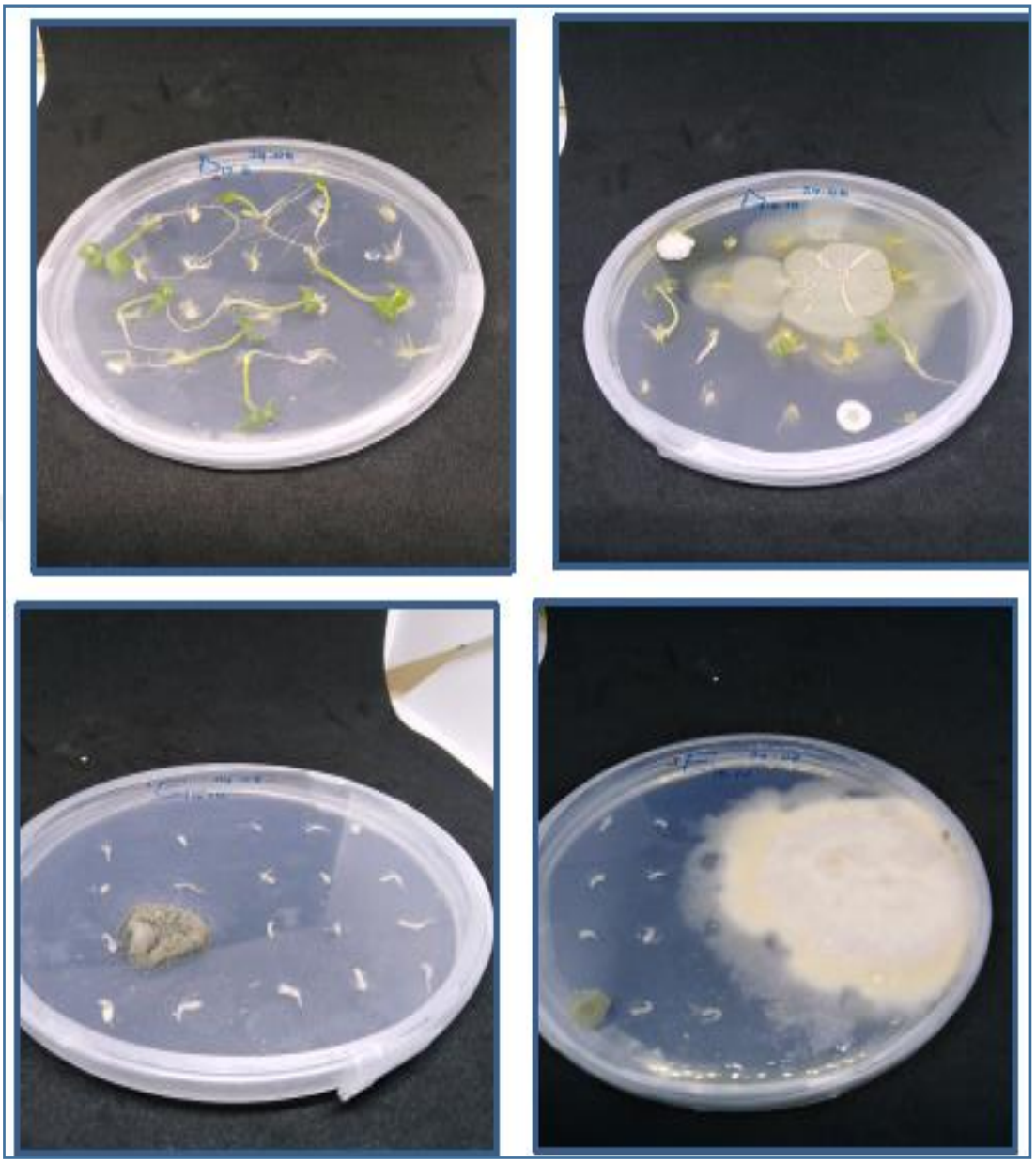


Figure 7. Different types of contamination on *in vitro* grown sesame explants.

2. AIMS AND SCOPE OF THE STUDY

This study investigates responses of *in vitro*-grown plantlets in regards to salt stress, sucrose, plant growth regulators and sterilization methods with the main objectives being;

a) To study the effect of different sucrose concentrations on germination and determine the suitable condition for sesame growth and development under *in vitro* conditions,

b) To investigate the effect the of plant growth regulator GA_3 on germination and the different responds between BAP and KIN on root induction under *in vitro* conditions,

c) To investigate salinity levels and attempt sesame's regeneration capacity after being exposed to salinity stress under *in vitro* conditions,

d) To investigate the optimal time interval best effective for seed and tissue sterilization.

It is anticipated that the results obtained in this study will contribute or assist other researchers or projects aimed at solving abiotic stress regarding sesame, as it is the major stress that limits crop production, thus resulting in severe losses in the agricultural, economical and scientific growth of this crop.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant materials

S. indicum L. seeds from two cultivars, Muganlı-57 and Baydar-2001, obtained from TTSM (Ministry of Agriculture and Forestry, Variety Registration and Seed Certification Center, Turkey) were used as genetic material for this study.

3.1.2 Culture media

The basal media used in all the experiments was Murashige and Skoog (1962) supplemented with sucrose and growth regulators. Its PH is balanced to 5.8 and autoclaved at 105 kpa and 121 ^{o}C for 15 minutes.

3.1.3 Growth regulators

The plant growth regulators used in this study are as follows: 0.5 mg/l KIN, 0.5 mg/l 6-benzylaminopurine (BAP) was used individually on *in vitro* derived shoots for shoot elongation and rooting after 14 days of germination. 0.5 mg/l gibberellic acid (GA_3) was used on seedling for germination.

3.2 Method

3.2.1 Test run

20 seeds from the each of the two cultivars were placed in pots filled with soil and 20 seeds on filter paper in petri plates, moisturized with water and placed in room temperature for germination and growth control purposes. Seeds were not surfaced sterilized as a test run.

3.2.2 Sterilization

Regular sterilization method entails seeds placed in a beaker containing 5% NaOCl with 3-4 drops of tween for 15 minutes. Seeds are then thoroughly rinsed with distilled water until the NaOCl is washed off, then sterilized with 70% ethanol

for 5-6 minutes before being placed on MS media. In the case of this experiment, seeds were just surface sterilized with just 70% ethanol for 5-6 minutes before being placed on MS media.

3.2.3 Tissue culture

3.2.3.1 Plant growth regulators vs control media

Sterilized seeds were placed on two types of MS media (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose and 8 g/L agar, and MS media containing 30 g/L sucrose with 8 g/L and 0.5mg/l GA_3. The pH for both medium were adjusted to 5.8 and autoclaved at 105 kpa and 121 ^oC for 15 minutes before seed culture. Samples were incubated at room temperature under light conditions for growth observation.

3.2.3.2 Subculture of shoots for root induction

Plantlets were subculture 14 days after germination into two different MS media for root induction. The first MS media contained 30 g/L sucrose, 8 g/L agar and 0.5 mg/l BAP with the other was supplemented with 30 g/L sucrose, 8 g/L agar and 0.5 mg/l KIN

3.2.3.3 Sucrose concentration treatments

MS media containing 8 g/L agar and different sucrose concentrations, (0 g, 5 g, 7.5 g, 10 g and 15 g) whose pH was adjusted to 5.8 and autoclaved at 105 kpa and 121 ^oC for 15 minutes was used to culture surfaced sterilized seeds. Cultured seeds were incubated at temperature 25 ^oC for 7-14 days.

3.2.4 Salt pre-treatment

3.2.4.1 Sterilization and salt-pre treatment

2% sodium hypochlorite was used to sterilize seeds at different time intervals, 5, 10 and 15 minutes, and then rinsed with distilled water (dH_20) 6 times. Seeds were then sterilized with 70% ethanol for 3 minutes, placed on filter paper in petri dishes and moisturized with 3 mL solution containing different salt concentrations, 50 mM,

100 mM, 150 mM and salt free solution was used as a control. Samples were placed in light and dark conditions at temperature 25 ^{o}C. Number of germinated seeds, developed roots, regenerated shoots and total length of the plantlets were recorded.

3.2.4.2 Regeneration from cotyledon and hypocotyl explants

Three- or four-day old cotyledons obtained from seedlings pretreated with salt solutions at different concentrations and hypocotyl explants obtained from 14-day old seedlings pretreated with salt solutions at different concentrations were cultured on MS media containing 30 g/L sucrose, 8 g/L agar and 0.5mg/l KIN. The pH of the media was adjusted to 5.8 and autoclaved at 105 kpa and 121 ^{o}C for 15 minutes for pre-culture. Samples were incubated in dark conditions for 3 days and then moved to light condition at room temperature (25 ^{o}C).

3.2.4.3 Subculture of shoots for root induction

Explants excised from control (salt-free) and salt-treated 14 day old seedlings were subcultured on media containing 30 g/l sucrose, 8 g/l agar and 0.5 mg/l KIN, and incubated at culture room in dark condition for 3 days and then moved to the light condition.

3.2.4.4 Data evaluation

All experiments were repeated three times and a total of 60 replicates per treatment were used. Mean values with standard errors were calculated for each treatment and indicated in the tables and graphs accordingly.

4. RESULTS AND DISCUSSION

4.1 Test Run

After day 6 of potting and being placed in petri dishes, germination rate for seeds cultured in light was observed with 90% with a 2.5% contamination rate. Plants were healthy, moist and had a green color. Seeds placed in dark conditions has 95% germination rate with no contamination. An average shoot height of between 1-1.5 cm and 2-2.5 cm root was recorded in seeds placed in light and dark conditions. Seeds placed in pots produced a 100% germination rate, with plantlets of 3-4 cm shoot length (**Figure 8**).

Figure 8. Pot grown Muganlı-57 (D) and Baydar-2001 (C) Sesame plant. a) day 14 Baydar-2001 plant grown in soil b) total plant height measurement c) plant leaf measurement and d) comparison of Baydar-2001 (C) and Muganlı-57 (D) growth.

The high germination rate of potted seeds is due to the nutrient found in the soil it was placed as compared to the seeds placed in the petri dishes, that had to use its stored nutrient for germination. Based on the results obtained; it can be concluded that light and dark conditions does not affect seed germination as there is no significant difference between the treatments.

Culturing the seeds in a complete dark condition seems to promote germination performance of several plant species (Rasmussen, 1995). Different suggestions could be withdrawn between these two parameters but most accepted one is that the effect of light in determining the success ratio of the germination process may not be so critical for the most of the plant species. Similar phenomena is also valid for vegetative growth of the plants in large plantations, especially under forest conditions as many terrestrial plants grow better under shaded environments than their light-grown counterparts due to less access of the light over the habitat floor (Rasmussen and Rasmussen, 1991). It was also reported that a significant reduction in germination ratio was evident when *Spiranthes odorata* seeds were kept, even for a short period of time, under light conditions Zettler and Hofer, 1997). In their studies, germination under full darkness for 3 weeks was found more effective than under conditions of different dark/light regimes, like one week of 8/16 h or 14/10 h photoperiod, which was followed by a transfer to darkness for 2 more weeks (Zettler and Hofer, 1997).

4.2 Effects of Plant Growth Regulators

Explants in media without hormone produced 88% germination rate in the first control and 92% germination rate in the second control at day 7 with a 29% and 19% shoot and root formation, respectively. The mean germination rate is 90±2,56% for seeds cultured in hormone free medium. Explants in hormone containing media produced a 95% germination rate in the first control and 92% germination rate in the second control with a 21% and 20% root formation, respectively. Cultured seeds on GA_3 containing medium produced a 94±1,69% mean germination rate (**Figure 9**). Leaves of explants in control media exhibited a darker green pigmentation.

Root formation at day 23 after germination was higher in control media explants and exhibited root length of 10-30 cm while explants in GA_3 containing media

produced lengths between 2-4 cm. Although GA_3 hormone produced a higher germination rate in explants compared to control media, the higher plant growth rate was observed in the control media. Therefore, we can conclude that GA_3 increases the seed germination rate but slows the growth of sesame shoots.

According to literature GA_3 plays a role in seed germination, leaf expansion, stem elongation flowering and flower development (Yanmaguchi and Kamiya, 2002). Aké et al. (2006) reported in his study that GA_3 not only promoted the frequency but also accelerated germination when compared with the control and a similar deduction was reported with seeds of other palm species such as *Aiphanes erosa*, *Arenga microcarpa*, *Hyphaene schatan*, *Sabal palmetto*, and *Phoenicoforium borsiqianum* (29–72 μM, Odetola 1987) and *Howea forsteriana* (290 μM, Chin et al., 1988). GA_3 also promoted germination of non-palm species such as *Zea mays* (seeds, 10–100 μM, White and Rivin, 2000) and *Panax ginseng* (somatic embryos, 2.9 μM, Kwang-Tae et al., 2000). In general, GA_3 treatment results in a higher seed germination and our results are in agreement due to the obtained results.

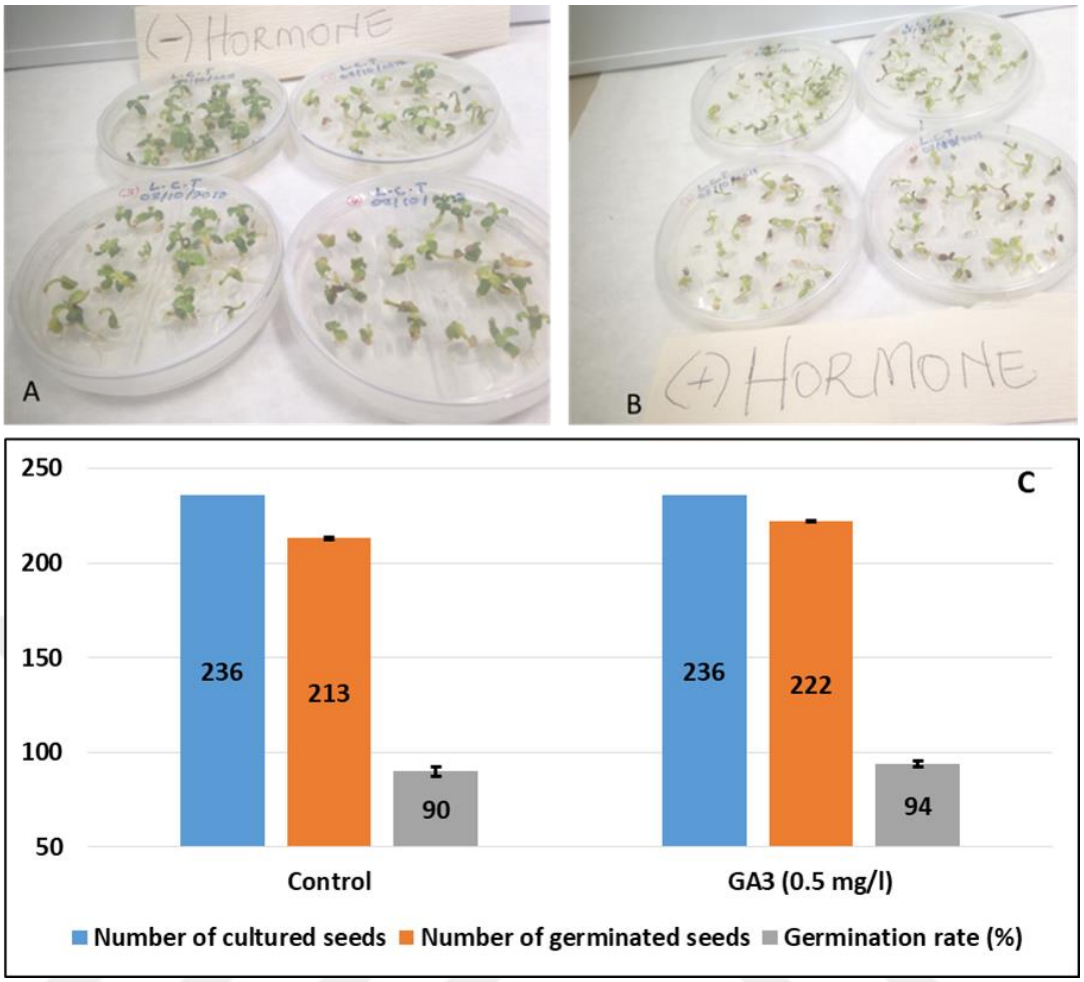


Figure 9. Germinated seeds on hormone free (control) (A) and on GA_3 (0.5 mg/l) containing medium (B). The effect of GA_3 on germination of sesame seeds (C). Error bars represent standard errors.

Subculturing:

Explants in both BAP- and KIN-containing media had a respond and the data was collected on day 7, 10 and 14 (**Figure 10**; **Table 5**). Explants in BAP containing media exhibited a higher growth rate/reaction rate in root formation at 45%, 88% and 64%, while KIN exhibited a 28%, 72% and 59%, respectively (data not provided). The number of shoots formed in explants in KIN containing media was higher at pigmentation in the two controls.

While the number of mean shoots obtained per explant was 8.17 in media containing KIN (0.5 mg/l), the number of shoots obtained in media containing BAP (0.5 mg/l) was 6.67. Shoots cultured on media containing produced longer roots

(8.50 cm) then roots (3.50 cm) obtained from shoots on media containing KIN. The difference was statistically significant for the shoot number and root length in terms of BAP and KIN. The shoot length obtained from both KIN (2.15 cm) and BAP (2.20 cm) containing medium were not significantly different (**Figure 10**; **Table 5)**.

The successful establishment of regenerated plants in soil in *in vitro* depends on the production of plantlets with profuse rooting. Cytokinins has a major role in plant development and growth, including shoot formation and multiplication (Kieber and Schaller, 2014; Mok and Mok, 2001). Consistent with our findings many studies have reported the positive effect of cytokinins on multiple shoot induction (Faiss et al., 1997; Asghari et al., 2012; Amer et al., 2017). Even though BAP is considered to be the most effective cytokinin for stimulating efficient shoot induction in sesame (Yadav et al., 2010; Baskaran et al., 2006; Were et al., 2006; Taskin and Turgut, 1997), it is in contrast with the findings in our experiment and this can be due to genotype and plant regulator concentration among other factors which resulted in different response of shoot induction medium, and according to literature different cultivars elicit different regeneration efficiency (Seo et al., 2007; Were et al., 2006).

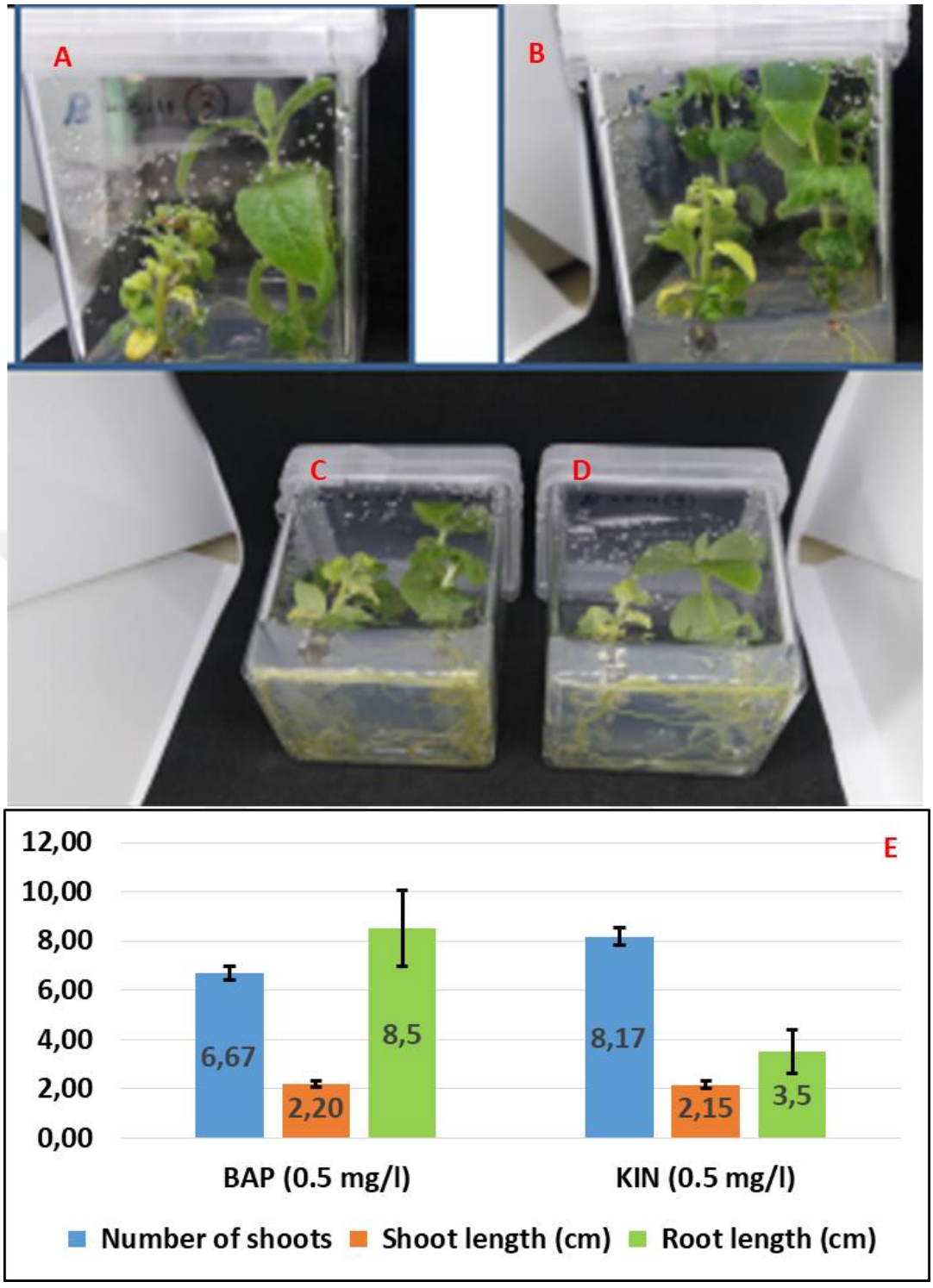


Figure 10. The effect of BAP (A, C) and KIN (B, D) on shoot regeneration and root formation after 14 days of subculture. The comparison of number of shoots per explant, shoot and root length (cm) for BAP and KIN at 0.5 mg/l concentration. Error bars represent standard errors.

Table 5.The effect of BAP and KIN on shoot number, shoot and root lenght of subcultured sesame plantlets.

TREATMENT	EXPLANT NUMBER	SHOOT NUMBER	SHOOT LENGTH (cm)	ROOT LENGTH (cm)
BAP (0.5 mg/l)	1	6	2	8
	2	6	2	12
	3	8	3	1
	4	6	2	13
	5	6	2	12
	6	6	2	5
	7	8	2	8
	8	8	2	5
	9	6	2	5
	10	6	3	11
	11	8	3	20
	12	6	2	2
	Mean±SE	**6,67 ± 0,28**	**2,20 ± 0,13**	**8,50 ± 1,55**
KIN (0.5 mg/l)	1	10	3	0
	2	9	2	0
	3	8	2	6
	4	9	2	5
	5	10	2	10
	6	8	1	6
	7	8	2	3
	8	8	2	2
	9	8	3	0
	10	6	2	3
	11	6	2	2
	12	8	2	5
	Mean±SE	**8,17 ± 0,36**	**2,15 ± 0,15**	**3,50 ± 0,87**

4.3 Sucrose Treatment

This experiment includes treatments in which seeds are placed in different sucrose concentration to study the effect on germination and growth of the plant (**Figure 11; Table 6**). Data for germination was collected on the day 7. An increase in germination is observed with an increase of sucrose until 7.5 and germination rate drops at a concentration greater than 7.5 g (10 g and 15 g). The contamination rate increased with the increasing sugar concentrations. The lowest contamination rate (7%) was obtained in control medium. Highest germination rate (37%) was obtained at 5 g and lowest (26%) at 15 g. Contamination rate was highest at 10 g (27%) and in the control treatment (7%). For growth, a linear graph indicates that an increase in sugar decreases the rate of growth and contamination.

This study validated that the absence of sucrose in culture medium improves the morphology and physiology of a plant, in fact the positive effect a sucrose-free medium in plants have been extensively studied by Kozai (2010). Kozai and Kubota (2005) who reported several advantages of the photoautotrophic systems, such as the promotion of growth and photosynthesis. An increase of the shoot length, leaf number and leaf area per plant was observed in a sucrose-free medium in *Solanum tuberosum* L. (Badr et al., 2011; Hoang et al., 2017). An increase in sesame growth was evident in the treatment of sucrose-free (control) and the opposite negative results of an increase sucrose concentration Yaseen et al. (2013) reported that a negative response of shoots cultivated in sucrose treatment is possibly associated with the increased sugar concentration, which induces osmotic stress, which can affect *in vitro* plant growth. Low chlorophyll content in shoots is one of the most common physiological response induced by high concentrations of sucrose (Regueira et al., 2018). Studies made in *Billbergia zebrina* (Herbert) Lindley, also showed that elevated sucrose concentration decreased the chlorophyll content, which affected the physiological response of the plants (Martins et al., 2016).

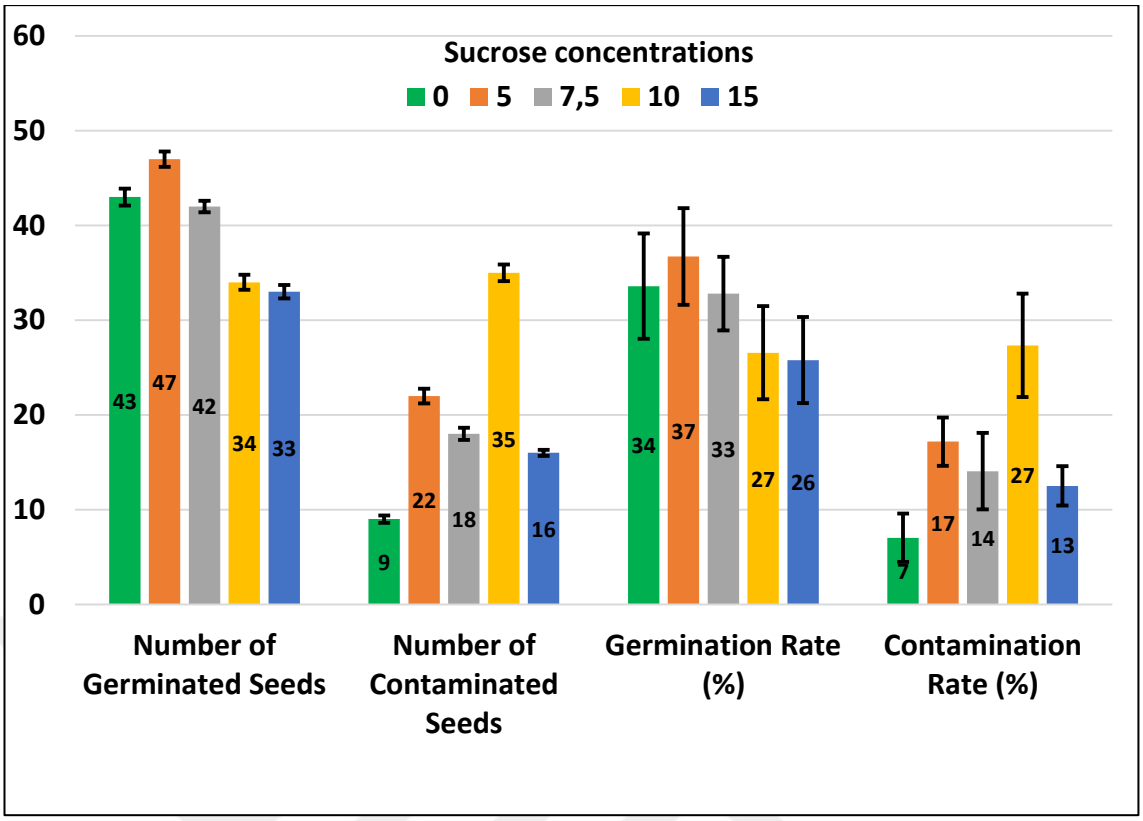


Figure 11. Mean number of germinated and contaminated seeds cultured *in vitro*. Error bars represent standard errors.

Table 6. The effects of different sucrose concentrations on germination and contamination of sesame seeds cultured *in vitro* (data recorded 7 days after culture). Datas were given as mean values ± standard error.

Sucrose concentration (g)	Number of Seeds Used	Number of Germinated Seeds	Number of Contaminated Seeds		Germination Rate (%)	Contamination Rate (%)
0	16	10±0,13	3±0,10		62,5	18,8
	16	4±0,11	1±0,06		25,0	6,3
	16	5±0,12	0±0,00		31,3	0,0
	16	8±0,13	2±0,09		50,0	12,5
	16	3±0,10	0±0,00		18,8	0,0
	16	4±0,11	2±0,09		25,0	12,5
	16	3±0,10	0±0,00		18,8	0,0
	16	6±0,13	1±0,06		37,5	6,3
Total	**128**	**43±0,89**	**9±0,40**	**Mean**	**33,6±5,56**	**7,03±2,55**
5	16	10±0,13	0±0,00		62,5	0,0
	16	6±0,13	0±0,00		37,5	0,0
	16	4±0,11	2±0,09		25,0	12,5
	16	6±0,13	3±0,10		37,5	18,8
	16	6±0,13	2±0,09		37,5	12,5
	16	8±0,13	5±0,12		50,0	31,3
	16	3±0,10	6±0,13		18,8	37,5
	16	4±0,11	4±0,11		25,0	25,0
Total	**128**	**47±0,81**	**22±0,77**	**Mean**	**36,7±5,11**	**17,2±2,55**
7,5	16	4±0,11	0±0,00		25,0	0,0
	16	4±0,11	0±0,00		25,0	0,0
	16	8±0,13	3±0,10		50,0	18,8
	16	4±0,11	5±0,12		25,0	31,3
	16	5±0,12	1±0,06		31,3	6,3
	16	5±0,12	2±0,09		31,3	12,5
	16	4±0,11	4±0,11		25,0	25,0
	16	8±0,13	3±0,10		50,0	18,8
Total	**128**	**42±0,62**	**18±0,65**	**Mean**	**32,81±3,88**	**14,1±4,05**
10	16	5±0,12	2±0,09		31,3	12,5
	16	4±0,11	9±0,13		25,0	56,3
	16	2±0,09	5±0,12		12,5	31,3
	16	2±0,09	4±0,11		12,5	25,0
	16	7±0,13	3±0,10		43,8	18,8
	16	3±0,10	3±0,10		18,8	18,8
	16	8±0,13	2±0,09		50,0	12,5
	16	3±0,10	7±0,13		18,8	43,8
Total	**128**	**34±0,80**	**35±0,89**	**Mean**	**26,56±4,92**	**27,34±5,46**
15	16	4±0,11	2±0,09		25,0	12,5
	16	4±0,11	2±0,09		25,0	12,5
	16	0±0,00	2±0,09		0,0	12,5
	16	3±0,10	2±0,09		18,8	12,5
	16	4±0,11	2±0,09		25,0	12,5
	16	6±0,13	4±0,11		37,5	25,0
	16	6±0,13	1±0,06		37,5	6,25
	16	6±0,13	1±0,06		37,5	6,25
Total	**128**	**33±0,72**	**16±0,33**	**Mean**	**25,8±4,55**	**12,5±2,08**

4.4 Effects of NaOCl Exposure on Seeds Germination and Plant Growth

The most vital treatment prior to culture initiation is the sterilization process. There is a wide range of literature on the effect of NaOCl concentration and temperature on germination (Badoni and Chauhan, 2010; Maina et al., 2010; Colgecen et al., 2011), but in this study, NaOCl exposure period sterilization time interval was tested. 70% ethanol was used as a control. Controls were placed in light and dark condition to also test the effect of light on germination after sterilization in part A of the experiment different NaOCl concentrations (5%,10% and 15%) at different time intervals (5, 10 and 15 minutes) were tested. Germination and seeds for both Muganlı-57 and Baydar-2001 were negatively affected by NaOCl as no germination of seeds was observed for 5%, 10% and 15% NaOCl concentration at all time intervals. Day 14 of control produced an 85% germination of seed at light condition and 70% in dark conditions for Muganlı-57. Baydar-2001 exhibited a 100% seed germination in both light and dark condition and therefore leads as the highest (**Figure 13**). 2% NaOCl at all time intervals produced the best response of 100% for germination in sesame seeds for both Baydar-2001 and Muganlı-57, the rate of survival of seeds was good and no contamination was observed (**Figure 12)**.

The highest shoot number (8,35) for Baydar-2001 were obtained from 5 min treatment and followed by 15 min (8.18) and 10 min (7.55) treatments. The shoot length were between 1.70-2.05 cm for 5, 10 and 15 min durations, respectively. The difference was not significant for the shoot number and shoot length. 5 min (6.38 cm) and 15 min (6.43 cm) treatments resulted in longer roots when compared to 10 min (5.50 cm). The Muganlı-57 variety gave the highest shoot number (9.0) and root length (6.37 cm) at 5 min treatment. There was no seed germination on 15 min sterilization **(Figure 12)**.

The most widely used surface sterilization of plant explants from different sources is sodium hypochlorite (Badoni and Chauhan, 2010; Maina et al., 2010; Colgecen et al., 2011). Research shows a cumulative germination percentage of conifer seeds increased by sterilizing with bleach (Wenny and Dumroese, 1987). Hsiao et al. (1984) deduced that sodium hypochlorite (NaOCl) at high concentration negatively affect seed germination, seedling growth, and the viability of the tissue in

in vitro while it was ineffective for sterilization of tissues at low concentration. Other reports on different sodium hypochlorite concentration for sterilization of *F. religiosa* explants indicate the highest rate of sterilization (80%) for fruit of *F. religiosa* when the treatment contained 2% sodium hypochlorite for 30 min (Parasharami et al., 2014; Deshpande et al., 1998; Gill and Siwach, 2009).

Parasharami et al. (2014) and Pierik (1987) also added that not only did regeneration capacity of tissue gets negatively affected by higher concentrations but also longer application periods of disinfectants and, therefore, advisable to aim for lowest concentration of disinfectant for the shortest time in sterilization process under *in vitro* conditions. In validation with our study, Telci et al. (2011) reported that seed-borne contamination increased gradually by decreasing concentrations and application periods of NaOCl below 3.75% and 15 min. The best results were obtained from 3.75% NaOCl concentration and 15 min application period.

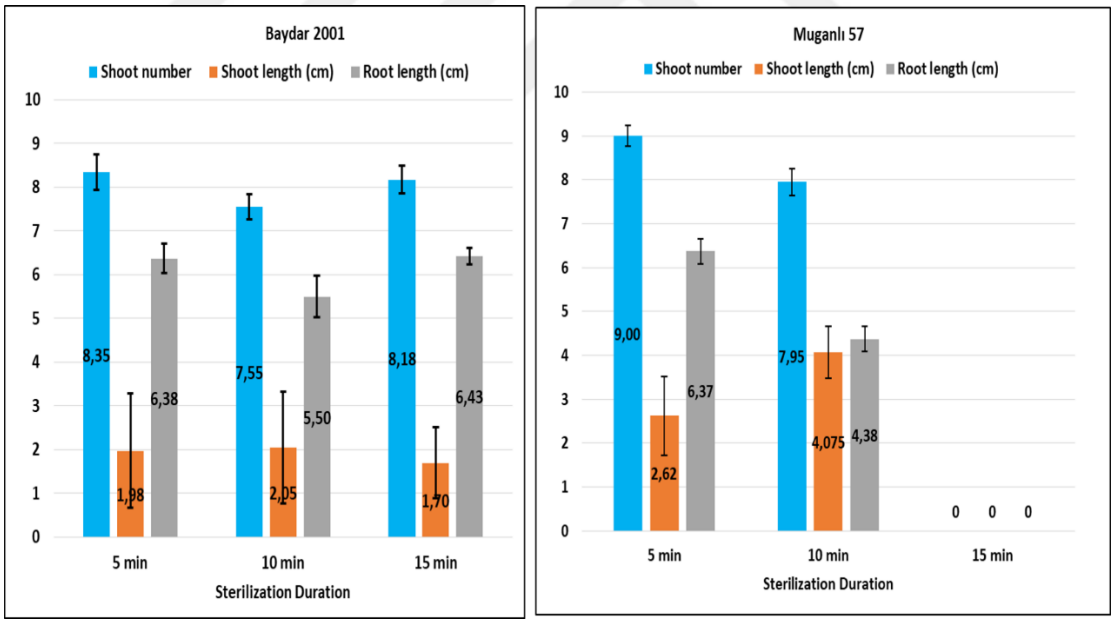


Figure 12. The effect of different sterilization durations at 2 % NaOCl treatment on shoot number, shoot and root length of sesame seedlings germinated *in vitro*.

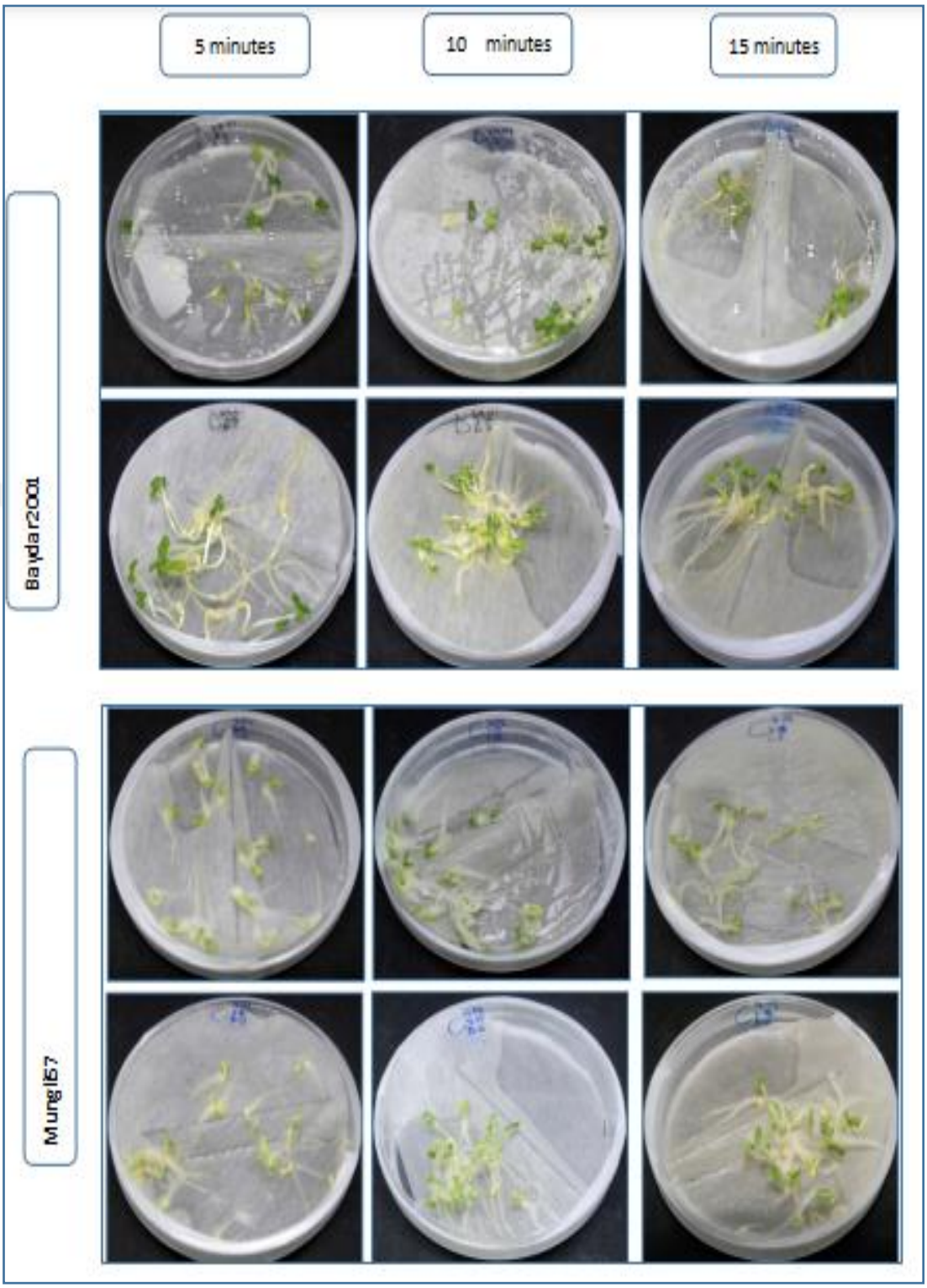


Figure 13. Germinated sesame varieties under different *in vitro* sterilization for 5, 10 and 15 minutes.

4.5 Salt Pre-treatment

In this experiment, 20 seeds per petri were exposed to different NaCl concentrations (0 mM, 50 mM, 100 mM and 150 mM) for 3-4 days in sterile conditions for germination purposes (**Figure 14, Figure 15, Figure 16**). 2% bleach at different time intervals (5, 10 and 15 minutes) was used to sterilize the seeds in order to identify the best result producing protocol. Of all the NaCl exposed samples, control and 50 mM treatment had the highest responsive rate in all two cultivars at both light and dark conditions. A 100% germination rate in control was observed for the two cultivars. Germination was inhibited starting from 100 mM to 150 mM or was very low at 100 mM. It was noted by the results obtained that sterilization period combined with NaCl exposure affects in plant growth. Seeds sterilized for 5 minutes produced stable amount of germinated seed for both cultivars in both light and dark conditions and both had a 10% germination rate at 100 mM in dark condition. For seeds sterilized for 10 minutes inhibition was observed at 100 mM or very low at 5% in light condition for Muganlı-57 and dark condition for Baydar-2001. Germination rate was 40% at 50 mM in all conditions for Muganlı-57 while Baydar-2001 produced a 75% germination in dark conditions. There was no reaction rate/germination for Muganlı-57 seeds sterilized using the 15 minutes protocol. Comparing the two cultivars, Muganlı-57 had the highest and best results using the 5 minutes sterilization protocol but inhibition was observed at 50 mM NaCl using the 15 minutes protocol. From this we can deduce that an increase in the duration of sterilization using bleach and increase in NaCl concentration is inversely proportional to germination rate. From this result, we can also conclude that genotype (cultivar) also plays a role in sesame salt tolerance.

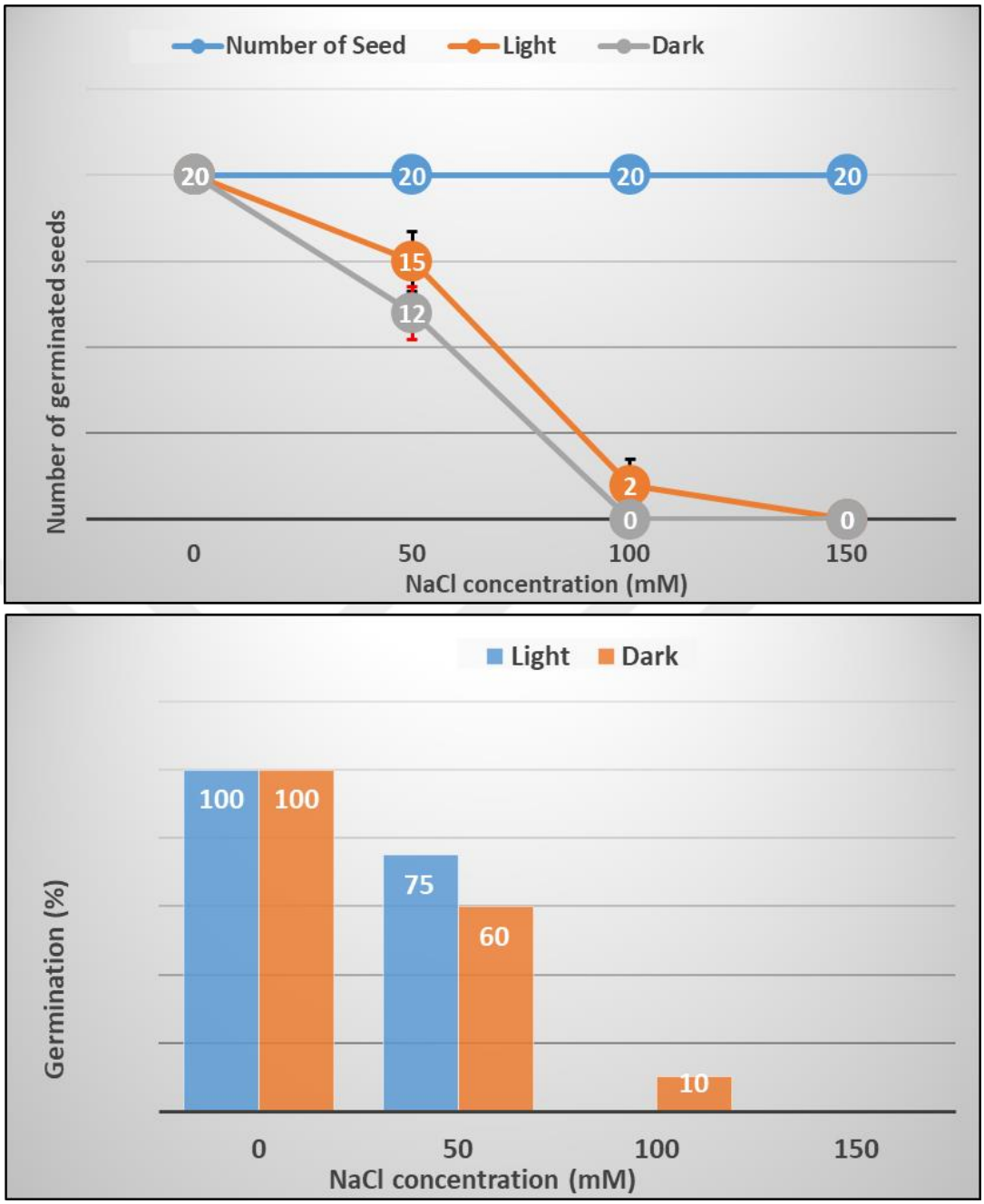
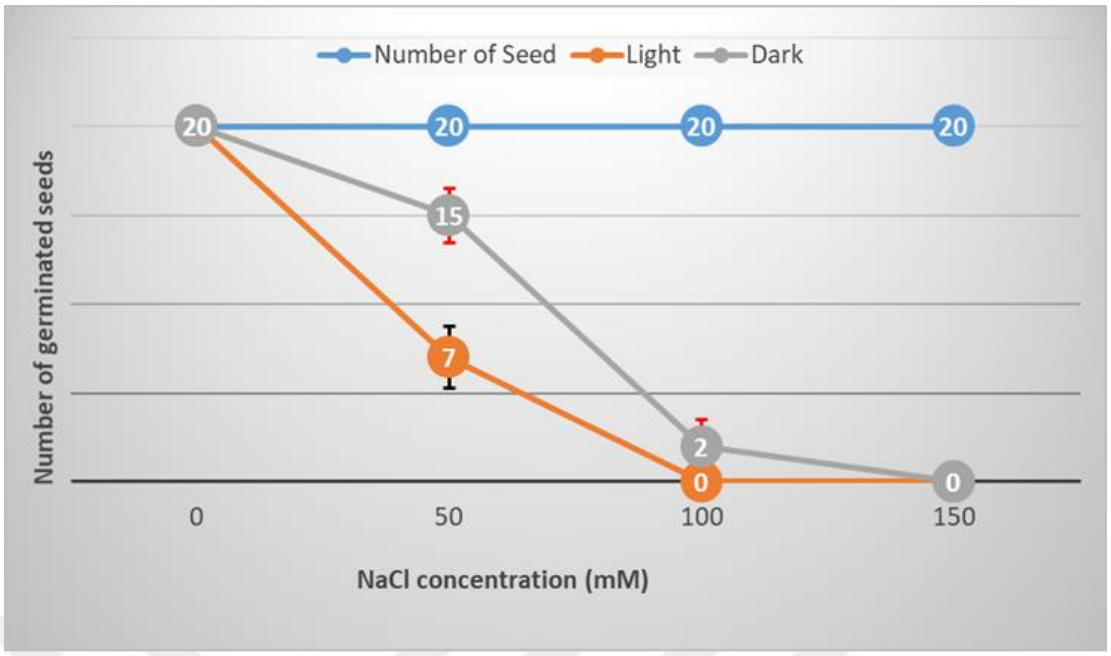


Figure 14(a). Germination and growth ability of the cultivar Baydar-2001 plants under salt stress, following by sterilising with 2% NaOCl for 5 min.



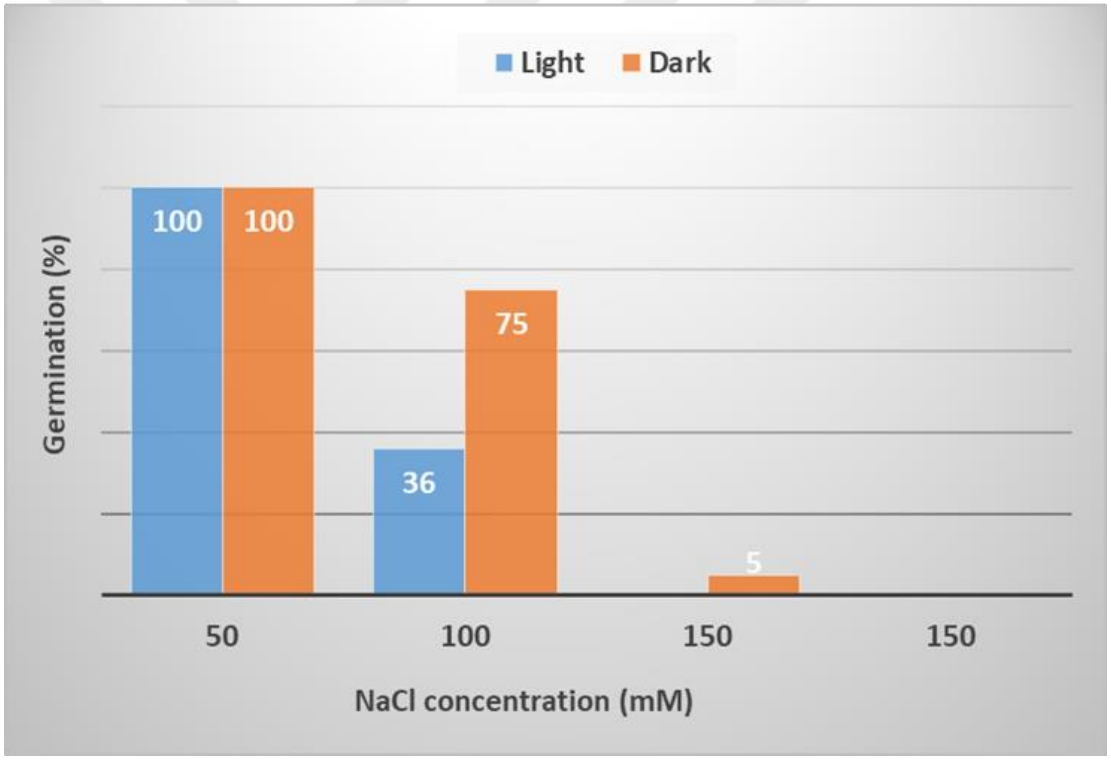


Figure 14(b). Germination and growth ability of the cultivar Baydar-2001 plants under salt stress, following by sterilising with 2% NaOCl for 10 min.

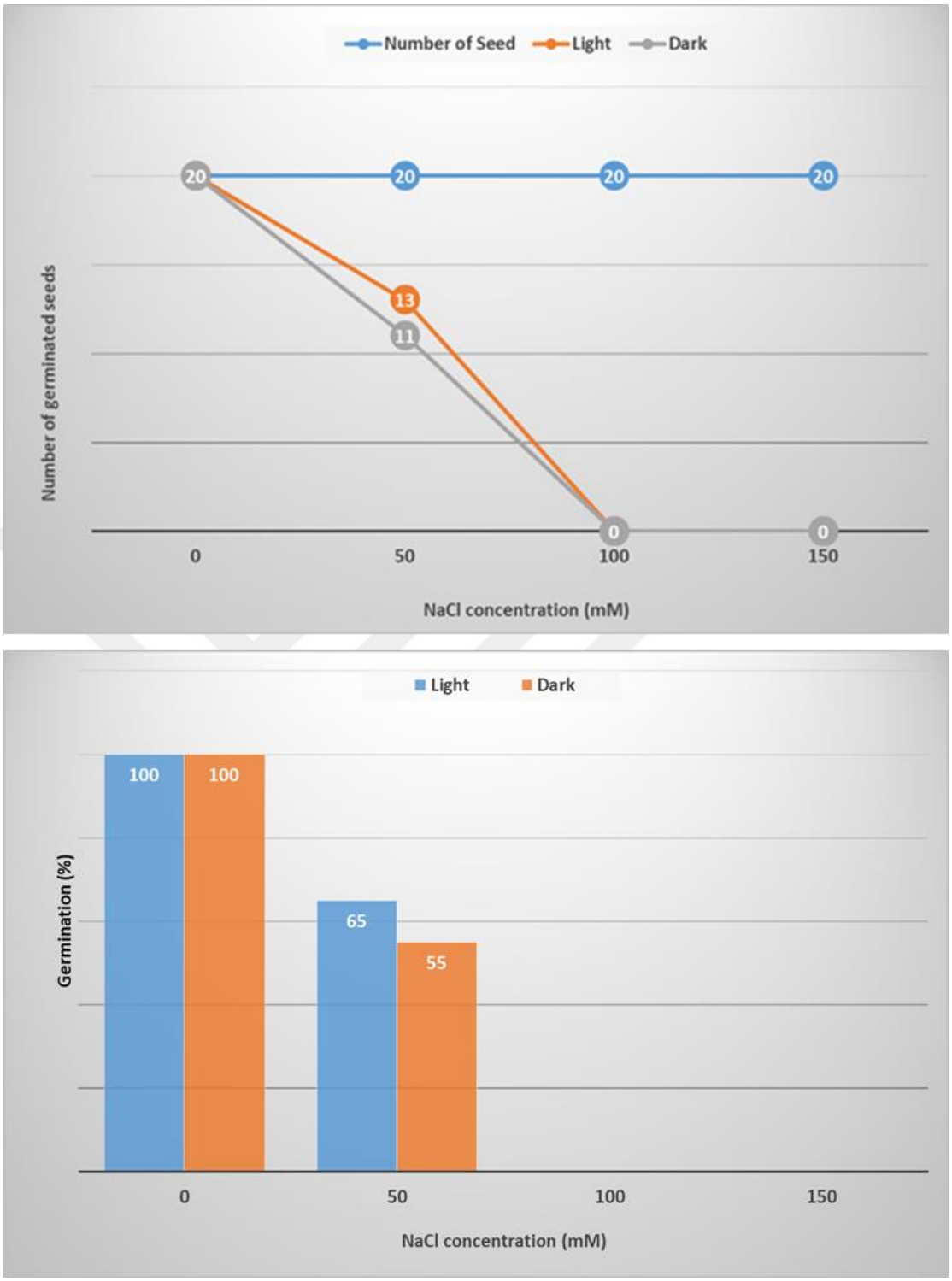


Figure 14(c). Germination and growth ability of the cultivar Baydar-2001 plants under salt stress, following by sterilising with 2% NaOCl for 15 min.

Genetic potential for salt tolerance of a seed is its ability to germinate and emerge under salt stress (Tejovathi et al., 1988), and because NaCl hinders normal metabolic pathways, it causes osmotic changes in ion toxic effect (Tafouo et al., 2010), which leads to a lower rate of absorption and/or ion toxicity that result in reduced germination rate (Huang and Redmann, 1995). The data obtained in this experiment corresponds with El-Harfi et al. (2016)'s findings on reduction of germination rate by increased salinity and Bahrami and Razmjoo (2012)'s who identified salinity adverse effects on sesame seed. Similar to our study, sesame seeds exposed to 100 mM NaCl obtained limited reduction in germination rate (Gehlot et al., 2005). A number of studies in other species such as safflower (Khodadad, 2011), wheat (Hampson and Simpson, 1990) and chickpea (Murillo-Amador et al., 2002) shows that NaCl stress reduces germination rate.

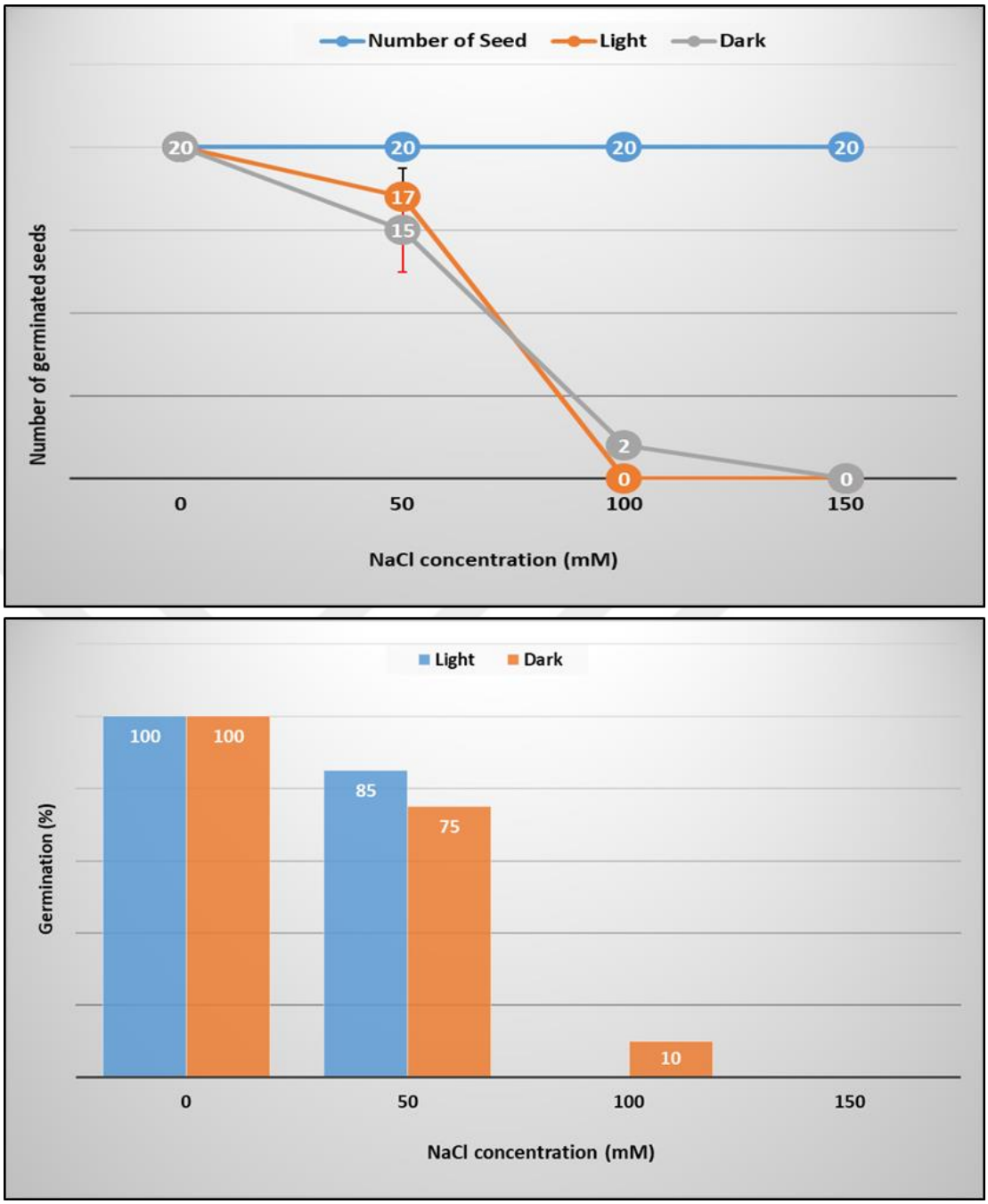


Figure 15(a). Germination and growth ability of the cultivar Muganlı-57 plants under salt stress, following by sterilising with 2% NaOCl for 5 min.

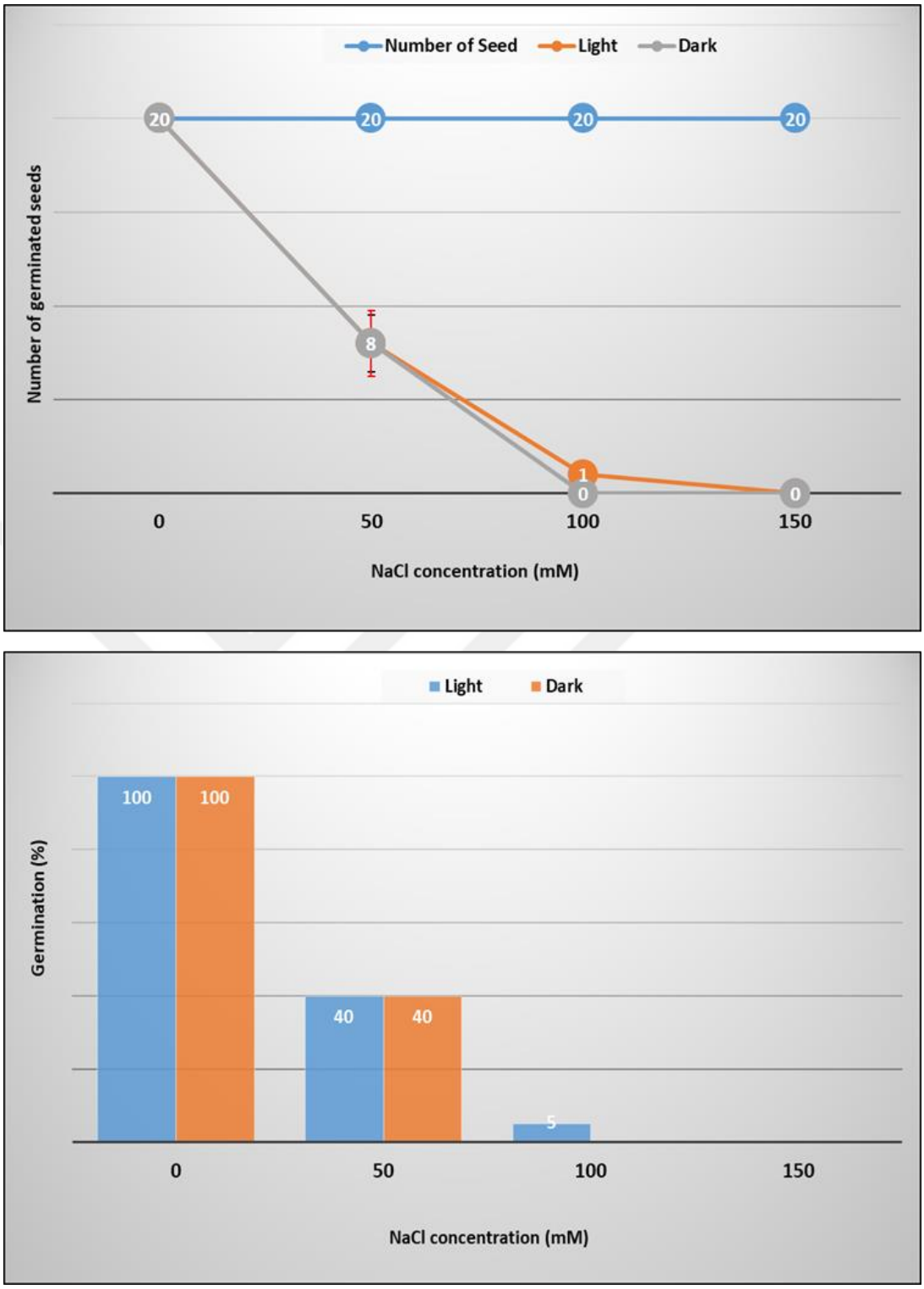
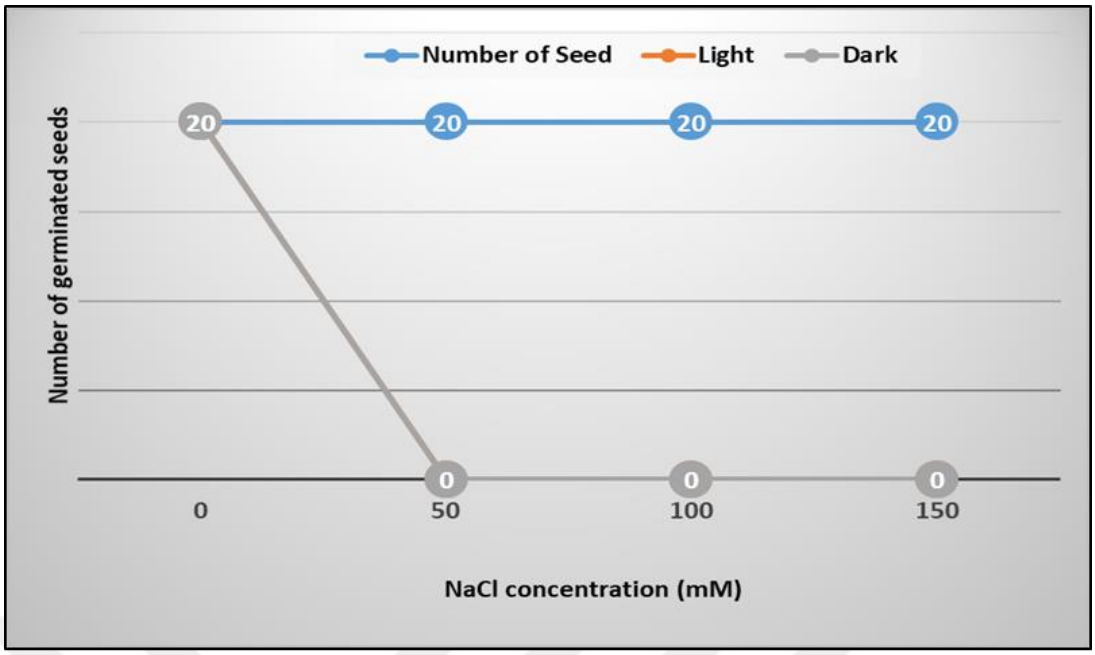


Figure 15(b). Germination and growth ability of the cultivar Muganlı-57 plants under salt stress, following by sterilising with 2% NaOCl for 10 min.



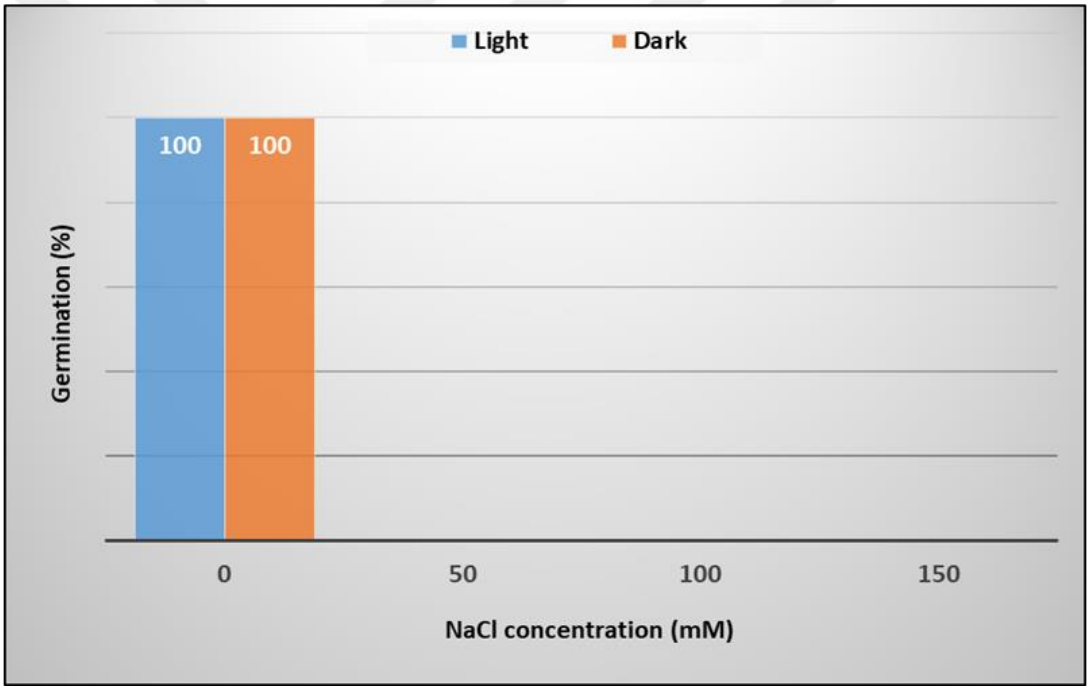


Figure 15(c). Germination and growth ability of the cultivar Muganlı-57 plants under salt stress, following by sterilising with 2% NaOCl for 15 min.

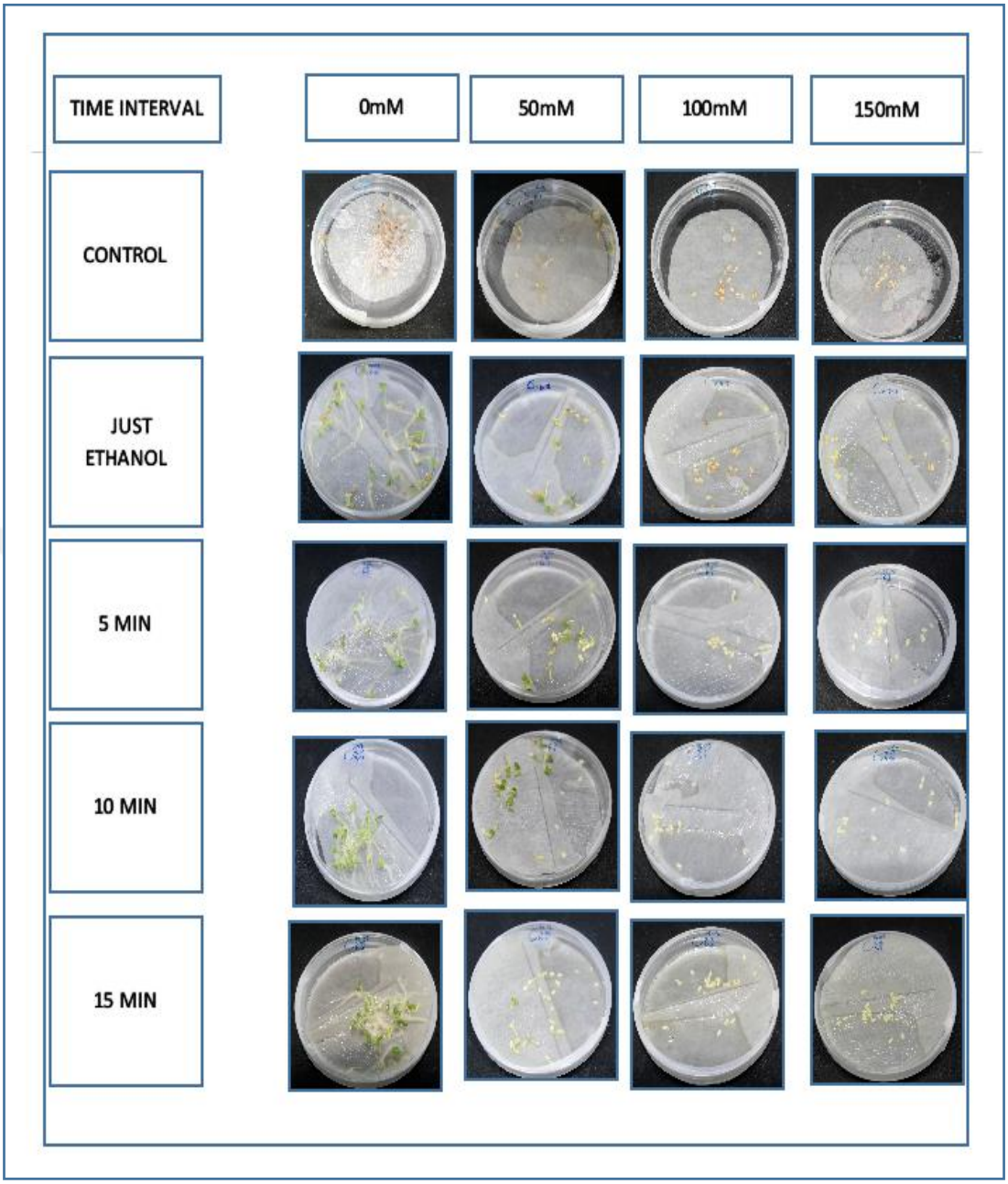


Figure 16. Germination ability of sesame seeds under salt stress for different lengths of time.

4.6 Regeneration from Cotyledons and Hypocotyl Explants

The initial aim of this study was to induce callus from sesame hypocotyl and cotyledons of different cultivars in which the seed they were obtained from underwent salt stress treatment. No cotyledon and hypocotyl were obtained from seeds that were treated with 100 mM and 150 mM salt concentration as no response from seeds were observed. The data provided in **Table 7** and **Table 8** represents the

results obtained from those of salt-free (control) and 50 mM treatment of the different sesame cultivars. No callus induction was observed in all hypocotyl and cotyledon of both Baydar-2001 and Muganlı-57 from the 0 mM and 50 mM treatments. However, root and shoot regeneration were observed in both cultivars (**Figure 17**). A 75% response rate was obtained on Baydar-2001 hypocotyl in both light and dark condition for control and 50% response rate in light conditions and 58% response rate in dark conditions for Muganlı-57. 50 mM Baydar-2001 explants produced an 25% response rate for light conditions at 5 min while Muganlı-57 didn't show any response in both light and dark conditions. All the responded hypocotyl of both cultivars formed roots at day 14 and growth into a plant was very slow. Control (salt-free) treatment resulted in a 58% mean response rate for the 5, 10 and 15 min salt treatment on Muganlı-57 cotyledon in both light and dark conditions whereas Baydar-2001 produced a 67% for all the treatment durations in light and 25% for 5 min treatment in dark conditions. Both Baydar-2001 and Muganlı-57 resulted in 33,3 % cotyledone response rate in light condition for 50 mM but differentiated in dark conditions with Muganlı-57 results at 25% and Baydar-2001 at 0% for all treatment durations. Some cotyledons in both cultivars formed roots at day 14 and progressed to grow shoots which then later became whole plants while others produced leaves first at day 14 then progressed to shoot and whole plants.

In order to achieve a high regeneration ability for the induction of shoots in sesame and other oil seed crops (more precisely groundnut), de-embryonated cotyledons, single or sliced or both can be used (Seo et al., 2007; Lokesha et al., 2012; Shafeay et al., 2011). There are very few literatures on direct hypocotyl regeneration but there are reports that only cotyledon explants were responsive to organogenesis in the variety *Mtwara-2* (Beatrice et al., 2006; Gupta et al., 1990), in other 10 different varieties (Taskin and Turgut, 1997). However, regeneration could be achieved only from the hypocotyl explant on lower KIN concentration on the other hand cotyledon explant completely failed to regenerate in all cytokinin concentrations and combinations tested (Rao and Honnale, 2011), they also reported that hypocotyl explants proved to be better explants for the induction of multiple shoot and cotyledon explants resulted in inducing callus. Reports on shoot regeneration only from cotyledons (Rao and Vaidyanath, 1997; Taskin and Turgut,

1997; Younghee, 2001) which was achieved using cotyledons that were 3-4 days old (Were et al., 2006), 8-10 days old (George et al., 1987; Rao and Vaidyanath, 1997) and 1-2 weeks old (Seo et al., 2007), while regeneration from cotyledons obtained from germinated seeds drastically declined (Seo et al., 2007).

Table 7. Regeneration of cotyledon and hypocotyl explants isolated from salt pre-treated germinated sesame seeds of cultivar Baydar-2001.

Cultivar	Salt Concen-tration	Duration	No. of Hypocotyl Explants Used	Response Rate (%)		No. of Cotyledons Explants Used	Response Rate (%)	
				Light	Dark		Light	Dark
Baydar-2001	0 mM	5 min	12	75	50	12	100	25
		10 min	12	100	75	12	50	0
		15 min	12	50	100	12	50	0
	50 mM	5 min	12	25	_	12	100	_
		10 min	12	_	25	12	_	_
		15 min	12	_	_	12	_	_

Table 8. Regeneration of cotyledon and hypocotyl explants isolated from salt pre-treated germinated sesame seeds of cultivar Muganlı-57.

Cultivar	Salt Concen-tration	Duration	No. of Hypocotyl Explants Used	Response Rate (%)		No. of Cotyledons Explants Used	Response Rate (%)	
				Light	Dark		Light	Dark
Muganlı-57	0 Mm	5 min	12	50	0	12	50	0
		10 min	12	75	100	12	75	75
		15 min	12	25	75	12	50	100
	50 Mm	5 min	12	0	0	12	100	75
		10 min	12	_	_	12	_	_
		15 min	12	_	_	12	_	_

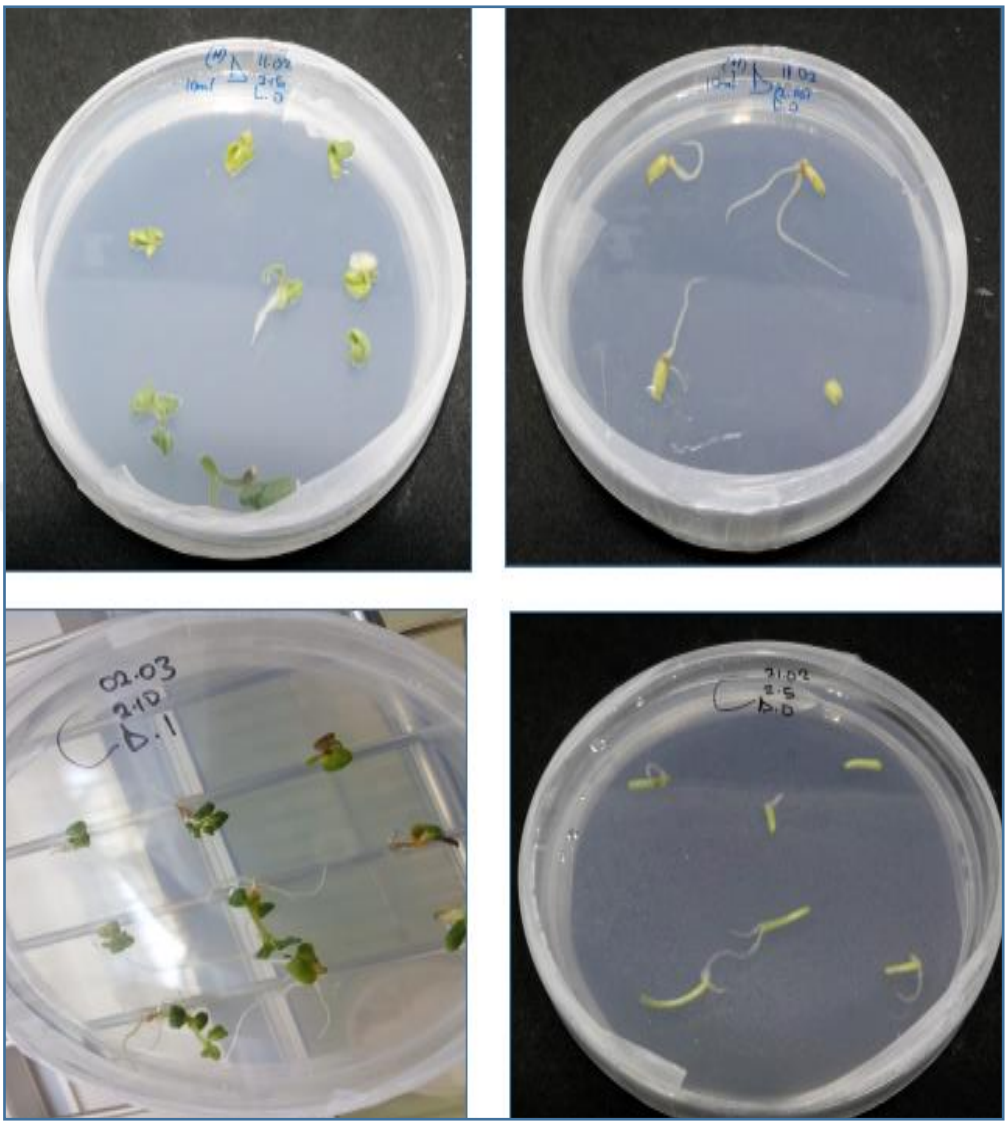


Figure 17. Regeneration from cotyledon and hypocotyl explants of cultivars Baydar-2001 and Muganlı-57 after pre-salt stress treatments. A) Muganlı-57 cotyledon explants with root formation (*top-left*), Muganlı-57 hypocotyl explants with root formation (*top-right*), Baydar-2001 cotyledon explants with root, shoot and leaf formation (*bottom-left*), D) Baydar-2001 hypocotyl explants with root formation (*bottom-right*).

5. CONCLUSSIONS

In accordance to this study;

- Sucrose-free medium is sufficient for sesame seed germination. Sucrose in medium is directly proportional to the contamination rate.
- GA_3 at 0.5 mg/l produces roots and promotes growth in sesame.
- The best plant growth regulator for sesame subculture and regeneration is KIN at 0.5 mg/l as compared to 0.5 mg/l BAP
- 2% NaOCl concentration for 5 minutes is effective at reducing contamination of *S. indicum* L. Concentration for a longer time interval (10-15 minutes) inhibits seed germination.
- Growth rate slows down at 50 mM salt, and germination and seedling growth is inhibited at 100 mM-150 mM.
- Regeneration of hypocotyl and cotyledons was achieved using KIN (0.5 mg/l) and was more responsive with hypocotyl explants producing roots and cotyledons forming shoots.
- Pre-salt treatment inhibits or reduces regeneration in sesame, therefore, we can conclude that salt concentration in a plant is directly proportional sesame regeneration and that light plays no role in affecting regeneration capacity.
- Experiments was done on two different cultivars to test their responses based on the published literature and we can conclude that indeed sesame growth and germination is highly genotype-dependent as Muganlı-57 produced higher rates in all experiments when compared to Baydar-2001, but Baydar-2001 had higher regeneration ability as compared to Muganlı-57.

6. REFERENCES

Amer AM, Mohamed GM, Hussein MH, Sedik MZ, Aly UI. (2017). Effect of some of the natural organic sources on rice tissue culture. Egypt Pharmaceut J., 16:152–156.

Asghari F, Hossieni B, Hassani B, Shirzad H. (2012). Effect of explants source and different hormonal combinations on direct regeneration of basil plants. AJAE, 3:1-12.

Badoni A., J.S. Chauhan. (2010). *In vitro* sterilization protocol for micropropagation of Solanum tuberosum cv. Kufri Himalini. Academia Arena, 2: 24–27.

Badr A, Angers P, Desjardins Y. (2011). Metabolic profiling of photoautotrophic and photomixotrophic potato plantlets (*Solanum tuberosum*) provides new insights into acclimatization. Plant Cell Tissue Organ Cult., 107(1): 13–24. https://doi.org/10.1007/ s11240-011-9951-5

Baskaran P, Jayabalan N. (2006). *In vitro* mass propagation and diverse callus orientation on *Sesamum indicum* L.- an important oil plant. Journal of Agricultural Technology, 2: 259-69.

Baydar H, Turgut I, Turgut K. (1999). Variation of certain characters and line selection of yield, oil, oleic, and lonoleic, acids in the Turkish sesame (*Sesamum indicum* L.) populations. Turkish Journal of Agriculture and Forestry, 23: 431-441.

Baydar H. (2001). Recent progress in sesame culture and breeding [Abstract in English]. Ekin, 17: 90-97.

Beatrice WA., Samuel G, Augustino O, Onkware A, Carlsson A, Welander A. (2006). *In vitro* regeneration of Sesame (*Sesamum indicum* L.) from seedling cotyledon and hypocotyl explants. Plant Cell, Tissue and Organ Culture, 85(2): 235-239.

Bedigian D. (1985). Sesame or flax? Bulletin on Sumerian Agriculture, 2: 159-178.

Bedigian D, Seigler DS, Harlan JR. (1985). Sesamin, sesamol and the origins of sesame. Biochemical Systematics and Ecology, 13: 133-139.

Bedigian D., Harlan JR. (1986). Evidence for cultivation of sesame in the ancient world. Economic Botany, 40(2): 137-154.

Bedigian D, Smyth CA, Harlan JR. (1986). Patterns of morphological variation in sesame. Economic Botany, 40: 353-365.

Bedigian D. (1998). Early history of sesame cultivation in the Near East and beyond. In A.B. Damania, J. Valkoun, G. Willcox and C.O Qualset, eds., The Origins of Agriculture and Crop Domestication. The Harlan Symposium. 93-10 ICARDA, Aleppo, Syria.

Bedigian D. (2000). Sesame. In K.F. Kiple and C.K. Ornelas-Kiple, eds., The Cambridge World History of Food Vol. I. 411-421. Cambridge University Press, New York.

Bedigian D. (2004). History of lore of sesame in southwest Asia. Economic Botany, 58: 329-353.

Chin HF, Krishnapillay B, Alang ZC. (1988). Breaking dormancy in kentia palm seeds by infusion technique. Pertanika, 11: 137–141.

Colgecen H, Koca U, Toker G. (2011). Influence of different sterilization methods on callus initiation and production of pigmented callus in *Arnebia densiflora* Ledeb. Turkish Journal of Biology, 35: 513– 520.

Deinlein U., Stephan AB, Horie T, Luo W, Xu G, Schroede JI. (2014). Plant salt-tolerance mechanisms. Trends in Plant Science, 19: 371–379.

Demir I. (1962). Studies on sesame landraces grown in Turkey for morphological, biological and cytological characters. [in Turkey]. Ege Üniversitesi Ziraat Fakültesi Yayınları: 53, s. 160, Izmir.

Deshpande S, Josekutty P, Prathapasenan G. (1998). Plant regeneration from axillary buds of a mature tree of *Ficus religiosa*. Plant Cell Ret., 17: 571-573.

DIE. (2002). Agricultural structure. State Institute of Statistics, Prime Ministry, Republic of Turkey.

Donnarumma F, Capuana M, Vettori C, Petrini G, Giannini R, Indorato C, Mastromei G. (2011). Isolation and characterization of bacterial colonies from seeds and *in vitro* culture of Fraxinus spp. from Italian site. Plant Biology, 13(1): 169-76. doi:10.1111/j.1438-8677. 2010.00334.x

El Harfi M, Hanine H, Rizki H, Latrache H, Nabloussi A. (2016). Effect of drought and salt stresses on germination and early seedling growth of different color-seeds of sesame (*Sesamum indicum*). Int. J. Agric. Biol., 18(6): 1088-1094

Faiss M, Zalubilova J, Strnad M, Schmulling T. (1997). Conditional transgenic expression of the ipt gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. Plant J, 12: 401–415.

FAO. 2018. http://faostats.fao.org/site/340/default.aspx

Furat S, Uzun B. (2010). The use of agro-morphological characters for the assessment of genetic diversity in sesame (*Sesamum indicum* L.). Plant Omics Journal, 3: 85–91.

Gehlot HS, Purohit A, Shekhawat NS. (2005). Metabolic changes and protein patterns associated with adaptation to salinity in *Sesamum indicum* cultivars. J. Cell Mol. Biol, 4: 31–39.

George L, Bapat VA, Rao PS. (1987). Ann Bot, 60: 17-21.

George L, Bapat V, Rao PS. (1987). Plant Regeneration invitro in different cultivars of sesame (*Sesamum indicum* L.). Plant Science, 99(2): 135 – 137.

Gill AR, Siwach P. (2009). *In vitro* culture establishment and shoot regeneration of *Ficus religiosa* L. Haryana J. Hortic. Sci., 38:173-177.

Gupta V, Agnihotri A, Jagannathan V. *(1990). Plant regeneration from callus and protoplasts of Brassica* nigra (IC 217) through somatic embryogenesis. Plant Cell Reports, 9: 427-430

Haberlandt G. (1902). Culturversuche mit Isolierten Pflanzenzellen. Sitz-Ber. Mat. Nat. Kl. Kais. Akad. Wiss. Wien, 111: 69-92.
Haberlandt G. (1913). Zur physiology der zellterlung. Sitzungsber Akad Wiss, Berlin Phys Mathematic c1: 318-345.

Hampson CR, Simpson GM. (1990). Efects of temperature, salt, and osmotic potentials on early growth of wheat (*Triticum aestivum*). I: Germination. Canadian. J. Bot., 68:524-528.

Hampson CR, Simpson GM. (1990). Efects of temperature, salt, and osmotic potentials on early growth of wheat (*Triticum aestivum*). II: Early seedling growth. Canadian J. Bot., 68(11):529- 532

Hasegawa PM, Bressan RA, Zhu JK, Bohnert H J. (2000). Plant cellular and molecular response to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology, 51: 463-499.

Hazarika BN. (2006). Morphological disorders in *in vitro* culture plants. Sci Hort, 108: 105-120.

Hiltebrandt VM. (1932). Sesame (*Sesamum indicum* L.) Bulletin of Applied Botany and Plant Breeding, Series IX No. 2: 1-114.

Hoang NN, Kitaya Y, Morishita T, Endo R, Shibuya T (2017). A comparative study on growth and morphology of wasabi plantlets under the influence of the micro-environment in shoot and root zones during photoautotrophic and photomixotrophic micropropagation. Plant Cell Tissue Organ Cult., 130(2): 255–263. https:// doi.org/10.1007/s11240-017-1219-2

Hsiao AI, Quick AW. (1984). Action of sodium hypochlorite and hydrogene peroxide on seed dormancy and germination of wild oats, *Avena fatua* L. Weed Research, 24: 411- 419.

Huang J, Redmann RE. (1995). Salt tolerance of Hordeum and Brassica species during germination and early seedling growth. Canadian Journal of Plant Science, 75:815–819.

Kawaide H. (2006). Biochemical and molecular analysis of gibberellin biosynthesis in fungi. Bioscience, Biotechnology and Biochemistry, 70: 583-590.

Khodadad M. (2011). An evaluation of safflower genotypes (*Carthamus tinctorius* L.), seed germination and seedling characters in salt stress conditions. Afr J. Agri. Res., 6(7): 1667-1672.

Kieber JJ, Schaller GE. (2014) Cytokinins. Arabidopsis Book; 12:e0168. 27.

Kotuby-Amacher J, Koenig K, Kitchen B. (2000). Salinity and plant tolerance. Available at https://extensio.usu.edu/files/publications/publication/AG-SO-03.pdf.

Kozai T, Kubota C. (2005). Concepts, definitions, ventilation methods, advantages and disadvantages. In: Kozai T, Afreen F, Zobayed SMA (eds) Photoautotrophic (sugar-free medium) micropropagation as a new propagation and transplant production system. Springer, Dordrecht, pp 19–30.

Kozai T. (2010) Photoautotrophic micropropagation-environmental control for promoting photosynthesis. Propag. Ornam. Plants, 10(4):188–204.

Kwang-Tae CH, Deok-Chun Y, Yong-Eui CH. (2000). Plant production via somatic embryogenesis from preplasmolysed cotyledons of Korean ginseng (*Panax ginsen* C. A. Meyer). American Society of Plant Biologists, 23: 2000.

Larcher W. (1980). Physiological plant ecology. In 2nd totally rev. Edition ed., (pp 303). Berlin and New York: Springer-Verlag.

Lee I, Park YH, Park YS, Kim BG. (1985. Korean, J. Breed, 17: 367-372.

Letham DS. (1973) Cytokinins from Zea mays. Phytochemistry, 12: 2445-2455.

Lokesha R, Rahimansab J, Ajjanavara R, Pallavi S. (2012). Whole plant regeneration via adventitious shoot formation from de-embryonated cotyledon explants of sesame (*Sesamum indicum* L.). World J. Sci. and Tech., 2 (7):47-51.

Maggio A, Hasegawa PM, Bressan RA, Consiglio MF, Joly RJ. (2001). Review: Unraveling the functional relationship between root anatomy and stress tolerance. Functional Plant Biology, 28(10): 999-1004.

Maina SM, Emongor KK, Sharma ST, Gichuki MG, De Villiers SM. (2010). 'Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (*Arachis hypogea* L.) varieties adapted to eastern and Southern Africa'. African Journal of Biotechnology, 9: 2866–2871.

Martins JP, Verdoodt V, Pasqual M, De Proft M (2016). Physiological responses by Billbergiazebrina (Bromeliaceae) when grown under controlled microenvironmental conditions. Afr J Biotechnol, 15(36):1952–1961. https://doi.org/10.5897/AJB2016. 15584

Miyazaki H, Tsuchiyama T, Terada H. (2011). Examination of radioactive contamination in food. J. Food Hygienic Soc. Japan., 54:156-164. doi: 10.3358/shokueishi.54156.

Mok DW, Mok MC. (2001). Cytokinin metabolism and action. Annual review of Plant Physiology and Plant Molecular Biology, 52: 89-118.

Munns R, Schachtman D, Codon A. (1995). The significance of a Two-Phase Growth Response to Salinity in Wheat and Barley. Functional Plant Biology, 22(4): 561-569.

Munns R. (2002). Comparative physiology of salt and water stress. Plant Cell and Environment, 25(2): 239-250.

Munns R, Tester M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651-681.

Murashige T, Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum, 15:473-479.

Murillo-Amador B, Lopez-Aguilar R, Kaya C, Larrinaga-Mayoraland J, Flores Hernandez A. (2002). Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. J. Agron. Crop Sci., 188: 235–247

Noguchi T, Ikeda K, Sasaki Y, Yamamoto J, Yamori Y. (2001). Effects of vitamin E and sesamin on hypertention and cerebral thrombogenesis in stroke-prone spontaneously hypertensive rats. Hypertense Res, 24: 735-742.

Odetola JA. (1987). Studies on seed dormancy, viability and germination in ornamental palms. Principes, 31: 24–30.

Parasharami V, Yadav P, Mandkulkar S. (2014). '*Ficus religiosa* L.: Callus, suspension culture and lectin activity in fruits and *in vitro* regenerated tissues'. British Biotechnology Journal, 4(2): 215-227.

Pech y Aké AA, Souza R, Maust B, Santamaría J, Oropeza C. (2204). Enhanced aerobic respiration improves *in vitro* coconut embryos germination and culture. In Vitro Cell Dev Biol Plant, 40: 90–94.

Pierik RIM. (1987). In-vitro culture of higher plants. Dordirectit, Martinus Nishoff publishers.

Rao KR, Vaidyanath K. (1997). Callus induction and morphogenesis in sesame (*Sesamum indicum* L.). Advances in Plant Sciences, 10: 21-6.

Rao S, Honnale NH. (2011). Callus Induction and Organogenesis in Sesamum indicum L. Curr. Trends in Biotech Pharm., 5(4):1462-1468

Rasmussen HN. (1995). Terrestrial Orchids from Seed to Mycotrophic Plant, Cambridge University Press, Cambridge, UK, 456 pp.

Rasmussen HN., Rasmussen FN. (1991). Climactic and seasonal regulation of seed plant establishment in *Dactylorhiza majalis* inferred from symbiotic experiments *in vitro*. Lindleyana, 5: 221-227.

Regueira M, Rial E, Blanco B, Bogo B, Aldrey A, Correa B, Vidal N. (2018). Micropropagation of axillary shoots of Salix viminalis using a temporary immersion system. Trees, 32(1): 61–71. https:// doi.org/10.1007/s00468-017-1611-x

Sakar D, Sambandam G, Ramakrishina R M, Pugalendi K. (2005). Modulation of blood pressure, liqid profiles and Redox status in hypertensive patients taking different edible oils. Clin Chem. Acta, 355: 97-104.

Seo HY, Kim YJ, Park TI, Kim HS, Yun SJ, Park KH, Oh MK., Choi MY, Paik CH, Lee YS, Choi YE. (2007). High frequency plant regeneration via adventitious shoot formation from deembryonated cotyledon explants of *Sesamum indicum* L. In Vitro Cellular and Developmental Biology-Plant, 43:209-14.

Serrano R, Culianz-Macia F, Moreno V. (1999). Genetic engineering of salt and drought tolerance with yeast regulatory genes. Scientia Horticulturae, 78: 261-269.

Shafeay A, Ibrahim AS, Mohamed RN, Mohamed ST. (2011). Establishment of regeneration and transformation system in Egyptian sesame (*Sesamum indicum* L.) cv Sohag 1. G M Crops, 2(3): 182-192.

Steward FC, Mapes MO, Mers K. (1958). Growth and organized development of cultured cells. ii. Organization in culture growth from freely suspended cells. Am. J. Bot., 45: 705-713.

Tafouo VD, Wamba OF, Youmbi E, Nono GV, Akoa A (2010). Growth, yield, water status, and ionic distribution response of three bambara groundnut landraces (*Vigna subterranean* (L.) Verdic.) grown under saline conditions. International Journal of Botany, 6: 53-58.

Taskin KM, Turgut K. (1997). *In vitro* regeneration of sesame (*Sesamum indicum* L.). Turkish Journal of Botany, 21:15–18.

Telci C, Yildiz M. (2011). The effect of surface-disinfection process on dormancy-breaking, seed germination, and seedling growth of *Lathyrus chrysanthus* Boiss under *in vitro* conditions. Propagation of Ornamental Plants, 11: 10-11.

Tejovathi G, Khadeer MA, Anwer SY. (1988). Studies on certain enzymes in salt tolerant and sensitive varieties of sunflower. Indian J. Bot., 11:113–117.

Provin TL, McFarland ML. The Texas A&M Agrilife Extention: https://agrilifeextension.tamu.edu/library/gardening/essential-nutrients-for-plants/.

Uzun B, Arslan C, Furat S. (2008). Variation in fatty acid compositions, oil content and oil yield in a germplasm collection of sesame (*Sesamum indicum* L.). Journal of the American Oil Chemists' Society, 85: 1135–1142.

Weiss EA. (1983). Oilseed Crop. 282-340. Longman, New York.

Wenny DL, Dumroese RK. (1987). Germination of conifer seeds surface-sterilized with bleach. Tree Planters Notes, 38: 18-21

Were BA, Gubu S, Onkwara AO, Carlsson AS, Welander M. (2006). Plant Cell, Tisssue Organ Culture, 85: 235-239.

Were BA, Gudu S, Onkware AO, Carlsson AS, Welander M. (2006). *In vitro* regeneration of sesame (*Sesamum indicum* L.) from seedling cotyledon and hypocotyl explants. Plant Cell, Tissue and Organ Culture, 85:235-9.

White CN, Rivin CJ. (2000). Gibberellins and seed development in maize. II. Gibberellins synthesis inhibition enhances abscisic acid signal in culture embryos. Plant Physiol., 122: 1089–1098.

Yadav M, Chaudhary D, Sainger M, Jaiwal PK. (2010). *Agrobacterium tumefaciens*-mediated genetic transformation of sesame (*Sesamum indicum* L.). Plant Cell, Tissue and Organ Culture, 103:377-86.

Yamaguchi S, Kamiya Y. (2002) Gibberellins and light-stimulated seed germination. Journal of Plant Growth Regulation, 20: 369–376.

Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA (2013). Role of carbon sources for *in vitro* plant growth and development. Mol Biol Rep 40(4):2837–2849.https://doi.org/10.1007/s11033-012-2299-z

Yildiz M, Avci M, Ozgen M. (1997). Studies on sterilization and medium preparation techniques in sugarbeet (*Beta vulgaris* L) regeneration. In: Ulbrich P. (Ed.). Turkish-German Agriculture Research Symposium V, Akdeniz University, Antalya, Turkey 125-130.

Yildiz M, Er C. (2002). The effect of sodium hypoclhorite solution on *in vitro* seedling growth and shoot regeneration of flax (*Linum ustitatissimum*). Naturwissenschaten, 89: 259-261.

Younghee K (2001). Effects of BA, NAA, 2, 4-D and AgNO3 treatments on the callus induction and shoot regeneration from hypocotyl and cotyledon of sesame (*Sesamum indicum* L.). J of the Korean Soc for Horticul Sci, 42: 70-74.

Zettler LW, Hofer CJ. (1997). Sensitivity of *Spiranthes odarata* seeds to light during *in vitro* symbiotic seed germination. Lindleyana, 12: 26-29.

11- İntihal beyan formu

T.C.
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ MÜDÜRLÜĞÜ

ÖĞRENCİ ve TEZ DANIŞMANI İNTİHAL BEYAN FORMU

Anabilim Dalı	Biyoloji		
Öğrencinin Adı ve Soyadı	Lebogang Charlotte THEMBA		
Numarası	18261010104		
Lisansüstü Programı	Tezli Yüksek Lisans ☑	Doktora ☐	
Tez Sınavına Girdiği Eğitim Yılı ve Dönemi	2020 / 2021	☑ Güz	☐ Bahar
Tez Sınavına Girdiği Tarih	31.11.2020		
Tezin Sayfa Sayısı	72		
Tez Başlığı (Tr)	Susam bitkisinin (Sesamum indicum L.) in vitro koşullarda çimlendirilmesi ve büyütülmesi		
Tez Başlığı (En)	In vitro germination and growth of sesame (Sesamum indicum L.)		

Teze ilişkin 08 / 12 / 2020 tarihinde yapılan Turnitin adlı intihal tespit programından enstitü müdürlüğünce belirlenen filtrelemeler uygulanarak alınmış olan benzerlik raporuna göre, tezin benzerlik oranı % 23 olarak tespit edilmiştir.

Bu tez çalışmasının herhangi bir intihal içermediğini; aksinin tespit edilmesi durumunda doğabilecek her türlü hukuki sorumluluğu kabul ettiğimizi ve yukarıda vermiş olduğumuz bilgilerin doğru olduğunu beyan ederiz.

Lebogang Charlotte THEMBA
Öğrenci
(Adı Soyadı, tarih ve İmza)

Doç. Dr. Songül GÜREL
Tez Danışmanı Ünvanı
(Adı, soyadı, tarih ve İmza)

Açıklamalar:
1-Tez savunma sınavı sonucunda başarılı bulunan öğrencinin tez çalışması onaylanmak üzere enstitüye teslim edeceği tez son hali için benzerlik raporu alınır. "Öğrenci ve Tez Danışmanı İntihal Beyan Formu"nu eklenir.
*2- Bu form öğrenci tarafından **elektronik ortamda** hazırlanmaktadır ve tez savunması sonrası "tez teslim formu (tez sınavı sonrası)" ile birlikte öğrenci danışmanına imzalatmak üzere tez danışmanına tarafından EABİS aracılığı ile Enstitüye teslim edilmektedir.*