PLANT-ON-A-CHIP DEVICES FOR SEED SCREENING

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Ali YETGİN

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We approve the thesis of **Ali YETGİN**

Examining Committee Members:

Prof. Dr. Devrim PESEN OKVUR
Department of Molecular Biology and Genetic, Izmir Institute of Technology

Assoc. Prof. Dr. Kerem CANLI
Department of Biology, Dokuz Eylül University

Prof. Dr. Ergin Murat ALTUNER
Department of Biology, Kastamonu University

Prof. Dr. Hüseyin Çağlar KARAKAYA
Department of Molecular Biology and Genetic, Izmir Institute of Technology

Prof. Dr. Anne FRARY
Department of Molecular Biology and Genetic, Izmir Institute of Technology

16 July 2019

Prof. Dr. Devrim PESEN OKVUR
Supervisor, Department of Molecular Biology and Genetic, Izmir Institute of Technology

Assoc. Prof. Dr. Kerem CANLI
Co-Supervisor, Department of Biology, Dokuz Eylül University

Assoc. Prof. Dr. Engin ÖZÇİVİCİ
Head of the Department of Biotechnology and Bioengineering

Prof. Dr. Aysun SOFUOĞLU
Dean of the Graduated School of Engineering and Sciences

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ABSTRACT

PLANT-ON-A-CHIP DEVICES FOR SEED SCREENING

Thanks to their roots, plants take the required molecules from the soil, therefore their research is important. The fact that absorption takes place from the root hair makes the observations necessary. The inadequacy of traditional methods requires the development of new methods to ensure their observations, especially during root studies. The fact that chip systems have the features that can overcome the problems has caused plant researches by using this method.

The fact that environmental factors can be imitated in chip systems paves the way for abiotic stresses to work. Although abiotic stresses have been studied extensively on plants, seed germination and subsequent root extension and root hair formation have not been studied in detail except for the model organism (*Arabidopsis thaliana*).

The use of chip system for plant studies of scientists have begun the 21st century is also reveals the need to do more work in this area. Researchers show that they are excited about the advantages they bring to the system and that necessary studies will be carried out in other side branches. It is thought that more research will be done by using chip systems after a short time.

By using chip systems, a suitable environment for seed growth can be provided and environmental conditions can be simulated. By creating the desired controlled environment, it is possible to create a system which results in less time and less cost in order to replace the inconvenient and expensive method.

ÖZET

TOHUM TARAMASI İÇİN YONGA-ÜSTÜ-BİTKİ AYGITLARI

Bitkiler kökleri sayesinde gerekli molekülleri topraktan alırlar, bundan dolayı araştırmaları önemlidir. Özellikle emilimin kök kıllarından gerçekleşiyor olması bunların gözlemlerini gerekli hale getirir. Geleneksel yöntemlerle yapılan çalışmaların yetersiz olması, özellikle kök çalışmaları sırasında onlara zarar vermeden gözlemlerini sağlayacak yeni yöntemlerin geliştirilmesini gerektirmektedir. Yonga sistemlerinin sorunların üstesinden gelebilecek özelliklere sahip olması, bu yöntemi kullanarak bitki araştırmalarının yapılmasına neden olmuştur.

Çevresel faktörlerin yonga sistemlerinde taklit edilebiliyor olması abiyotik streslerin çalışabilmesinin önünü açmaktadır. Abiyotik stresler bitkiler üzerinde yoğun bir şekilde çalışılmış olmasına rağmen tohum çimlenmesi ve bundan sonra meydana gelen kök uzaması ve kök kılı oluşumları model organizma (*Arabidopsis thaliana*) dışında detaylı olarak çalışılmamıştır.

Bitki çalışmaları için yonga sistemlerin kullanılması 21. yy da başlamış olup bilim insanlarının bu alanda daha çok çalışma yapılmasının gerekliliğini ortaya koymaktadır. Araştırmacılar sistemin getirdikleri avantajlar konusunda heyecanlı olmaları diğer yan dallarda da gerekli çalışmaların yapılacağını göstermektedir. Kısa süre sonra yonga sistemleri kullanarak daha çok araştırmalar gerçekleştirileceği düşünülmektedir.

Yonga sistemleri kullanarak tohumun büyümesi için gereken uygun ortam sağlanabilir ve çevre koşulların taklidi sağlanabilir. İstenilen kontrollü ortam yaratılarak normalde zahmetli ve pahalı olan yöntemin yerini daha kısa sürede ve az maliyetle sonuç veren bir sistemin oluşturulması mümkündür.

To my family…

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CHAPTER 1

INTRODUCTION

1.1. Plant

Plants provide adaptation to environmental factors by controlling the rate and pattern of root growth (Lux and Rost, 2012). The determination of this is usually carried out in the first millimeter of the root apex (ZepedaJazo, 2016). Changes due to stress affect the growth and development performance of the plant. Studies are being carried out on how the differences have affected the life cycle of the plant (Khan et al., 2009).

The rhizosphere is an environment with a different ecosystem and structural complexity. Factors such as soil structure, mineral content and oxygen level affect the adaptation of the root structure to micro-environmental conditions (Gruber et al., 2013). The local microenvironment is changed due to biotic and abiotic conditions, so that the rhizosphere conditions is maked suitable for itself. The structure of lateral roots and root hairs can be activated or inhibited in response to environmental stimuli. Soil conditions, such as water, nutrient and salt concentration, cause specific root architecture changed with root hairs length and density (Rellán-Álvarez et al., 2016).

Some agricultural plants, which are heavily influenced by environmental factors, can be enlarged especially in controlled environments and their productivity is increased. The production of tomatoes is increased with the traditional method of cultivation. However, only the temperature can be controlled by this method. Stabilization of soil content and biological pathogens is carried out with the contribution of chemical fertilizers (Bargaz et al., 2018). The widespread use of such a method threatens to decrease in soil quality and threatens human health. Therefore, it has become a necessity to investigate a system that can be replaced.

The development of microfabricated devices with fast production and high yielding quantitative data and resolution is essential for plant root research. To compensate people's needs, plants are extensively researched in medical, agricultural and other subjects (Crotty et al., 2015). The fact that laboratory applications do not

comply with the latest technological developments cause plant root studies to be a laborious task.

1.2. Agriculture

Agricultural production starts with the transition of people to settled life 10,000-12,000 years ago. It is also regarded as the first scientific and technological revolution in the world of science. In the 21st century, the global scarcity of water resources originated from environmental pollution and salinization. The increase in the human population and the reduction of cultivable land are happen two main threats to agricultural sustainability (Shahbaz and Ashraf, 2013). Severe winds, extreme temperatures, soil salinity, drought, and flood are among the most destructive environmental stresses that cause major reductions in crop yield and quality.

Fertilization programs are still indispensable today for the purpose of feeding the growing world population. Since we depend on fertilizer consumption, it is not possible to develop a production model without using them. Despite the negative social, economic and environmental problems in agricultural production, the use of fertilizers is inevitable. This leads us to reconsider fertilization programs and re-evaluate them with a new perspective.

Traditional agriculture is based on regular applications of mineral fertilizers, especially those containing basic plant nutrients such as nitrogen, potassium and phosphorus. Mineral nitrogenous fertilizers are produced from atmospheric nitrogen, which is converted into ammonia by the energy-intensive Haber-Bosch process (Kermeli et al., 2017). Phosphorus fertilizers are produced by treating mineral phosphate rock with sulfuric acid. These fertilizers are produced with limited natural resources and have high energy costs. Only half of nitrogenous fertilizers and 20% of phosphate fertilizers are taken up by crops (Good and Beatty et al., 2011). Most of the unused fertilizers mix into the groundwater and gaseously to the sky. This leads to global warming and over-fertilization of natural ecosystems.

Because of the mistakes made in agriculture, they have been one of the main sources of environmental pollution. In addition, the increasing world population and the impact of climate change contribute to the acceleration of this negative situation. Agricultural production is responsible for 85% of global water consumption (Lobell et

al., 2008). Due to wrong practices in agriculture, fertile soils have been decreasing for 50 years. Together with the latest developments in biotechnology, nanotechnology and materials sciences, these problems are quickly identified, and solutions can be produced (Scognamiglio et al., 2015). The use of microfluidic technology to support sustainable agriculture requires the application of the last 10 years of literature developments.

1.3. Abiotic Stress

Tomato (*Solanum lycopersicum L.*) is one of the most important horticultural plants in world trade (Ronga et al., 2017). It is a tropical plant species and is well adapted to almost all climatic zones. However, environmental stresses are the primary constraints on yield and quality of tomato potential (Ronga et al., 2018).

Biotic or abiotic stress that negatively affect the growth and development of plants are considered as stress factors. Stress first begins with deterioration in metabolic and physiological mechanisms (Hasanuzzaman et al., 2013). It can cause damage to plant organs, a decrease in product quality and even death. Biotic stresses include pathogens and other organisms. Abiotic stresses include heat, water, salt, radiation, chemicals, sound, electricity and pressure.

Factors related to abiotic stress in plants are an important issue worldwide; because they prevent the efficiency and distribution of species. Salt and arid soils affect important parts of plants, therefore reduce overall plant productivity (Chinnusamy et al., 2005). Many of the issues such as how the roots grow, how react to changes in soil conditions are still not deeply understood. Adverse conditions, such as drought and salt stress, target the PIN2 auxin transporter expressed in the root transition region. Therefore, their relation to each other should be considered. In addition, salt ions such as Na and Cl can be readily absorbed by plants. Ion toxicity caused by high levels of Na^+ ion accumulation causes deterioration on biochemical reactions and prevents seed germination (Aydın, 2015).

As it is known, in salty conditions, plant growth and development are negatively affected. Since the osmotic pressure of the environment increases in saline conditions, it prevents water intake and therefore, metabolic events related to germination cannot be initiated (Srivastava, 2002). In plants grown under salty conditions, while the total leaf area decreases, the rate of photosynthesis slows down by the closing of the stomata.

However, the negative effect of salt on growth and development is greatest during germination. When all these effects are combined, the growth and development of plants are negatively affected and in some cases the plant dies before completing the life cycle (Alian et al., 2000).

In the photosynthetic apparatus and total antioxidant amount changes at different salt concentration. High ion levels create osmotic pressure by reducing the water uptake of the roots, and increased excess ion uptake adversely affects physiological and cellular processes (Hunsche et al., 2010). Plant regulates cell biophysics that promote leaf stomatal closure to prevent water loss (Rigano et al., 2014). Besides, chloroplasts can influence plant photosynthesis associated with inhibition of growth by triggering the formation of reactive oxygen species (Tripathy, Oelmüller, 2012).

It is expected that product will decrease further with possible climate changes. Abiotic stresses such as salinity, drought and temperature decrease the productivity of agricultural crops and cause more hunger in the world (Wang et al., 2003). As abiotic stresses become more and more dangerous, food production is in great difficulty. Therefore, studies aimed at reducing stress factors increase its importance. It was stated that the use of biostimulants would increase agricultural production against abiotic stresses. Biostimulants have the characteristics that can be used during organic farming because they are reliable for people's health and environment

1.4. Fertilizer

The use of chemical fertilizers during agricultural production adversely affects the balance of the natural environment. The accumulation of fertilizers in the soil causes the next generations to leave unproductive soils. These fertilizers, which pass from soil to water resources, accelerate the death of microorganisms in the ecosystem.

In addition, the passage of chemical residues to human during agricultural consumption increases the prevalence of important health problems. The fact that agricultural crops produced by traditional means become dependent on chemical fertilizers necessitates the expansion of new production methods.

It is estimated that product efficiency will decrease further with possible climate changes. Abiotic stresses such as salinity, drought and temperature decrease the productivity of agricultural crops and cause more hunger in the world. The use of

fertilizers with natural ingredients has become important in recent years to solve such problems. Although the chemical fertilizers are considered to beneficial in increasing the agricultural yield, it is necessary to use more fertilizers in the long term because they cause the soil to become barren.

1.5. Biostimulant

Today, the share of organic-based chemicals in the global fertilizer industry is increasing rapidly. In order to protect the limited natural resources in the world, it is necessary to manage the supply-demand relationship of the rapidly growing world population in agricultural production. In our country, the need for effective use of rapidly developing technologies with industry and the development of new products for the evaluation of organic resources has emerged.

Biostimulants are offered as an alternative to chemical fertilizer sold intensively in the agricultural market. However, if the use of chemical fertilizers was not at these levels, it would not be possible to produce enough crops to feed the world. The main reason for this is the need to recycle the components to the soil. The development of biologically based preservatives instead of chemical pesticides makes it necessary for agricultural production to continue in this way. These products of natural origin are biodegradable and non-toxic for consumers. These formulations can be obtained from macro or microorganisms by methods such as extraction or homogenization.

Although the classifications of biostimulants are not entirely clear, some researchers have identified important categories. These categories are; amino acids and other nitrogenous compounds, humic and fulvic acids, seaweed and plant extracts, chitin and chitosan-like polymers, inorganic compounds, beneficial fungi and bacteria. Biostimulants have positive effects on plant growth, nutrition, product quality, yield and the resistance to stress. They are applied to foliar, soil or seed, and is organic compounds. It is consumed in Turkey since 2002. The global market is expected to reach 2.91 billion $ in 2021.

Plant metabolism is classified as primary and secondary. While primary metabolites are necessary for the maintenance of plant cells, secondary metabolites are significant for defense of plants against improper conditions (Kliebenstein et al., 2012).

Biostimulants are products with high potential in modern agriculture. However, their presence in the market will further improve with the removal of legal barriers. Difficulties in identifying the active ingredient in the registry should be eliminated. The elimination of these official barriers will increase the frequency of biostimulant products appear on the market.

Biostimulants increase nutrient uptake by soil and increase plant growth and vitality (Crouch et al., 1990). It also has improved lateral root formation in root development and increase the total volume of the root system (Mancuso et al., 2006). In addition, it has increase early seed germination and resistance to abiotic stress. Biostimulants used not only help the plants grow, but also affect the biological, chemical and physical structure of the soil.

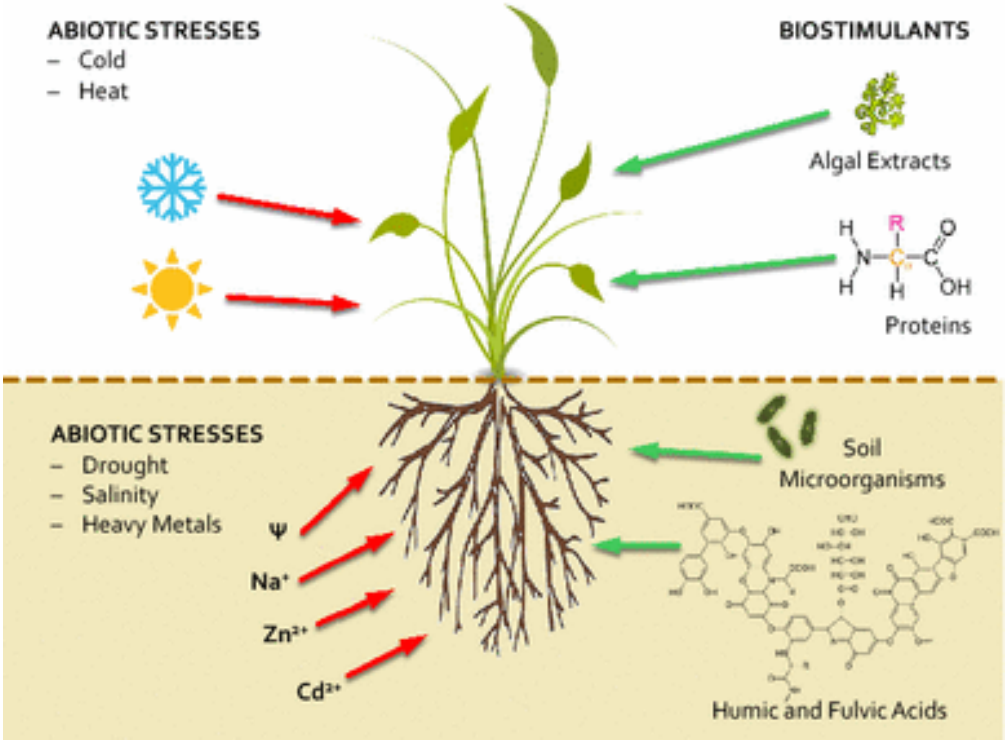

Figure 1.1. Effects of biostimulants on abiotic stresses (Source: Oosten et al., 2017).

Biostimulants do not have precisely defined content, such as chemical fertilizers. There are many different compounds based on synergy. Because of this, the mechanism of biostimulants is not fully known and necessary investigations should be carried out. Biostimulite increases the root growth of agriculturally important plants. When applied in the early growth stage, it demonstrates similar properties with auxin and supports

root growth significantly. High concentration extract (1:100 seaweed extract: water) was prevented tomato root growth but its stimulating effect was observed in low concentrations (1:600) (Finnie ve van Staden, 1985). When applied a research about tomato leads to a long-term gain in productivity.

The effectiveness of these products can be realized by creating experimental environments for the root measurement, thanks to comparing the control products with the reference products. The effect of biostimulants used in abiotic stress conditions is very clear and is used for plant protection. If the stress factor is not effective at the stage of observation, the differences may not be determined.

Research about biostimulant have increased rapidly since 2011. The main reason for this trend is the fact that the long-term harm of human and environmental health of chemically produced fertilizers is understanding in the community, so that consumption became to organic agriculture. Although usage amounts increase day by day, more research should be applied to suitable them widely used worldwide.

1.6. Plant on a Chip

Chips have some advantages that cannot be achieved by conventional methods. They have been developed for small volumes and prevents the use of unnecessary chemicals; control and manipulation of the environment increases the accuracy of plant chemical stimulation; microscope observations can be achieved in high resolution without causing plant stress; the ability to develop automated systems requiring little effort ensures that the research is based on a standard; high-throughput data generation provides a convenient study for quantitative analysis (Elitaş et al., 2017).

In order to explain the interactions of plants with the environment, studies with classical methods have been insufficient (Lüttge, 2012; Bertolli et al., 2014). The integration of chip systems into root studies is necessary in order to eliminate such information deficiencies (Stanley et al., 2016).

Microfluidic chip systems were used to investigate germination and early growth on different hormone concentrations in Arabidopsis seed (Jiang et al., 2017). Plant phenotyping studies have been carried out more efficiently, accurately and effectively thanks to this system.

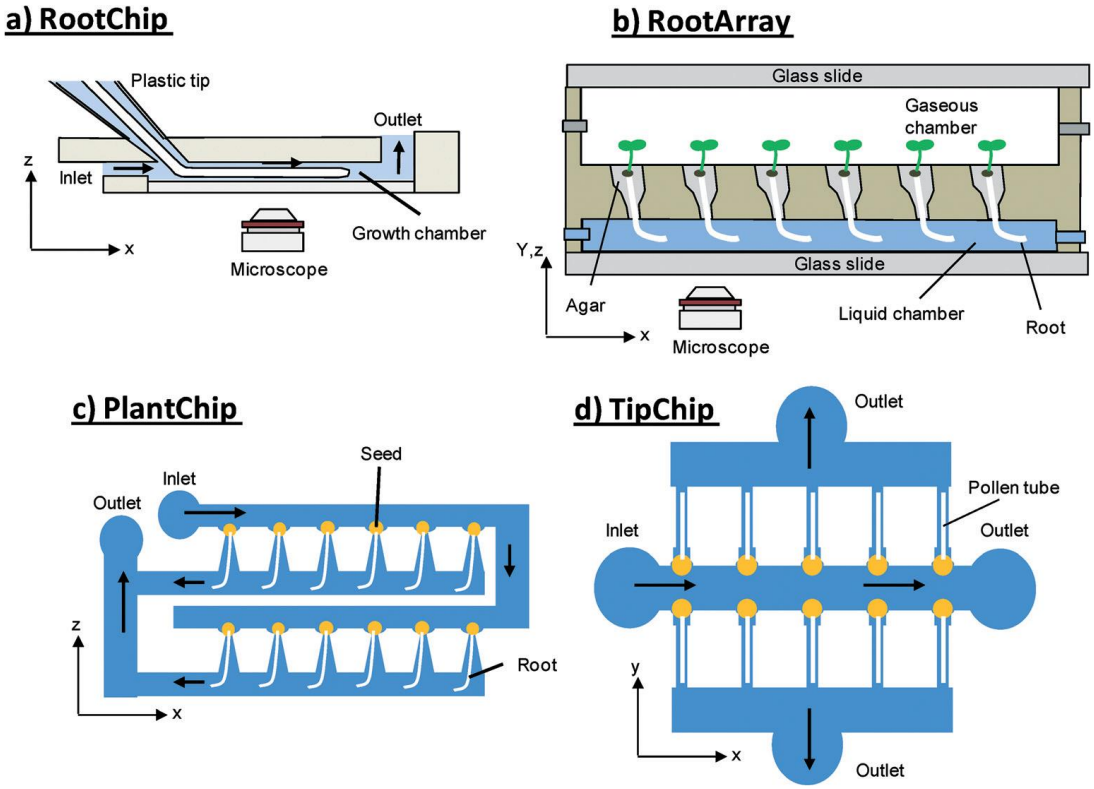

Figure 1.2. Microfluidic platforms for plant research (Source: Nezhad et al., 2014).

Many studies can be performed with this system, including biochemical studies such as drug delivery, environmental observation and chemical concentration. Recently, several microfluidic devices have been reported that characterize the growth of the model plant Arabidopsis thaliana (Agudelo et al., 2013). Even if studied for plant phenotyping, it is not easy to observe chemical and hormone concentrations in different growth regions on the chip.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant Culture

Tomato (*Solanum lycopersicum L.*) seeds were used. Growth chamber was processed in 25 ºC 60% humidity and 12/12 light/dark.

2.1.1 Preparation of Medium

½ MS (Murashige and Skoog) was used in the study. Murashige and Skoog Basal Medium (Sigma Aldrich) measurements were used as a mixture (Table 1.) 30 grams of sucrose was added per liter. The pH was fixed at 6 using a meter. Prepared medium 121^0 autoclave for 15 minutes.

Table 1. Sigma-Aldrich MS content.

Components	mg/L
Ammonium nitrate	1,650.0
Boric acid	6.20
Calcium chloride (anhydrous)	332.20
Disodium EDTA dihydrate	37.260
Ferrous sulfate heptahydrate	27.80
Glycine	2.0
Magnesium sulfate (anhydrous)	180.70
Manganese sulfate monohydrate	16.90
myo-Inositol	100.0
Potassium iodide	0.830
Potassium nitrate	1,900.0
Potassium phosphate monobasic	170.0
Sodium molybdate dihydrate	0.250
Zinc sulfate heptahydrate	8.60

2.1.2 Seed Sterilization

Seeds were added to beakers and 20% bleach and a few drops of Tween (0.1%) were added. After waiting 10 minutes, it was washed 5 times with sterile dH2O and placed on a filter paper to dry.

2.1.3. Seed Cultivation

Tomato seeds were added to sterile upH2O for 96 well plate at first stage. Seeds were placed gently with sterile tweezers. After 72 hours for the second stage, seeds germinated and root elongation starting (0.75 ± 0.5 mm) was placed in chips containing ½ MS.

2.2. Chip Preparation

The design of the plant on a chip device was done with AutoCAD application on computer. This design was used for fabrication with 3D printer.

2.2.1. PDMS Molding and Cleaning

A 10:1 ratio of PDMS and current agent was added and mixed until a white color was obtained. Vacuum was applied until the bubbles were removed. It was added to the mold to be used for the study.

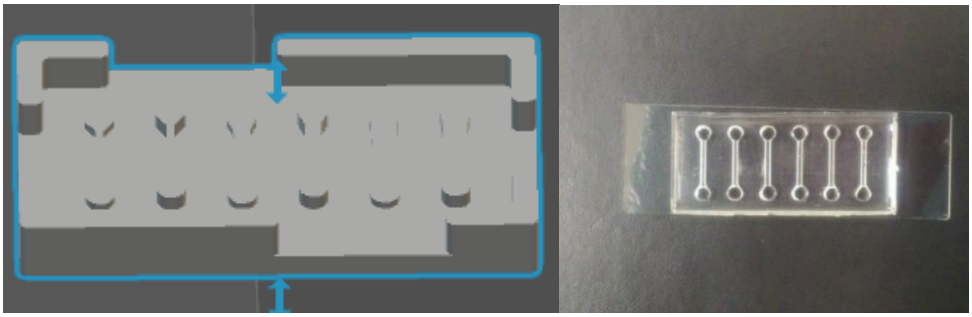

Figure 2.1. Plant chip design (left) and view (right).

The mold was cleaned and treated with developing agents. The hair dryer was used for drying of the molds and the PDMS was added. The polymerization process was maintained at firstly 37 ° C for 1 hour at then 60 ° C for 17 hours.

The prepared PDMS was punched with 3.5 mm and then cleaned. PDMS cleaning: Rinse with 1x upH2O-70% EtOH-upH2O then sonicate for 10 min in upH2O. After rinse with 1x upH2O, sonication was performed in 70% EtOH for 5 min. Finally, wait at 5 minutes in 70% EtOH and rinse with 1x upH2O.

Figure 2.2.Image of vacuum pump (left) and sonicator (right).

The water drops were dried using a vacuum pump and the chips were placed in sterile glass petri dishes. Waiting for the PDMS to dry to 1 day.

2.2.2. Permanent Bonding

Bonding process took place in the fume hood. Uv ozone was made inside the device for 5 minutes with the PDMS figure facing up. PDMS and glass slide UV exposed sites were adhered to each other and standing on 100^0C in 15 min heater. The prepared chips were sterilized by exposure to UV in the cabin.

2.3 Fertilizer Preparation

K, P and S coded 3 commercial fertilizers were used in our study. When K and P coded fertilizers are in solid state, S coded is in liquid state.

Table 2. List of chemicals.

Code	Brand name	Product name
K	Genta agriculture	Sprinter Plus
P	Dripfert	NPK fertilizer
S	Genta agriculture	Biosoil

Table 3. K coded fertilizer content.

Guaranteed Content (w/w)	
Total Nitrogen (N)	6%
Ammonia Nitrogen (N)	6%
Water Soluble Phosphorus Pentaoxide (P_2O_5)	30%
Water Soluble Zinc (Zn)	4,5%
Chelated Zinc with EDTA (EDTA-Zn):	2,2%
EDTA-chelated Zinc (EDTA-Zn) with stable pH	6-8

Table 4. S coded fertilizer content.

Guaranteed Content (w/w)	
Total (Humic + Fülvik) Acid	15%
Organic Matter	15%
Water Soluble Potassium Oxide (K_2O)	3%
pH	9-11
Raw Material Used in Production	Leonardit

Table 5. P coded fertilizer content.

Guaranteed Content (w/w)	
Total Nitrogen (N)	16%
Nitrate nitrogen (NO_3-N)	6%
Ammonium nitrogen (NH_4-N)	7%
Urea nitrogen (NH_2-N)	3%
Water Soluble Phosphorus Pentaoxide (P_2O_5)	8%
Water Soluble Potassium Oxide (K_2O)	24%
Stable pH range	2,5-8,5

Solid form fertilizers were weighed 0.1 g, then1 ml of sterile upH2O was dissolved. 0.1 ml of the liquid fertilizer was dissolved in 0.9 ml of sterile upH2O. After the mixture was obtained, it was filtered for remove microorganisms. Fertilizer mixes in the ratios of 1: 1, 10: 1, 100: 1, 1000: 1 and 10000: 1 are prepared for chip studies.

2.4. Plant Extract Preparation

The plant extracts were prepared by the ethanol extraction method and after the measurement step, the solution was dissolved with sterile upH2O.

Figure 2.3. Image of shaker (left) and UV ozone (right).

Table 6. List of the names of plant extracts.

Code	Turkish name	Latin names	English name
B1	Sarımsak	*Allium sativum*	Garlic
B2	Zerdeçal	*Curcuma longa*	Curcuma
B3	Keçiboynuzu	*Ceratonia siliqua*	Carob
B4	Bozdağ adaçayı	*Sideritis spilia*	Sage
B5	İt üzümü	*Solanum americanum*	Grape

2.5. Abiotic Stress Applications

The tomato seeds placed on the chip were exposed for 2 days in different salinity stresses and their changes and elongation levels were observed. Different contents of fertilizers were added, and changes were examined.

Figure 2.4. View of the growth chamber (left) and the working of the chips (right).

2.6. Statistical Analysis

The 8mp phone camera and 5x microscope images were used to display the results of the study. The lengths of the roots were measured using the image j program. The roots do not grow completely straight direction in a channel, but it can be a bending. Therefore, this was taken into consideration during the measurement. The segmented line in image j program was selected and the length was determined. The ruler was used to determine the reference length. The ruler was used at each photo.

Rio manager data was recorded in order to check the results in later studies. In addition, the results were recorded as length and transferred into excel program. The results were defined by using descriptive statistics and t test in excel program.

The significant differences were identified and used for planning later in the study. In the experiments where significant differences were not reached, the study was repeated by adding different features. In the main study, the experiment was repeated with at least three times biologically and nine times technically. In preliminary studies, repeat was applied at two times biologically and six times technically.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Plant on a Chip (POC) Preparation

In the first "plant on a chip" studies consisting of different widths of 1,2 and 3 mm and a depth of 1 and 2 mm, the chanel with width and depth of 1 mm were found to be more suitable for tomato seeds. The 2 and 3mm width was wide for the root, causing the bending to occur during elongation. The width and depth of 1 mm were determined to be suitable for straight progress. Each chip consists of 6 channels and there are 12 mm length. In this way a standard system for working tomato seeds has been established.

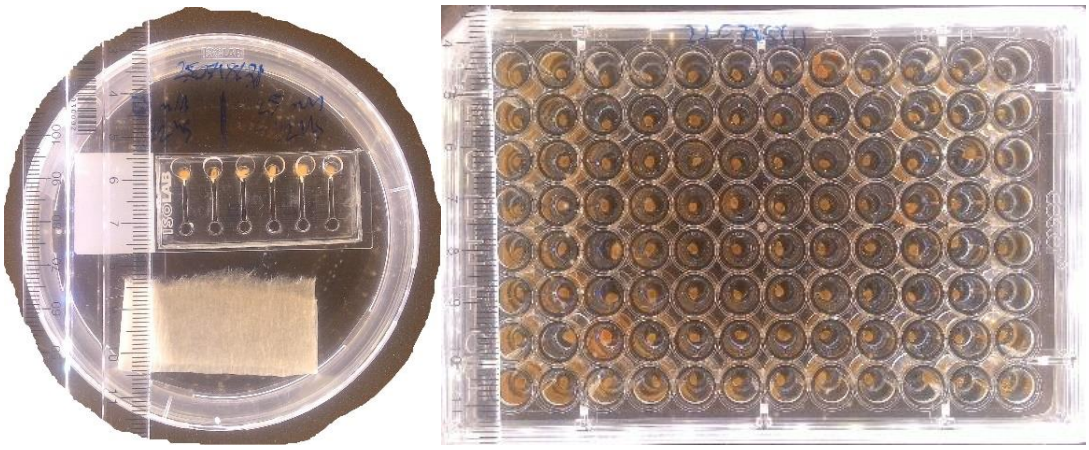

Figure 3.1. Research images of chip (left) and 96 well plate (right).

The production of PDMS in a certain thickness is important for the roots of the seeds to reach the channels in the correct direction. In the experiments, 3.5 ± 0.5 mm PDMS thickness was determined as appropriate.

Temperature should be considered as an additional stress factor. The best temperature for tomato seeds was determined as 25^0C before starting the chip experiment. The intake of nutrients by the roots are reduced at low temperatures. The main reasons for these are the lower solubility of mineral nutrients and the increased

viscosity of water (Wan et al., 2001). Therefore, experimental studies were carried out under suitable temperature conditions.

3.2. Workflow Determination

In order to standardize the seed germination beginning time, methods with many different parameters were tested. For this purpose, the seeds were kept in the refrigerator for 1,2,3 and 4 days separately. In addition, the covering of the petri dish with aluminum foil was performed to see the effect of light as another parameter. The most positive result of them is to wait in the dark for 3 days at the refrigerator and then work in the growth chamber. However, this did not provide the standard germination beginning time. Therefore, the idea of putting seeds in the fridge was abandoned. Instead, germination was initiated by placing it directly on the growth chamber.

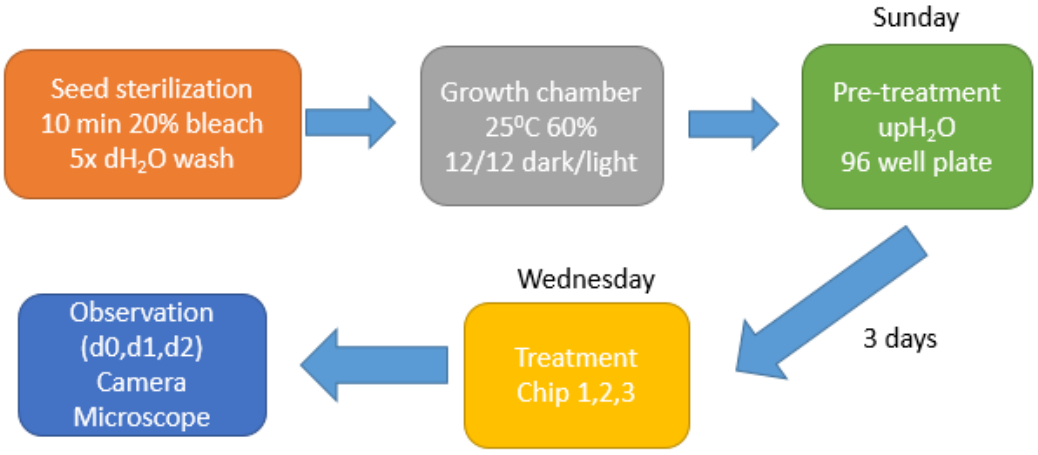

Figure 3.2. Weekly work flow.

In the early studies, many different growth platforms were tested before 96 well plates. The 60 mm plastic petri dish was first tested for seed germination. The seeds were fluctuated during observation, so they were required to immobilize. The seeds were fixed by using well and then 8 well plates were used as the first study. 8 well plates were produced using a similar method to chip production. They are more expensive than plastic well plates and they cause time loss due to production yourself. The use of plastic 24 well plates made the work faster and provided a suitable space for germinating to the seeds. Because the number of seeds was increase at used

simultaneously, there was needed a faster and cheaper method. The 96 well plate was tested then the study became optimum. At the end of the preliminary study, 20 seeds of the same standard were obtained using 96 well plates for chip experiment.

In order to make the experiment workflow suitable, it was tested to start the study on different days. The most appropriate of workflow was determined as experiment starts on Sunday and ends on Friday. When this work is continued until Monday instead of Friday, entanglement occurs due to excessive elongation in the root. The chip was caused stress due to inadequate nutrition and environment, therefore the continuation of the experiment was not observed. The design needs to be reorganized and improved for longer experiment.

The seeds reaching the root length of 0.75 ± 0.5 mm were placed on the chip. Photographs were taken at 0, 6, 24, 30, 48 and 54 hours. In addition, 5x images were taken at 0, 24 and 54 hours with zeis microscope. The lengths of the roots were measured by Image J program. Descriptive statistics and t-test were performed in Excel program.

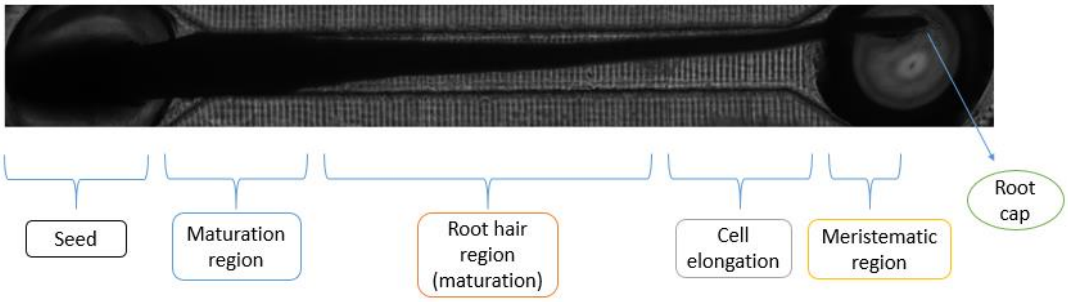

Figure 3.3. The image of the root under the microscope (5x).

When the tomato seeds are placed on the chip after germination, the root structure can be observed in detail under the microscope without the use of stain. Being able to examine the root hair helps us to investigate the root easily.

3.3. Plant on a Chip (POC) Experimentation

When we look at the root hair under the microscope at 40x, the molecular flow can also be observed without using stain. When fertilizer is applied, observations allow us to understand the effect on root hair.

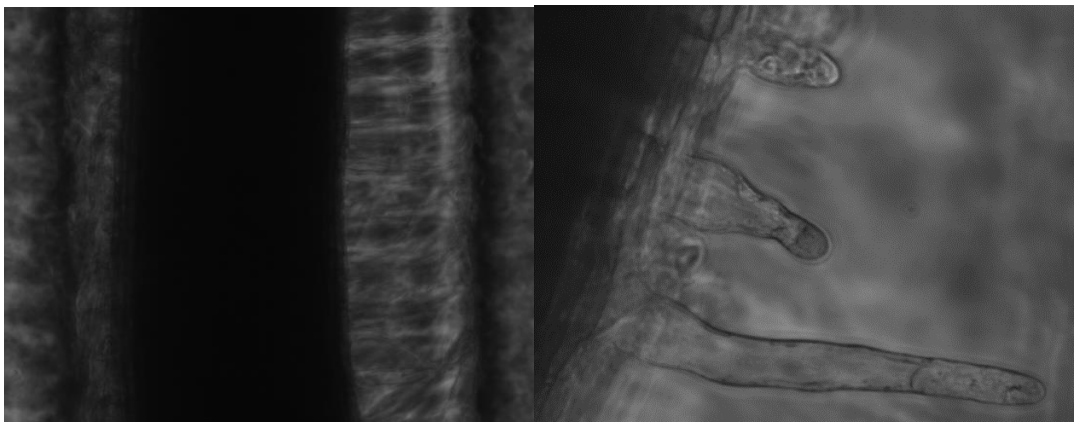

Figure 3.4. Image of root hair under microscope (5x: left, 40x: right).

The fact that the root lengths can be taken at d0, d1 and d2 under the microscope helps to observe the time-dependent change and movement. In this way, the root studies of agricultural plants without damaging can be done in detail. Image J is combined with overlapping images to show time-dependent change in root extension.

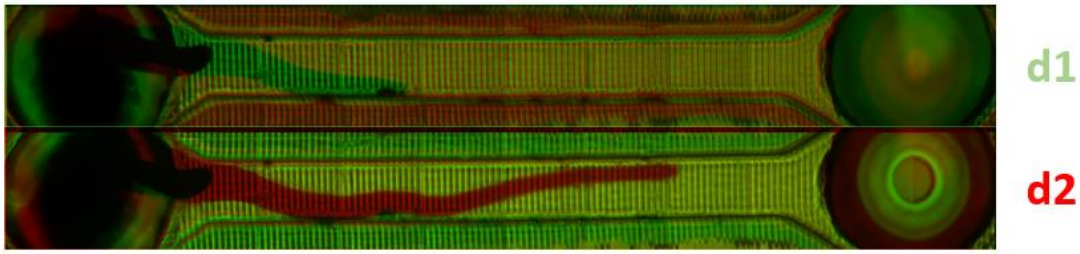

Figure 3.5. Root extension under a microscope (d0: black, d1: green, d2: red).

Photographs at 0. (d0), 24. (d1) and 48 (d2) hours shown us to understand how they affect root elongation in different fertilizers. Using the Image J program, the change in time-dependent root elongation was shown. By Excel analysis, it was determined statistically whether the used chemicals cause change in root elongation.

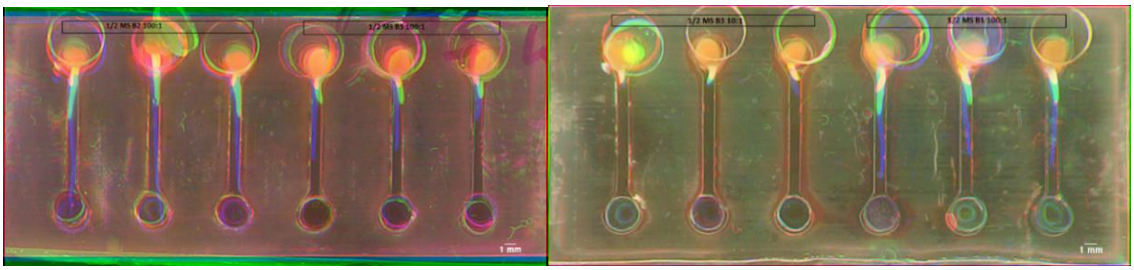

Figure 3.6. Photo image of the elongation of the root (d0: yellow, d1: green, d2: blue).

Even if the root hairs were observed under the microscope, it was not possible to standard measurement and to obtain reliable results. For this purpose, root hair studies should be developed with fluorescent or confocal microscope. As a preliminary study, root hair structure was taken with confocal microscope but not applied to all studies due to cost and time pressure. When positive results could be achieved in biostimulant and fertilizer studies, a detailed study on this should be done. However, the inability to reach the desired data resulted in the lack of further study on this subject.

3.4. Effect of NaCl Concentration

When NaCl concentration was increased, decrease in root elongation was observed. As time progresses, there is a steady increase in root elongation. As the upper limit of the chip (11 mm) was reached at the root, there was a slowdown in elongation. While the negative effect of each salt was observed gradually, it was determined that the elongation at 300 mM NaCl stopped completely. 125 mM NaCl has a significant ($p<0.05$) reduction effect on the root.

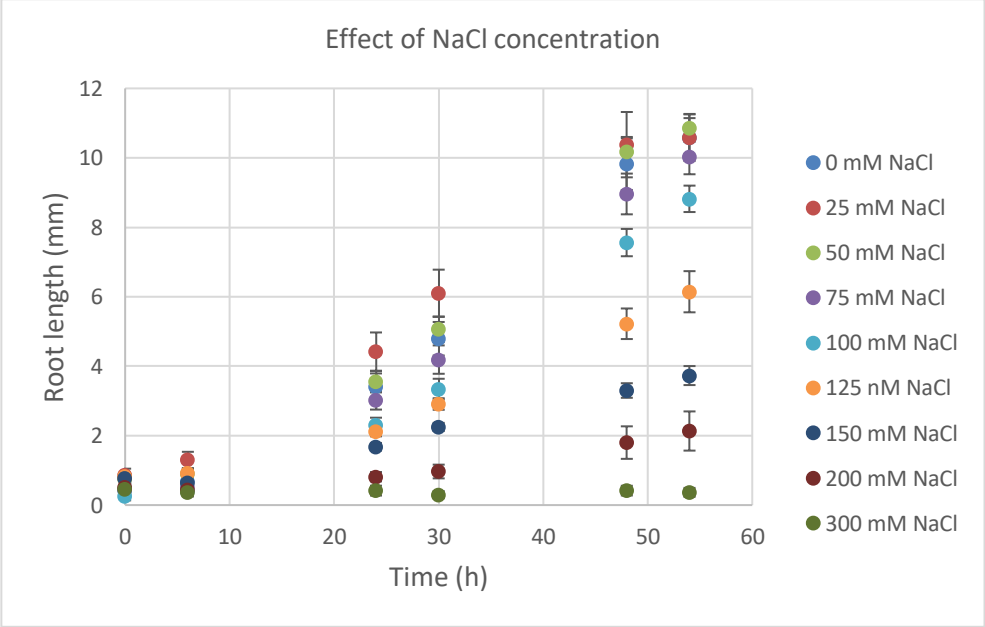

Figure 3.7. Effect of different NaCl concentrations on root elongation.

Salt stress has been reported to have negative effects on plant root and stem length by many researchers (Akbarimoghaddam et al., 2011). In our study, increasing

salt concentrations significantly increased the inhibition of root length. For this reason, it is very important to define and understand the specific physiological characteristics that provide salt tolerance in the development of field studies. The difficulty in using saline irrigation water will be overcome by obtaining more information on salt tolerance.

In the research of Çolak et al., it has been determined that increasing salt concentration has negative effects on each parts seedling development. Significant reductions occurred in all sections examined. It has been suggested that germination at tomato has never occurred for above 150 mM, because high salt levels cause high toxicity in embryo tissue. The decrease in root length occurred at higher salt concentrations than the decrease in stem length. This may be an adaptation developed by the plant to maintain water uptake (Kökten et al., 2010).

Because the natural environment of the root is the darkness under the soil, light causes negative stress on the seed. Evolution has occurred in order to protect the root from the light stress and to reach the dark environment. The petri dish covered in aluminum foil is the most accurate method to create darkness determined in the preliminary study. However, it was determined that the darkness at 0, 125 and 200 mM NaCl for 24 hours did not cause any change in root elongation. In the subsequent experiments, the studies were carried out with remove the light factor by using cover the aluminum foil.

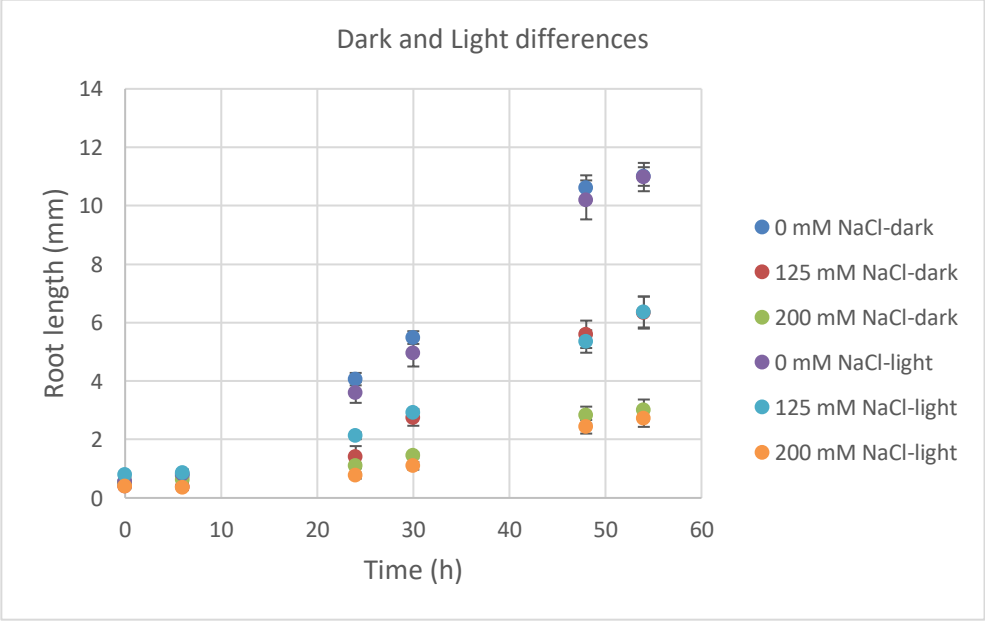

Figure 3.8. Effect of darkness on root elongation at different NaCl concentrations.

3.5. Effect of Fertilizer on Root Elongation

½ MS has all molecules for plant growth. upH2O was used as the solvent to determine whether fertilizers were effective. Fertilizers were added at 1: 1, 10: 1 and 100: 1 ratios for preliminary study and negative effect was obtained. These ratios are not suitable for experiment shows that the use of fertilizers in NaCl studies.

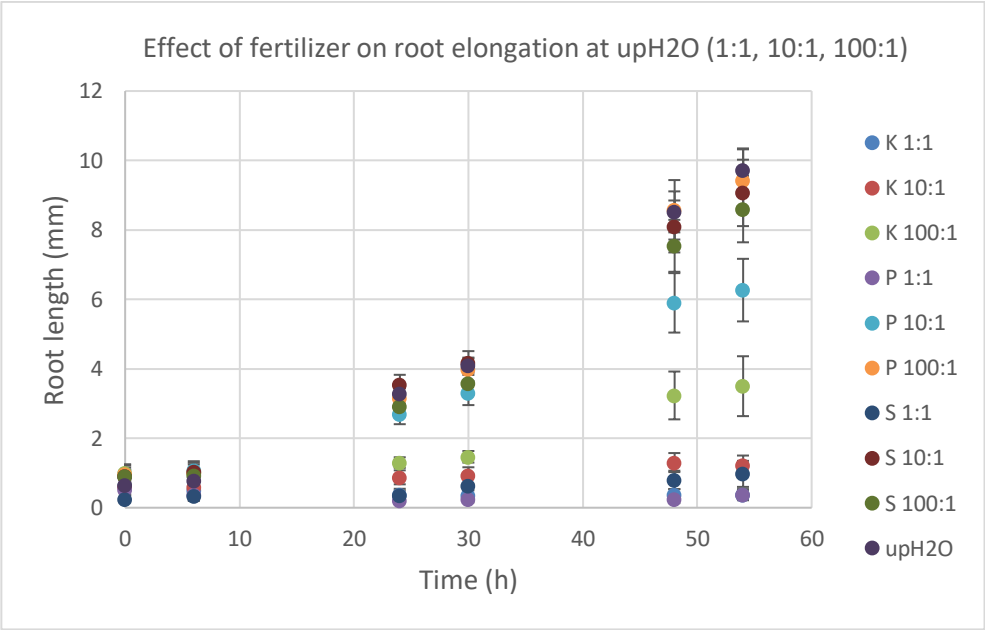

Figure 3.9. Effect of different fertilizers on root elongation (1:1, 10:1, 100:1).

Fertilization is one of the most common agricultural practices. In this context, biostimulant are considered as an organic fertilizer that has the potential to prevent nutrient losses due to slow release N, P and K (Coppens et al., 2016). As foliar application, 80 g L-1 compost, NPK fertilizer and biostimulant were applied separately every 15 days. It was concluded that biostimulant treated pepper yield had more effect compared to organic compost but less yield than inorganic NPK fertilizer (Aly and Esawy, 2008).

When we look at the 1000:1 and 10000:1 ratios, we cannot reach the positive effect. However, achieving similar effect with upH2O ratio of 1000: 1 fertilizer rate has been determined that this is the most suitable fertilizer rate. After seed germination, it was determined that there was no need for fertilizer for the elongation of the root at the first root elongation.

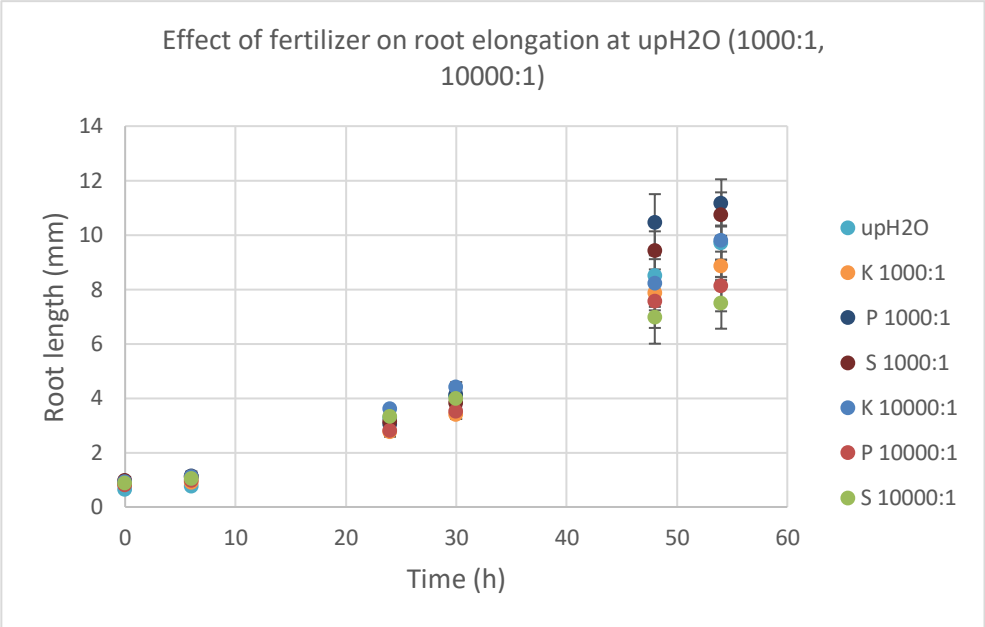

Figure 3.10. Effect of different fertilizers on root elongation (1000:1, 10000:1).

In our preliminary study we did not have a positive effect in the ratio of 100:1, 1000:1 and 10000:1 at 150mM NaCl. However, the ratio of 1000:1 the most suitable fertilizer ratio against salt stresses. Finally, 100000:1 and 1000000:1 ratios were tested. Similar result was observed when we look at this experiment. It was determined that the fertilizers used did not have a positive effect on salinity stress at this stage.

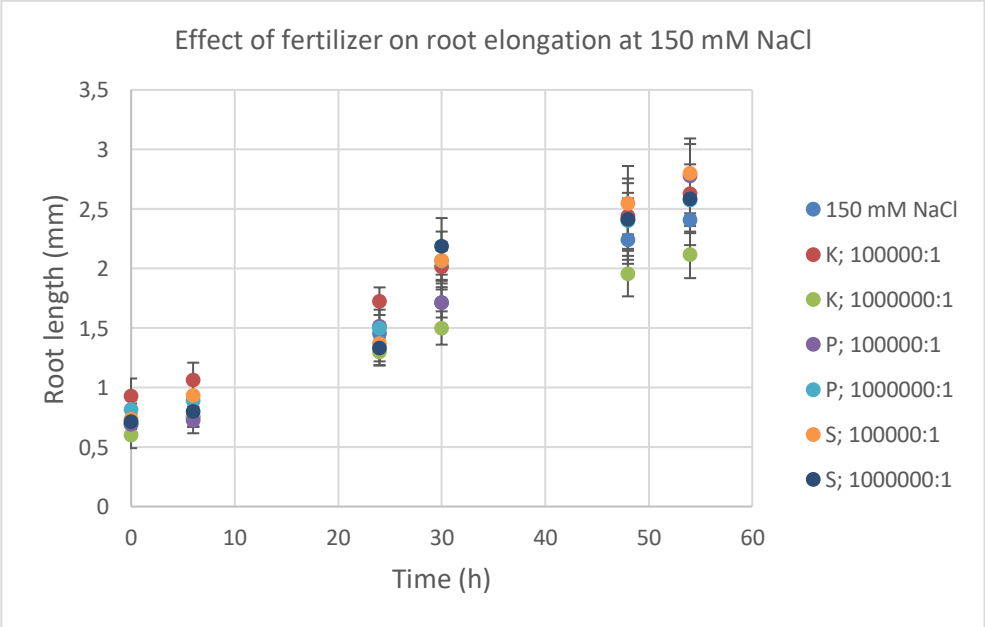

Figure 3.11. Effect of different fertilizers on tomato root elongation at 150 mM NaCl.

Humic and fulvic acids are the main organic components of lignites and soil. They are produced by biodegrading organic matter, and they include acid containing phenolate and carboxyl groups. Fulvic acids are humic acids with higher oxygen content and lower molecular weight (Bulgaria et al., 2015). These substances have the potential to improve abiotic stress tolerance in plants.

3.6. Effect of Biostimulant on Root Elongation

B2-coded biostimulant was added to 100 mM ½ MS at different concentrations and the effect on root elongation was observed. hen the concentration level increased, decreasing root elongation concluded in a negative effect on plant growth at high concentrations. The 200: 1 ratio has a higher root elongation than the others. The rates of 200: 1 and 100: 1 were therefore re-experimented. According to the results of the new study, 100: 1 ratio was chosen for biostimulants because there was no difference between them. The 10: 1 and 100: 1 ratios were determined to be suitable for their activities in biostimulants.

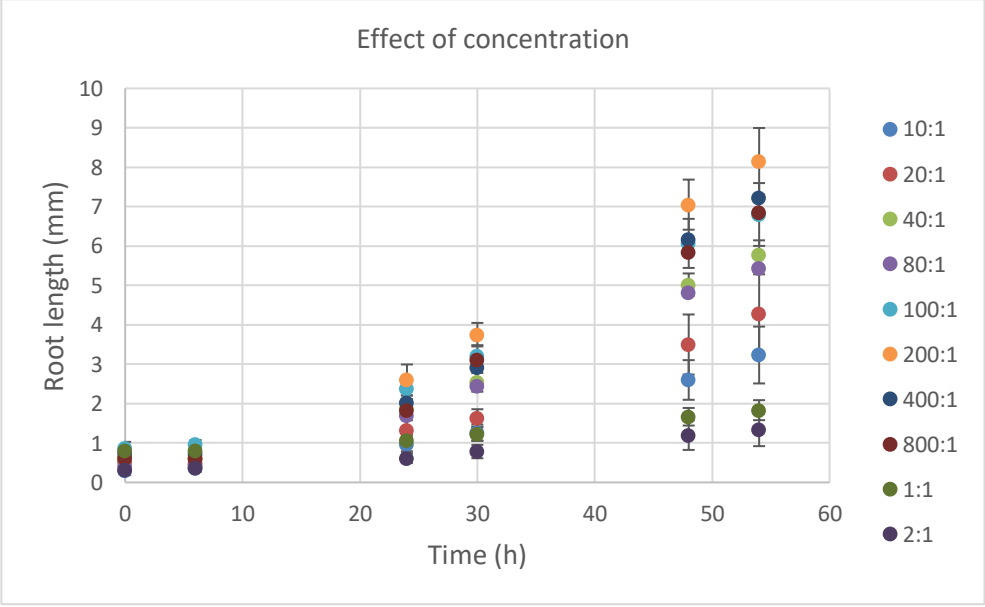

Figure 3.12. Effect of different *Curcuma longa* concentrations at 100 mM ½ MS.

The mechanism to overcome plant stress conditions needs to be understood, and hypotheses must be established and verified by experiments and observations. The

discovery of new biostimulants requires a comprehensive and systematic process using an interdisciplinary approach (Povero et al., 2016).

Plant secondary metabolism produces numerous compounds with pharmaceutical activity. These compounds are formed by different and specific cell types and cannot be produced biotechnologically. The microfluidic design allows the production of specific metabolites at different stages. Therefore, their cells can mimic in the microfluidic system.

B1, B2 coded biostimulants have no positive effect to reduce the stress of 100 mM NaCl. The lack of positive root elongation in fertilizers with lower ratios (1000: 1.1000: 1, etc.), has caused stoped work at biostimulants.

The economic feasibility of using biostimulant as renewable and environmentally friendly raw materials is based on handling their waste management. Various studies conducted for different species reveal the value of biostimulants (Baghel et al., 2015). Further studies are needed to make cost effective biostimulant as competitive raw material against inorganic fertilizers. In order to obtain the final or intermediate products economically, they must be produced under optimized conditions.

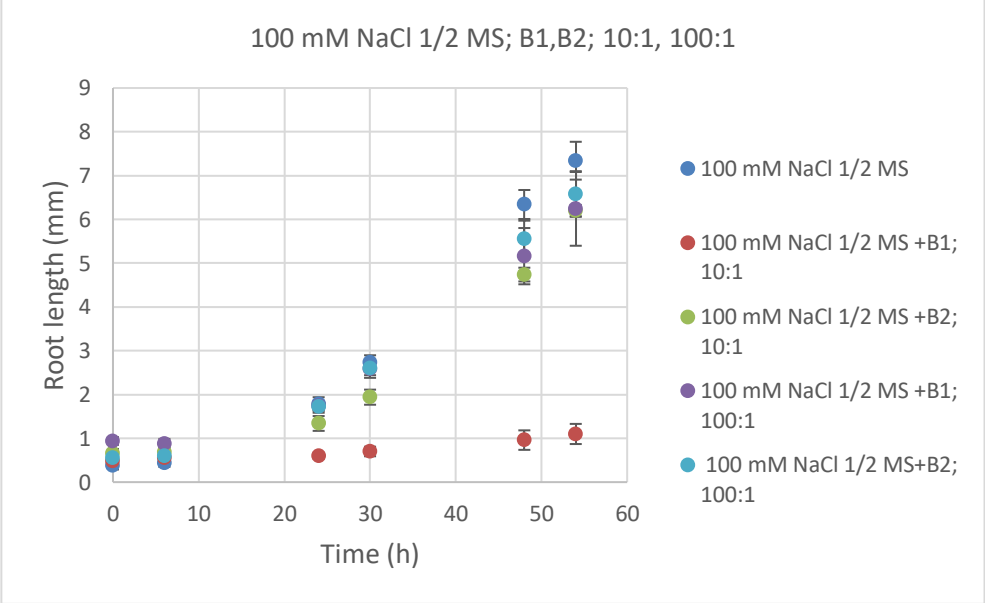

Figure 3.13. Effect of B1, B2 coded biostimulants at 100 mM NaCl ½ MS.

Similar results were found in B4 and B5 coded biostimulant and it was determined that the desired result could not be reached with this method no matter which plant extract it uses. Even though the study has allowed the desired results to be

achieved in the salt effect, the positive results could not be obtained when more detailed contents. It is concluded that the ratio to be used before agricultural production should be determined precisely.

Resistant species are not affected by salt using the selective permeability of the root cell membrane. When growing tomatoes in salty soils, it is necessary to give more weight to resistant species. It may be interesting to examine the similar method at different species and stresses. In this way, our research will lead to achieve feasible results and to create new research topics. Finally, we recommend using this method that the development of tomato species in greenhouse cultivation as a preliminary study.

This study focused on salinity stress, but this requires more extensive research on biostimulants and fertilizers. It identified a pioneering method for greenhouse or field trials. This method has been developed for agricultural plants and offers the possibility to experiment the physiological functions in detail.

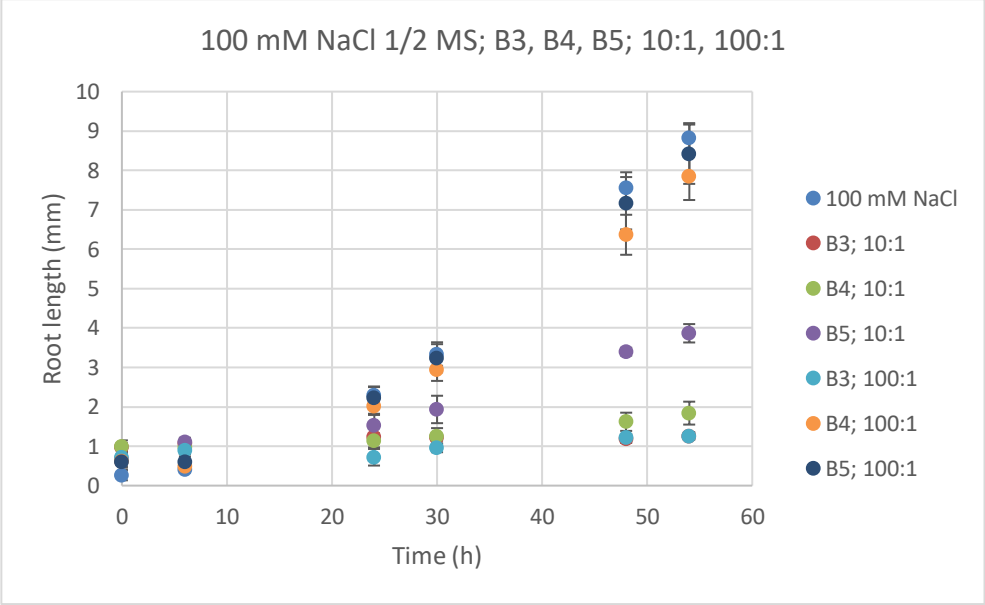

Figure 3.14. Effect of B3, B4, B5 coded biostimulants at 100 mM NaCl ½ MS.

Biotechnological developments enable the implementation of conservation and development strategies for plants and solutions to problems. Chips, which consist of the different materials such as glass and PDMS, are suitable for plant experiment. It will become a powerful tool for the analysis of fertilizers and biostimulants.

The following topics include research for the preparation and application of biostimulants from plants and their extracts:

1. There are many plant family and therefore different types of biostimulants can be recommended;
2. Various abiotic factors during plant production may affect the biochemical composition;
3. Even if the same plant species are processed, biostimulants with different biochemical contents can be obtained using different extraction methods;
4. Bioactive compounds in biostimulants can activate different mechanisms;
5. The frequency and amount of biostimulants applications may change;
6. Different crop types may respond differently to the same biostimulant.

Agronomic, physiological, biochemical and molecular studies are needed to better understand the changes caused by biostimulants in plant products (Bulgari et al., 2019). Collection and investigation of detailed information is required about the best preparation. All this information will allow the determination of precise protocols that can assist agricultural industry and farmers in plant production.

CHAPTER 4

CONCLUSION

Our project carried out a comprehensive screening of seeds. Initial germination and root phenotype observation was performed under a microscope without damaging the plant. The control and implementation of the parameters were made by the chips and more detailed information was obtained. It was developed with a novel method for carrying out laboratory experiments prior to land for fertilizer studies. The ease of use and the variety of designs allow for different work. Our study shows that plant on a chip can be used in fertilizers studies, so that they would be investigated innovative chemical.

As a result, a controlled environment was created to ensure the root extension of the tomato seeds after germination with the designed chip system. Stress-induced changes in the tomato root structure of NaCl in different ratios (25, 50, 75,100, 125, 150, 200 and 300 mM) were observed. 3 different fertilizer (NP, NPK and Humic+Fulvic acid) were prepared and used for tomato seeds. Besides, 5 differen plant extract (*Allium sativum*, *Curcuma longa*, *Ceratonia silique*, *Sideritis spilia*, *Solanum americanum*) was used as biostimulant for tomato seeds.

It is shown that the standardized experiment can be studied for different seed and stress factors. Observing the effect of combining different parameters in plant germination and first root formation makes possible the discovery of different mechanisms. The existence of such a system prior to the greenhouse and field studies constitutes preliminary information for these experiments.

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