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

**MICROPROPAGATION OF *Lycopersicon esculentum* Mill.
(YELLOW TOMATO)**

MASTER THESIS

PREPARED BY: Zana Abdalrahman JAWAD
SUPERVISOR: Prof. Dr. Musa TÜRKER

VAN-2016

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**Micropropagation of *Lycopersicon esculentum* Mill. (Yellow Tomato)**" presented by Zana Abdalrahman Jawad under supervision of Prof. Dr. Musa TÜRKER in the department of Molecular Biology and Genetics has been accepted as a M.Sc. thesis according to Legislations of Graduate Higher Education on …..../......./.......... with unanimity / majority of votes members of jury.

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THESIS STATEMENT

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

Signature

Zana Abdalrahman JAWAD

ÖZET

Lycopersicon esculentum Mill. (SARI DOMATES) BİTKİSİNİN DOKU KÜLTÜRÜNDE MİKROÜRETİMİ

JAWAD, Zana Abdalrahman
Yüksek Lisans Tezi
Moleküler Biyoloji ve Genetik Bölümü
Danışmanı: Prof. Dr. Musa TÜRKER
Eylül, 2016, 60 Sayfa

Bu çalışmada Kuzey Irak'ta ve Türkiye'de nadir olarak yetişen ve soyu tükenme tehlikesi altında olan Sarı Domates bitkisinin Doku Kültüründe yetiştirilmesi ile ilgili bir protokol geliştirilmeye çalışılmıştır. Besi ortamı, eksplant ve Bitki Büyüme Düzenleyicilerinin (BBD) bitkinin laboratuvar ortamında geliştirilmesi üzerine etkileri araştırılmıştır.

Besi ortamı olarak Murashige and Skoog (MS) kullanılmışır. Bitki tohumları Kuzey Irak'tan sağlanmıştır. Tohumlar steril, hormonsuz hazır ortamlarda çimlendirilmiştir. Çimlenen fidelerden gövde ucu, hipokotil, kotiledon, yaprak, nod ve internod bölgeleri eksplant olarak kullanılmıştır. Eksplantlardan direkt ve indirekt organogenez yolu ile farklı konsantrasyon ve kombinasyonlarda Bitki Büyüme Düzenleyicileri (BBD) kullanılarak sürgünler elde edilmiştir. Elde edilen sürgünler köklendirme ortamlarına transfer edilerek köklendirilmiş ve fideler elde edilmiştir. Fideler steril ortamda geliştitlerek toprak içeren saksılara aktarılmış ve steril olmayan dış ortama alıştırılmıştır. Saksılarda domates rejenere edilmiştir.

Sürgün eldesi en başarılı şekilde direkt organogenez yönteminde nod eksplantında, 0.5 mg/l BAP ve 2 mg/l BAP+1 mg/l NAA destekli farklı iki ortamda %100 oranında elde edilmiştir. İndirekt organogenez yönteminde hipokotil eksplantından 2 mg/l Kinetin eklenmiş MS ortamında %83.33 oranıda kallus ve kallustan sürgün elde edilmiştir. Elde edilen sürgünler 0.5 mg/l IBA + 0.5 mg/l IAA desteklenmiş MS ortamında %100 kök üretmiştir.

Anahtar kelimeler: Bitki büyüme düzenleyicileri, Eksplant, Mikroüretim, Sarı domates

ABSTRACT

MICROPROPAGATION OF *Lycopersicon esculentum* Mill. (YELLOW TOMATO)

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M.Sc. Thesis
Molecular Biology and Genetics Department
Supervisor: Prof. Dr. Musa TÜRKER
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In the present study an *in vitro* tissue culture protocol was developed on Yellow Tomato grown in Northern Iraq region and Turkey. The effects of Media, Plant Growth Regulators (PGR) and explant on *in vitro* regeneration of Yellow Tomato were investigated. Plant seeds were provided from Northern Iraq and germinated in PGR free MS and White media.

Shoot tip, hypocotyl, cotyledon, leaf, node and inter node were excised from germinated seedlings and used to be explant. Adventitious shoot regeneration was achieved in tissue culture from explants by applying different concentration and combination of different PGR via indirect and direct organogenesis. The regenerants were transferred to the different media conditions to be developed roots. The whole seedlings regenerated *in vitro* were transferred to soil and acclimatized in natural environment.

The most successful (100%) adventitious shoot regeneration was provided from node explant in MS medium supplemented with 0.5 Mg/L BAP and 2Mg/L BAP + 1Mg/L NAA with two different application via direct organogenesis. 83.33% adventitious shoot regeneration was achieved from hypocotyl explant in MS medium supplemented with 2Mg/L kinetin via indirect organogenesis from calli.

Adventitious shoots without root were transferred to MS medium supplemented with 0.5 Mg/L IBA + 0.5Mg/L IAA and (100%) root development were provided.

Keywords: Plant growth regulators, Explant, Micro propagation, Yellow tomato

ACKNOWLEDGMENT

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SYMBOLS AND ABBREVIATIONS

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

Abbreviations	Description
BA	Benzyl adenine
BAP	Benzyl amino purine
2,4-D	2,4-Dichlorophenoxyacetic acid
IAA	Indole acetic acid
IBA	Indole-3-butyric acid
KN	Kinetin
NAA	Naphthalene acetic acid
ST	Shoot tip
PGR	Plant Growth Regulator
WT	White medium
MS	Murashige and Skoog Medium
hr	Hour
N	Not used
HCL	Hydrogen Chloride
NaOH	Sodium Hydroxide
pH	Hydrogen Potential
NaOCl	Sodium hypochlorite
UV	Ultraviolet
Lab.	Laboratory

Symbols	Description
ml	milliliter
w/v	Weight/volume
$^{\circ}$C	Centigrade
mg/l	Milligram/ liter

1. INTRODUCTION

With the increase of the population in world; human requirements will also increase such as environment, space and nutrients. In some countries of the universe at the 21st century; hunger, famine, war, pestilence and mass death are being noted. Out as the most important and outstanding one of these issues is hunger problems. The hunger problem lies at the root of other problems mentioned above. The other points shall be reduced and solved after the hunger. The solution for this problem is believed to increase the harvest and stock output. Plants and plant products are a key function in sustaining the human life. Plants as a food source constitute directly 93% of the human diet, and 7% indirectly.

Plants are used as nutrient supplies, also medicines, fragrances, and baits. They are responsible for the production of many high-value natural products. In fact, at the western countries, plant-based prescription drugs are commonly used. Today, the location of the plants which are used for the derivation of food is really important. The studies prove the increase of the quality and quantity of products with the help of last 50 years developed breeding methods and appropriate cultivation techniques with modern technology. This is just as a consequence of the amalgamation.

Understanding the structure of DNA and bacterial genetics, the development of plant tissue culture and techniques, the onset of many plant genes revolution period are not applicable prepared. Plant tissue culture is the production of a whole plant cell, tissues or organs, such as new tissue from parts of plants, plants or plant products; under aseptic conditions, in an artificial nutrient medium. This develops new forms and produce a variety of genetic variation present in tissues. It is among the main objectives of the culture. Consequently, plant tissue cultures are genetically important. It has a big role in the improvement of work. Moreover, the species are being lost.

To enhance the production of plant products *in vitro*, to develop products using very little progress in biotechnology has been achieved. With this techniques it is possible to produce "*in vitro*" artificial food in the closed section and glass containers. The process includes obtaining all of the plants with fully grown bodies under the sterile conditions and environment. Replication in the tissue culture is also called "micro fabrication"

Many parts of the plant can form new plants; meristems, shoot tips, and knuckles between nodes, hypocotyls, leaves, stem parts, cotyledons, roots, buds, etc. with different explants used.

Plants regenerated via micro fabrication, the meristematic cells are derived from characteristic organized meristems. Methods available for propagating plants *in vitro* are much more extended from the already developed conventional propagation methods. *In vitro* techniques have the following advantages over traditional methods:

-Cultures start with very small pieces of plants (explants), and thereafter small shoots or embryos are propagated (hence the term 'micro propagation' to describe the *in vitro* methods).

-Only a small amount of space is required to maintain plants or to greatly increase their number.

-Propagation is ideally carried out in aseptic conditions (avoiding contaminations).

-Methods are available to free plants from specific virus diseases. Providing these techniques are engaged, or virus-tested material is used for initiating cultures, so certified virus-tested plants can be produced in large numbers. Terminology such as virus-free and bacteria-free should not be used, as it is impossible to demonstrate that a plant is free of all bacteria or viruses.

-A more flexible adjustment of factors influencing vegetative regeneration is possible, such as nutrient and growth regulator levels, light and temperature. The rate of propagation is therefore much greater than in macro propagation and many more plants can be produced in a given time.

-It may be possible to produce clones of some types of plants that are otherwise slow and difficult (or even impossible) to propagate vegetativelly.

-Plants may take a new temporary characteristic through micro propagation which makes them more suitable to grow in comparison with the conventionally-grown livestock. Production can be continued all the year round and is more independent of seasonal changes.

-Vegetativelly-reproduced material can often be stored over long periods.

-Less energy and space are required for propagation purposes and for the maintenance of stock plants.

-Plant material needs less attention between subcultures and there is no labor or materials required for watering, weeding, spraying etc.

One of the main conditions of applying the technique of *in vitro* cultivation of isolated organs, tissues, and cells of tomato is the availability of highly effective protocols for producing valid fertile regenerates.
Usually organogenesis require the labor-intensive crop production and the emergence of a low-frequency.

Clonal propagation (Shoot tip culture), on average, have higher multiplication rates. Because of this rate, it is used widely in the propagation of plants. During the multiplication with a pathogen free process; the possibility of recontamination risk is very low. Genetic variation between cultured plants in the shoot tip, has a very low frequency (Hu and Wang, 1983).

Unfortunately, clonal propagation (shoot tip culture) requires a lot of labor, expensive production.

It is a method like: each new stem end should be excised from the parent plant or rooting; or multiplication medium must be placed separately.
Tomatoes are an important crop, which occupies the first place in the world, among the products of vegetables as well as the second place citrus crops on a vitamin value. The global gross yield of marketable products of tomatoes in 2012 was 161.8 million tons, Russia accounts for about 2.5 million tons (~1.5%) on a cultivation area of 117700 hectares (FAO, 2014).

Tomato (*Lycopersicon esculentum* Mill.) is a vegetable crop of large importance throughout the world and it's an integral part of human diet worldwide. It is a member of the *Solanaceae* family and is a watery fruit containing 5-7 % dry matter. Although it contains relatively low concentrations of vitamin C, pro-vitamin A and minerals, compared to other fruit species, it is a major source of these nutrients because it is consumed in large quantities (McGlasson, 2003). Tomato is the second most important vegetable crop next to the potato. Present world production is about 100 million tons fresh fruits produced on 3.7 million hectares. Tomato production has been reported in 144

countries (FAOSTAT Database, 2004). Its annual production accounts for 152.9 million tons with a value $74.1 billion (FAO, 2009). Tomatoes are grown both under plastic covered greenhouses and in the open field.

Tomato (*Lycopersicon esculentum* Mill.) is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in almost every country of the world (Abu-El-Heba et al., 2008). Tomato (*Lycopersicon esculentum* Mill.) is considered to be an important vegetable crop and a model species for introduction of agronomical important genes in dicotyledonous crop plants (Wing et al., 1994). It is grown throughout the country where irrigation water and arable land are available and is mainly grown by small holders who employ relatively poor crop management practice (Abdelmageed et al., 2003).

Yellow tomatoes are roughly globular in shape and vibrant sun-gold in color. Their skin is thick and flesh, very weighty as they naturally have a high-water content. Yellow tomatoes are a low-acid type tomato with mild and sweet tomato flavor. Their flesh is both succulent and meaty. It contains edible seeds within the flesh's pulp, an indicator that it is a true fruit.

1.1. Seasons/Availability

Large Yellow Tomatoes are available year-round with a peak season during summer months.

1.2. Current Facts

The Yellow Tomato, scientific name *lycopersicon esculentum*, is a member of the nightshade family. It is the edible fruit of the vine of an herbaceous upright and sprawling plant.

1.3. Applications

For culinary purposes, Yellow tomatoes are considered a vegetable. Their first and foremost use is for fresh eating. Raw Yellow tomatoes are quintessential sandwich and salad tomatoes. They can be used in place of red tomatoes in any given recipe, fresh,

hot or cold. Yellow tomatoes can be processed and cooked to make ketchup, paste and jams. They can also be pureed and made into soups and granites. Pair Yellow tomatoes with citruses, especially lemon and lime varieties, seafood such as scallops, shrimp, crab and fish, grilled and roasted meats and poultry, mild and bitter salad greens, olive oil, vinaigrettes, young, milky cheeses, eggs, cream, hazelnuts, pine nuts, avocados, onions, basil, mint and cilantro.

1.4. Geography/History

Wild tomatoes are native to the Andean highlands of Chile, Ecuador and Peru. Though exact domestication dates are unknown, Yellow tomatoes were in fact the very first domesticated tomato variety. They were originally cultivated by the Aztecs of Central Mexico. Natural mutations and breeding led to the development of tomatoes of other colors and of course, thousands of new varieties. Crossbreeding tomatoes are so simple, it can happen naturally. The pollen of one tomato can be rubbed onto the stigma of another and within a month, a hybrid is created. The C.M. Rick Tomato Genetics Resource Center at the University CA Davis houses the world's largest repository of Yellow tomato seed varieties.

Yellow tomato is extremely rare, lemon-yellow colored, medium-small gooseberry tomatoes are very tasty with a good, sweet and mild flavor, and when immature has some faint striping similar to gooseberries, hence the name. A very old heirloom tomato that was popular in the 1800's and listed by Fearing Burr in 1865. It was also illustrated in the Album Vilmorin in 1868. (Cordon).

Yellow gooseberry tomatoes on vines that produce several hundred fruit per plant. Great flavor and excellent in salads.

The *in vitro* production of such an economically important crop is a quite serious issue due to the risk of extinction of the rare cultivar of tomato.

2. LITERATURE REVIEW

Plant biotechnology is the strategy to improve the plants for human uses by different techniques. Crop improvement by plant biotechnology improves the food quality, quantity, provide job, money, and research work for any country. Through plant biotechnology, crop or plants are being improved on genetic level.

We know that for many thousand years, farmers used selection and breeding techniques to improve crop plant and quality. But modern technology also called plant biotechnology to provide facility and study particularly on genes and its expression in crop plants. It is a speedup process in comparison with the traditional breeding method and it's also less from breeding drawbacks.

In short; plant biotechnology is the art, science, and application of knowledge gained from studies or researches to produce technological changes and improvement in crop plants for human benefit. For this aim; molecular techniques, propagations, tissue culture, and other basic strategies are used to improve plants.

Tomatoes (*Lycopersicon esculentum* Mill.) are an integral part of human diet worldwide. It ranks third in the world's vegetable production, next to potato and sweet potato, placing itself first as processing crop among the vegetables (BBS, 2007). It is a dicotyledonous plant and a member of the *Solanaceae* family and is a watery fruit containing 5-7 % dry matter. Although it contains relatively low concentrations of vitamin C, pro-vitamin A and minerals, compared to other fruit species it is major source of these nutrients because it is consumed in large quantities (McGlasson, 2003). The yield of tomato is variable according to the growing conditions, crop duration and the variety; it is between 60 – 120 ton ha-1 (Vural et al., 2000).

The optimum temperature for tomato production is between 20-27 °C. High and low temperatures cause a reduction in fruit setting. Tomato is not selective in terms of soil requirements, and it can be grown in every type of soil however in light soils production will be earlier than the heavy soils (Hanson et al., 2001).

The optimum temperature for tomato production is between 20-27 °C. High and low temperatures cause a reduction in fruit setting. The indicated species, traditionally

grown in the warm season, are adapted to average ambient temperatures ranging from 17 to 28 °C, with limits of 12 ºC (minimum) and 32 ºC (maximum).

2.1. Tomato Taxonomic Classification

Kingdom: *Plantae*

  Sub kingdom: *Tracheobiota*

    Division: *Magnoliopsida*

      Class: *Asteridae*

        Order: *Solanales*

          Family: *Solanaceae*

            Genera: *Solanum*

              Species: *S. lycopersicum*

                Variety: Gooseberry tomato

The tomato belongs to the *Solanaceae* family along with other economically important crops such as pepper, eggplant and potato. The tomato was classified by Miller as *Lycopersicon esculentum* and renamed by Child (1990) and Peralta and Spooner as *Solanum lycopersicum*. Tomato is a diploid species with $2n = 2x = 24$ chromosomes. The tomato genome is composed of approximately 950 Mb of DNA, more than 75% of which is heterochromatin and largely devoid of genes. The tomato was cultivated and consumed in Mexico well before the arrival of the Spanish. Its introduction and diffusion in Europe were accompanied by a trans domestication which developed different types associated with new uses and growing systems.

Tomato was among the first crops for which molecular markers (isozymes) were suggested for marker-assisted selection (MAS) in breeding (Rick and Fobes 1974; Tanksley and Rick 1980).

Tomato is rich in vitamins A and C and fibre, and is also cholesterol free (Hobson and Davies, 1971).   An average sized tomato (148 g) boasts only 35 calories. Tomato contains approximately 20–50mg of lycopene/100 g of fruit weight (Kalloo, 1991).

The center of origin of the genus *Solanum* section *Lycopersicum* (formerly genus *Lycopersicon*) is the Andean region that includes parts of Colombia, Ecuador, Peru,

Bolivia and Chile. All tomato wild relatives are native to this area (Rick, 1973; Taylor, 1986). Until the arrival of the Spanish explorers to America, the tomato was cultivated in the small vegetable orchards of the Mesoamerican area and was of little economic importance. The commercial tomato, *Lycopersicon esculentum* Mill, is an important vegetable crop whose yields are often limited by plant and fruit sensitivity to chilling injury, especially in northern climates. The commercial tomato is subject to chilling injury at all stages of development, and growth and productivity decrease markedly when night temperatures fall below 10°C (Handley and Sink, 1985).

The bibliography for this crop is very extensive, with the contributions of Kalloo (1991) and Nuez (1995) being some of the most prominent.
This review will focus mainly on plant tissue culture achievements in tomato, the applications of new biotechnologies in tomato breeding.

Tomato (*Lycopersicon esculentum* Mill.) is an important food crop, for which successful applications of *in vitro* regeneration and genetic transformation have already been implemented for genetic improvement (Lindsey, 1992). Shoot organogenesis has been achieved in tomato for explants from many different tissues: apical meristems, cotyledons, stems, petioles, leaves, anthers, and inflorescences (Young et al. 1987; Branca et al. 1990; Compton and Veilleux 1991).

Resulted from disinfected tomato seedlings, and they were suitable to induce different organogenic pathway under the influence of specific hormonal treatment (Jehan and Hassanein, 2013).

The method of the plant tissue culture plays a pivotal role in the second green revolution that gene modification and biotechnology can be used for improving the crop harvest and also superiority.

Tissue culture considered as an *in vitro* aseptic culture of cells, tissues, organs or whole plant exact nutritional and ecological circumstances (Thorpe, 2007). That can be used for manufacturing clones of plants.

Development of protocols for *in vitro* selection can provide new advances for the production of stress tolerant cultivars. Although, some information is available on the morphogenesis of tomato, the techniques have not been developed to a level at which they can be utilized in large-scale multiplication of commercially important cultivars. The morphogenesis response seems to be highly dependent PGRs used in the media. The use

of a combination of molecular and conventional breeding techniques could be the option for the development of cultivars resistant to biotic and abiotic stresses (Bhatia et al., 2004).

Tomato is considered as a prototypical plant for introduction of agronomically important genes (Wing et al., 1994). Developing a reproducible *in vitro* regeneration protocol has been a subject of research because of the economic importance of the crop and its responsiveness for further improvement via genetic manipulation (Evans, 1989). Plant cell/tissue culture, also referred to as *in vitro,* axenic, or sterile culture, is an important tool in both basic and applied studies as well as in commercial application (Thorpe, 2007).

Plant tissue culture techniques are recognized as useful instruments in crop improvement. *In vitro* culture is used in tomato in different biotechnological applications including production of virus free plants (Moghaieb et al., 1999) and genetic transformation (Park et al., 2003). The successful application of plant tissue culture presupposes the establishment of an efficient culture system, consisting of a competent genotype, explant source as well as optimal culture conditions (Plana et al., 2005).

Tomato regeneration has been previously reported via organogenesis in several articles using different explants, such as leaf (McCormic et al., 1986; Gaffer et al., 1997; Öktem et al., 1999) and cotyledon (VanRoekel et al., 1993). *In vitro* culture of the tomato has been successfully used in different biotechnological application including the clonal propagation of high-value commercial cultivars, virus-free plants, and genetic transformation (Namitha and Negi 2013; Hanus-Fajerska 2006; Li et al. 2011; Yarra et al. 2012). The Flavr Savr tomato (also known as CGN-89564) was the first commercially grown, genetically engineered food to be granted a license for human consumption. The Food and Drug Administration approved the Flavr Savr tomato in 1994. Unfortunately, the tomatoes had a bland taste and they also were very delicate, proving difficult to transport.

The most important contribution made through plant tissue culture (PTC) is the demonstration of the unique capacity of plant cells to regenerate full plants, via organogenesis or embryogenesis. Considerable work is being done to understand the physiology and genetics of embryogenesis and organogenesis using PTC systems.

Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. It includes techniques and methods used to research into many botanical disciplines and has several practical objectives. The process of de novo organ formation is called organogenesis or morphogenesis. The growth of higher plants depends on the organized allocation of functions to organs which in consequence become differentiated, that is to say, modified and specialized to enable them undertake their essential roles.

The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902 on his experiments on the culture of single cells (Haberlandt, 1902). He opined that to "my knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants have been made.

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cyto differentiation and organogenesis. (Mineo, 1990).

Plants used in tissue culture need to be healthy and actively growing. Stressed plants, particularly water-stressed plants, usually do not grow as tissue cultures. Insect and disease-free greenhouse plants are rendered aseptic more readily, *In-vitro* propagation comprises of various stages: selection of explants; aseptic culture establishment; multiplication of propagules; rooting and acclimatization of plantlets. Using a different approach Kotte (1922), a student of Haberlandt, and Robbins (1922) succeeded in culturing isolated root tips. This approach, of using explants with meristematic cells, led to the successful and indefinite culture of tomato root tips by White. Tissue culture is an important tool of biotechnology, which can be used to improve productivity of a crop via rapid availability of superior planting stock. However, cultures can progressively lose their characteristics with sub culturing (Henke et al. 1978, Bajaj and Dhanju 1981). Sometimes total loss can occur after two subcultures (Wu and Antonovics 1978).

The effect of different growth regulators on *in vitro* growth and plant regeneration of tomato (*Lycopersicon esculentum* Mill.) explants, derived from hypocotyls and cotyledons of aseptically grown seedlings, was studied. With regard to the regeneration

frequency, number of shoot primordia and shoots per explant, the best regeneration medium was the Murashige-Skoog (MS) medium (Gubis et al., 2004).

Tomato (*Lycopersicon esculentum* Mill.) is considered to be an important vegetable crop and a model species for introduction of agronomically important genes into dicotyledonous crop plants (Wing et al., 1994).

In vitro regeneration through organogenesis and somatic embryogenesis can be used for multiplication of genetically identical clones and it is an integral part of genetic transformation procedures. Plant cell, tissue and organ culture is a set of techniques designed for the growth and multiplication of cells and tissues using organic nutrients in an aseptic and controlled physical environment. The exact conditions required to initiate and sustain plant cells in culture, or to regenerate intact plants from cultured cells, are different for each plant species. Tissue culture practices have shown that three factors namely explant choice, medium composition and control of the physical environment, are important in successful cultures.

All living cells of a plant are capable of differentiating and dedifferentiating into whole plants.

Development of protocols for *in vitro* selection can provide new advances for the production of stress tolerant cultivars. Attempts have also been made to transfer the higher regenerative ability of wild varieties to cultivated tomatoes. The morphogenesis response seems to be highly dependent PGRs used in the media, which is again cultivar and genotypic specific (Bhatia et al., 2004).

Micro propagation is an important tool in the multiplication of genetically uniform plants that possess desirable traits for horticultural industry. Like many other technologies, it has gone through different stages of evolution that focused mainly on the production efficiency in terms of quantity.

A single explant can be multiplied into several thousand plants in a very short time. Plant tissue culture laboratories have been transferred to the developing countries with low labor costs. The artificial *in vitro* culture conditions should be closely related to the natural environments. Despite the meticulous efforts involved in growing plants *in vitro* and the advances made in plant tissue culture, the application of this technique is still hampered by various physiological and developmental problems. (Ruffoni and Savona, 2013). The *in vitro* morphogenic responses of cultured plant tissues are affected

by the different components of the culture media, and it is important to evaluate their effects on plant regeneration (Costa et al., 2000).

The movement of plants between *in vitro* and soil conditions represents an environmental switch which is important in plant conservation and micro propagation, but which is less well studied by molecular approaches (1991 Kluwer Academic Publishers). Plant tissue culture is recognized as an important tool to generate useful genetic variability for crop improvement.

Tomato can be propagated by plant biotechnology technique (plant tissue culture), micro propagation and other approach of micro propagations like organogenesis.

2.2. Organogenesis

"The unit of organogenesis" is an integral component of all dynamic models aimed at description of the production process in cultivated plants (Poluektov and Topazh, 2005).

The main objective in plant cultures is to regenerate a plant or plant organ from the callus culture. The regeneration of plant or plant organs only taken place by the expression of cellular totipotancy of the callus tissues. Scattered areas of actively dividing cells, known as meristematic centers, develop as a result of differentiation and their further activity results in the production of root and shoot primordia. These processes can be controlled by adjusting the cytokinins: auxin ratio in culture medium. The production of adventitious roots and shoots from cells of tissue is called organogenesis.

The development of adventitious organs or primordia from undifferentiated cell mass in tissue culture by the process of differentiation is called organogenesis. The formation of roots, shoots or flower buds from the cells in culture in manner similar to adventitious root or shoot formation in cuttings is called organogenesis

Organogenesis refers to the differentiation of organs such as roots, shoots or flowers. Shoot bud differentiation may occur directly from the explant or from the callus. The stimulus for organogenesis may come from the medium, from the endogenous compounds produced by the cultured tissue or substances carried over from the original explant. Organogenesis is chemically controlled by growth regulators. Skoog while

working with tobacco pith callus, observed that the addition of an auxin Indole Acetic Acid (IAA) enhanced formation of roots and suppressed shoot differentiation. Plant development proceeds as an interative process of organ initiation from meristems. Above-ground tissues originate from the shoot apical meristem (SAM), which initiates lateral organs in regular phyllotactic patterns. Organogenesis involves a subset of meristematic cells transitioning to determinate growth, requiring broad changes in cell physiology, transcriptional regulatory networks, and hormones. Cells that transition to organ initiation are within the peripheral zone (also called the morphogenetic zone) while cells that replenish the meristematic cells are in the central zone.

Organogenesis starts in the callus in response to the stimulation given by the chemicals in the medium. Organogenesis takes place in two stages, namely autogenesis or shoot initiation and rhizogenesis or root initiation. Both types of organogenesis are controlled by the hormones present in the medium. Skoog and Miller (1957) demonstrated that a high auxin:cytokinin ratio may induce shoot formation. In 1966, Torrey proposed the hypothesis of organogenesis. According to this hypothesis, organogenesis starts with the development of a group of meristematic cells called meristemoids, which initiate the formation of a primordium. Depending on the factors within the system, this primordium develops into either shoot, root or embryoid.

Mutant studies have been essential in teasing apart the various spatial and temporal interactions that regulate organogenesis. The first step in organogenesis is establishing where organ primordia will form. The Hofmeister principle states that new organs form in the location maximally distant from previous primordial (Sluis and Hake, 2015).

Organ culture is used as a general term for those types of culture in which an organized form of growth can be continuously maintained. It includes the aseptic isolation from whole plants of such definite structures as leaf primordia, immature flowers and fruits, and their growth *in vitro*. For the purposes of plant propagation, the most important kinds of organ culture are:

Leaves originate from stem cells located at the shoot apical meristem. The meristem is shielded from the environment by older leaves, and leaf initiation is considered to be an autonomous process that does not depend on environmental cues. Here we show that light acts as a morphogenic signal that controls leaf initiation and stabilizes leaf positioning. Leaf initiation in tomato shoot apices ceases in the dark but

resumes in the light, an effect that is mediated through the plant hormone cytokinin. Dark treatment also affects the subcellular localization of the auxin transporter and the concomitant formation of auxin maxima (Yoshida et al., 2011).

Plant tissues and organs are grown *in vitro* on artificial media, which supply the nutrients necessary for growth. The success of plant tissue culture as a means of plant propagation is greatly influenced by the nature of the culture medium used.

Plant regeneration achieved through organogenesis is affected by several factors such as genotype, explant source, age of explants, media composition and environmental conditions (Mamidala and Nanna 2011; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). There are many reports regarding tomato transformation and *in vitro* plant regeneration from different explants (including seed-cut cotyledons, hypocotyls, leaves, stem sections, pedicels, petioles and inflorescences) via organogenesis (Khoudi et al. 2009; Yasmeen 2009;Goel et al. 2011; Koleva Gudeva and Dedejski 2012; Rai et al. 2012; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014).

As mentioned above, scientists have used different types of explant, but it should be emphasized that the type of explants determines not only the frequency of the explants' organogenesis but also determines the number of shoots produced per explant (Bahurpe et al. 2013; Jehan and Hassanein 2013). Mamidala and Nanna (2011) reported that cotyledons explants showed organogenesis superiority over hypocotyls and leaf explants.

The transformation frequency was assessed in response to several different factors, including seed germination medium, seedling age, pre-culture duration, pre-culture and cocultivation media, inoculation medium, medium pH, washing medium in initial selection medium (Rai et al., 2012).

Organogenesis is controlled by a balance between cytokinin and auxin concentration i.e. it is their relative rather than the absolute concentration that determines the nature of differentiation. Differential response to exogenously applied growth regulators may be due to differences in the endogenous levels of the hormones within the tissue. Organogenesis is a complex process. Whereas in the cultured tissues of many species organogenesis can be demonstrated in this pattern, some plants, notably the monocots, are exceptions.

A plant contains many organs like meristem, cortex, phloem, epidermis are consist of structural unit called cell because an cell have to nature of create whole plant like any organ or tissue of plant also show same nature mean they also create to whole plant in *in-vitro* condition. If plant organs used in in-vitro conditions to generated new plant this process called organogenesis. In organogenesis of plant included two different stages first is de-differentiation (callus formation) and other is re-differentiation (budding on callus) of organ or explant. Organogenesis of plant is require to gain how a organ generated or developed to an whole plant. Organogenesis is method to regeneration of plant in for of organs from callus.

Plants can generate organs and tissues throughout their whole life (Birnbaum and Sanchez Alvarado, 2008; Dinneny and Benfey, 2008). Post-embryonic formation of organs initially arises from the shoot and root apical meristems, which are also known as primary meristems. Primary meristems are formed as a result of embryogenesis and upon activation during germination, they start generating main root(s), leaves and flowers (Peris et al., 2010; Besnard et al., 2011; Sozzani and Iyer-Pascuzzi, 2014). Formation of aerial organs in the shoot apical meristem occurs in a predictable pattern development of new aerial organs is initiated by the plant hormone auxin (Reinhardt et al., 2000; Hamant et al., 2010; Besnard et al., 2011).

Plant cells have high developmental plasticity and initial cell fate can be entirely changed during post-embryonic development (van den Berg et al., 1995). This developmental plasticity has also been observed upon hormonal treatments. Exogenous auxin application results in the production of new organs: leaves and flowers in the shoot and roots in the underground part of the plant (Reinhardt et al., 2000; De Smet et al., 2007). Growth and morphogenesis of plant tissue cultures can be improved by small amounts of some organic nutrients. These are mainly vitamins (including some substances that are not strictly animal vitamins), amino acids and certain undefined supplements. The amount of these substances required for successful culture varies with the species and genotype, and is probably a reflection of the synthetic capacity of the explant.

In vitro plant regeneration has been found to depend on many factors, of which most important are genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels

and vessel covers (Reed, 1999). Several studies have reported adventitious regeneration in tomato dealing with induction of shoots on hypocotyls, apical meristem, cotyledons, stems petioles, leaves, anthers and inflorescences explants (Young et al., 1987; Branca et al. 1990; Compton and Veillux 1991). Gubis et al. (2004) reported that the frequency of adventitious shoot regeneration differed, depending on the type of explants and both the type and concentration of growth regulators added to the regeneration medium. Moreover, the adventitious shoot formation is based on the type of explant used and plays a major role in plant growth regulation (Pana et al., 2005).

The majority of taxonomists as well as most plant breeders and other users have accepted the reintegration of tomatoes to *Solanum* (Caicedo and Schaal 2004; Fridman et al., 2004; Schauer et al., 2005; Mueller et al., 2009).

Growth, and shoot regeneration were strongly influenced by the genotype, explant source, and medium utilized (Carputo et al., 1995). Small tomato hypocotyl explants develop differentiated structures often resembling primitive ancestral reproductive organs. The *in vitro* culture of explants from many plant species (e.g., carrot, tomato, sunflower, eggplant, fruit trees and corn) gives rise to the formation of a meristem where cytological analysis proved the presence of meiosis like divisions leading to chromosome segregation (Nuti Ronchi, 1995).

Successful *in vitro* plant regeneration has been achieved from explants of different organs and tissues of the (Solanaceae.) such as tuber discs (Lam, 1977; Bragdo-Aas, 1977: Jarret et al., 1980a,b; Kikuta & Okazawa, 1984: Wang & Hu,1985; Smolenskaya, 1992), shoots (Patrascu, 1981; Maroti et al., 1982; Wheeler et al., 1985), and leaves (Roest & Bokelmann, 1980; Webb et al., 1983; Hulme et al., 1992).

Explants were transferred to an agar-solidified medium that was not supplemented with growth regulators or to a species-specific standard induction medium. Control explants from each species were incubated directly on the species-specific standard induction medium (Kintzios et al., 2002). The impact of different concentrations of auxins, cytokinins, additives, amino acids and sugars were evaluated for callus induction and shoot proliferation.

Shoot tip, or shoot cultures, started from excised shoot tips, or buds, larger than the shoot apices employed to establish meristem cultures, having several leaf primordia. These shoot apices are usually cultured in such a way that each produces multiple shoots.

Node cultures of separate lateral buds, each carried on a small piece of stem tissue; stem pieces carrying either single or multiple nodes may be cultured. Each bud is grown to provide a single shoot. Meristem cultures, in which are grown very small excised shoot apices, each consisting of the apical meristematic dome with or without one or two leaf primordia. The shoot apex is typically grown to give one single shoot.

In vitro regeneration of plants was achieved in cultivars of tomato (*Lycopersicon esculentum* Mill.). Regeneration through somatic embryogenesis and shoot organogenesis occurred in explants of all treatments, even on hypocotyls from seedlings grown on basal medium and was subcultured to medium without growth regulators (Newman et al., 1996).

2.2.1. Types of organogenesis

2.2.1.1. Direct organogenesis

Direct shoot; root formation from the explant, it means that after culturing explant in medium root system or shoot system was produced directly.

Other methods, such as direct organogenesis of shoots or somatic embryogenesis, are used much less frequently (New man et al., 1996; Chandel and Katiyar, 2000).

2.2.1.2. Indirect organogenesis

Explant cultured and callus was produced after that produced Meristemoid then Primordium. One of the main conditions of applying the technique of *in vitro* cultivation of isolated organs, tissues, and cells of tomato is the availability of highly effective protocols of producing valid fertile regenerates. Regeneration of tomato shoots from somatic cells is most often achieved by organogenesis with the previous stage of callus tissue formation (Bhatia et al., 2004; Jabeen et al., 2005; Rashid and Bal, 2010).

2.3. Callus induction

The culture of tomato callus tissues were carried on for about a month and afterwards subjected to regeneration through adventitious organogenesis.

Formation of organ in callus depends on medium constitutes because medium of tissue culture also contain growth regulators witch determine to shoot regeneration or root regeneration. For this auxin and cytokinin ratio used in an appropriate ratio witch responsible for callus regeneration. Different concentrations of these hormones regulate the balance between cell proliferation and differentiation (Dello Ioio et al., 2008). More concentration of cytokinin compare auxin generated to shoot part in callus and more quantity of auxin generated to roots in callus.

Further insight into the molecular mechanisms operating during de novo organogenesis upon hormonal induction come from studies performed on proliferating masses of cells, which are commonly designed as called (singular: callus). The term callus had been previously used to designate outgrowth of cells associated with callus accumulation and wounding (Ikeuchi et al., 2013).

Callus formation is controlled by the source of the explant, composition of the medium and environmental factors. Explants obtained from meristematic regions develop more rapidly than those of other tissues.

Plant growth regulators like auxin, cytokinin, gibberellin, ethylene, abscisic acid, etc. are affect to callus regeneration according ratio of them in medium. Callus formation requires dramatic changes in both cell identities and cell growth patterns. These changes have been shown to be accompanied by activation or repression of numerous genes across the genome (Atta et al., 2009; Sugimoto et al., 2010). Equimolar amounts of auxin and cytokinin stimulate cell division. Leads to a mass proliferation of an unorganized mass of cells called a callus. Requirement for support ensures that scale-up is limited (Ginseng saponins successfully produced in this way).

The culture of single cells (and small cell clumps) was achieved by shaking callus cultures of tobacco and subsequently placing them onfilter paper resting on well-established callus, giving rise to the so-called nurse culture (Muir et al., 1954, 1958). Later, single cells could be grown in medium in which tissues had already been grown, i.e., conditioned medium (Jones et al., 1960).

Intra and interspecific variability for callus proliferation and shoot regeneration has been widely reported in the genus *Lycopersicon* (Tal et al. 1977, Pratta et al. 1997). Dedifferentiation of leaf explants into a callus, either followed or not by shoot formation, was dependent on genotype, culture medium and physiological stage of the donor plants. Although genetic control of *in vitro* culture traits was investigated in various crops (Kuroda et al. 1998, Nestares et al. 1998, Ogburia 2003/4) there is not enough information about the inheritance of callus production and shoot formation in the cultivated tomato (*Lycopersicon esculentum* Mill.) (Frankerberger et al.1981, Pratta et al. 2003).

The influence of explant type as well as of the type of growth regulators and concentration on callus induction and somatic organogenesis of shoots was studied *in vitro* on four tomato genotypes. Cytological study of callus tissue was conducted. It was established that tomato varieties have a substantially greater ability to indirect shoot organogenesis compared with the F1 hybrid. The highest frequency of somatic organogenesis of shoots, as well as their number per explant, was observed for most of the genotypes studied during the cultivation of cotyledons on Murashige–Skoog culture medium (Khaliluev et al., 2014).

An important condition for the differentiation of plant cells and the formation of callus tissue is the presence of two classes of plant growth regulators, auxins and cytokinins, in the medium (Butenko, 1999). Morphogenic callus formation depends also on the type of explant and on the stage of its development.   At the same time, its origin, physiological age, and size are essential too. It is believed that young, poorly differentiated tissues containing an increasing number of competent cells have a morphogenetic ability (Chandel and Katiyar, 2000; Bhatia et al., 2004).

In vitro culture response was assessed in tomato (*Lycopersicon esculentum* Mill.) for optimum callus induction and plantlet regeneration. Callus induction was achieved within seven to ten days directly on the cut surfaces of both hypocotyls and cotyledon explants cultured on Murashige and Skoog (MS) basal medium supplemented with other hormones.

The *in vitro* morphogenetic responses of cultured plants are affected by different components of the culture media and therefore, it is important to evaluate their effects on plant callus induction and regeneration (Gubis et al, 2004). Primary callus culture derived from tissues with high contents of parenchyma or meristematic cells. In such explants,

mostly only a limited number of cell types occur, so a higher histological homogeneity exists than that in the entire organ. However, growth of the explant in the nutrient medium usually results in an unorganized mass or clump of cells a callus consisting largely of cells different from those in the original explant. Plant organs initiate from meristems and grow into diverse forms. After initiation, organs enter a morphological phase where they develop their shape, followed by differentiation into mature tissue.

Up to date there are several report in the literature considering micropropagation of tomato. However, studies on yellow tomato, a cultivar of tomato, is quite rare. *In vitro* nutrient and culture conditions differ from species to species even in the cultivar of same species grown in different location. Therefore, it is important to determine new cultural and PGRs desire of cultivars. For this reason the present study was designed and a regeneration protocol was developed for yellow tomato.

3. MATERIALS and METHODS

3.1. Plant Material

In this study, yellow tomato seeds (Gooseberry Tomato) were provided by Shex Jamal KRPCHNA who is a modern farmer in Northern Iraq. Yellow tomato has a good taste and small size shape. It was cultivated in glass greenhouses and plastic greenhouses and open field in Iraq.

Seeds were germinated in the Laboratory of Molecular Biology and Genetics Department. Mature plants with flower and fruit was identified by plant taxonomist in Biology Department. The plant was approved to be *Lycopersicon esculentum* Mill.

The calculation of regeneration percentage was carried out by multiplication of regenerant and hundred and dividing the sum to incubated explants.

3.2. Sterilization

The most important point in tissue culture studies is sterilization. Microorganisms can contaminate plant culture and may cause loss of labor, chemical and cultural force. Because of the nutrient medium for the cultured plant material it is quite favorable for the growth of microorganisms. Therefore, for tissue culture the basic condition for the success of the study is sterilization. Metal and glassware, nutrient medium, seeds and plant material has to be sterile. Workspace, laboratory materials, nutrient medium and the explants were sterilized.

3.2.1. Sterilization of glassware and equipment

All materials used in this experiment first were washed with detergent and water. All metal and glassware were packed in aluminum foil. Then all the equipment were incubated in autoclave at 121 ° C and 1.5 atmosphere. Sterilization was carried out in 25 minutes for media and 1 h for metal and glassware.

3.2.2. Seed sterilization

Each plant seeds may carry superficially fungi, bacteria and reproductive cells. The sterilization time and component are dependent on seed structure and size. Therefore, researcher modifies sterilization condition during studies by manipulating appropriate dose of disinfectant and sterilization time (Yıldız, 2000). There were some surface seeds sterilizer like mercury, hydrogen peroxide, antibiotics, and silver nitrate. In this study, most widely used commercial sodium hypochlorite (bleach) was used (Ozcan and Libby, 1996). 100% commercial bleach (NaOCl- ACE-Turkey) was purchased and, diluted to 5% and treated for 20 minutes on seeds. Then seeds were rinsed 3 times with sterile double distilled water. Seeds were incubated in MS and white medium. With such a sterilization methods very few incidence of contamination occurred.

3.2.3. Sterilization of nutrient media containing hormones

In this study, the MS and White medium (Table 3.1) were sterilized under 1.5 atmospheres pressure and 121 ° C for 25 minutes. The time period is changeable depending on media volume.

3.2.4. Working environment sterilization

Workplace, in Laminar Flow Cabin (LFC) was washed with 10% commercial sodium hypochlorite solution and then 70% alcohol at least for 10 to 15 minutes and a UV lamp inside the cabin was turned on for1 h away from plant material before starting tissue culture studies. Fluorescent light and airflow were turned on in LFC during the studies.

Table 3.1. White medium composition

Ingredients	milligrams/ litre
Potassium nitrate	80.00
Calcium nitrate	221.96
Magnesium sulphate	360.00
Sodium phosphate monobasic	18.98
Potassium chloride	65.00
Sodium sulphate	200.00
Manganese sulphate.H_2O	5.04
Boric acid	1.50
Potassium iodide	0.75
Molybdenum trioxide	0.001
Zinc sulphate.$7H_2O$	2.67
Copper sulphate.$5H_2O$	0.01
Ferrous sulphate.$7H_2O$	2.50
Myo- Inositol	100.00
Thiamine hydrochloride	0.10
Pyridoxine hydrochloride	0.10
Nicotinic acid (Free acid)	0.50
Glycine (Free base)	3.00
Sucrose	30000.00
TOTAL gm/litre	31.06

3.3. Growth Media and Culture Conditions

Nutritional environment is the most important factors of success in tissue culture studies. MS (Table 3.2) and White (Table 3.1) medium, sucrose, agar and Plant Growth Regulators (Auxins, Cytokinins and Kinetin) were used for plant regeneration and callus production. Media, sugar, agar (gelling agent) and PGRs concentration were arranged according to commercial instruction and literature. pH was adjusted to 5.8 with 1 M NaOH and 1 M HCl. Double distilled pure water was used for media and stock solutions. Plant Tissue Culture Media contains all necessary mineral salts, amino acids and vitamins.

3.4. Seedling culture

The seeds were rinsed three times with sterile distilled water and cultured at the rate of 5 seeds per jar dish (40 mm X 10 mm) containing 20 mL of appropriate media. Seeds were cultured on two different media treatments: MS (Murashige and Skoog 1962) basal medium (MS 0), and white basal medium (WM 0) hormone free.

Both treatments were supplemented 3% sucrose and were solidified with 0.6% agar. The pH was adjusted to 5.8 before autoclaving at 121 ° C for 25 min. The jars were sealed with Para film (American National Can, Greenwich, Conn.). The cultures were incubated in a growth chamber (Phytotron, Sanyo, Gellenkamp PLC, UK), maintained at 25 ±2° C, and a 16-h photoperiod and 8-h in dark, was provided by cool white fluorescent lamps; 500 micromol-2 sec. (Phillips Canada, Scarborough, Ont.). Several explants were taken from the seedlings 17 days after initial culture.

3.5. Taking explant from germinated shoot

Cotyledons, shoot tip, hypocotyls, leaves, nodes, inter nodes were excised from 17days-old seedlings and used as explants. Explants were cultured on MS basal medium supplemented with BAP, NAA, kinetin, and 2,4-D, and IBA, IAA in various concentration.

After 3 to 4 weeks adventitious shoots were developed from explants. Then shoots were transferred to different medium to proliferate roots.

Generally, auxins and cytokinins alone and in combination caused callus production. Adventitious shoot and root were developed from callus. *In vitro* regenerated plantlets were transferred to plastic pots containing sand and soil (Figure3.1). The plastic pots were covered with polythene bags to maintain high (70 to 80%) humidity levels. Pots were kept in ambient condition in the laboratory.

These conditions were maintained for 2 weeks. After 2 weeks polythene bags were removed and pots were kept in the shade for one week. Plantlets were exposed gradually to full sunlight on the window of the laboratory, and then plantlets were taken to the field condition.All the experiments were repeated at least three times and standard errors of the means were calculated. Data were analyzed by using MS Excel computer program.

Table 3.2. Basic Murashige and Skoog (1962) (MS)

Medium Compound	Concentration (mg / l)
CaCl2.2H2O	440
KNO3	1900
NH4NO3	1650
KH2PO4	170
mgso47h2o	370
mnso4. 4h2o	22.3
znso4. 7h2o	8.6
H3BO3	6.2
K	0.83
CuSO4.5H2O	0.025
na2moo4. 2h2o	0:25
cocl2. 6h2o	0.025
na2edta. 2h2o	37.3
feso4. 7h2o	27.8
Glycine	2.0
Nicotinic Acid	0.5
Thiamine-HCl	0.1
Pyridoxine HCl	0.5
Myo- inositol	100
Total GM/l	4.736

Figure 3. 1.Culturing plantlets in the pot after growing in *in vitro*.

4. RESULTS

4.1.Seed germination

Yellow Tomato Seeds were cultured in two different hormone free media: MS (Murashige and Skoog 1962) basal medium MS, and White basal medium WM.   In White medium, seeds were germinated 83.33 %, but in MS Medium seeds were germinated 67.6 % (Figure 4.1). After the germination, explants were excised from seedlings and incubated in MS medium supplanted with PGRs for adventitious shoot regeneration.
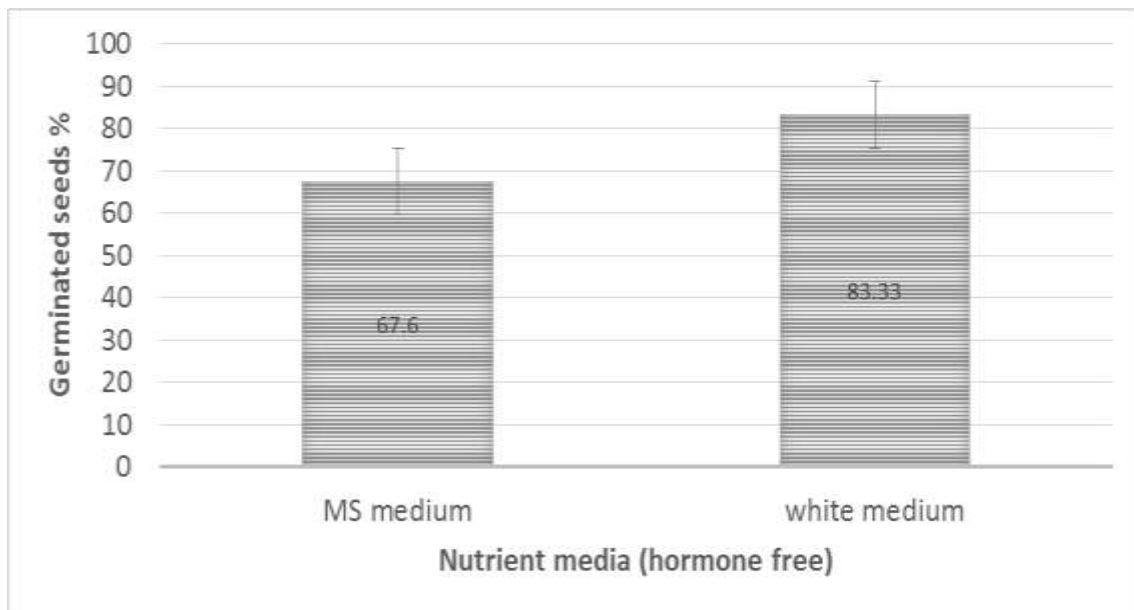

Figure 4. 1.Germination percentage of yellow tomato in MS and White medium.

4.2. Shoot Regeneration (Direct organogenesis)

İn vitro morphogenetic responses of cultured plants are affected by different components of the culture media and therefore, it is important to evaluate their effects on plant callus induction and regeneration (Gubis et al. 2004). After 17 days from seedling

some part of plantlets were isolated as an explant: shoot tip, hypocotyl, cotyledon, leaf, node and inter node. Adventitious shoots were produced from node explant only within 8-10 days from taking explant and callus was initiated within 10 - 13 days directly on the cut surfaces of the hypocotyl, cotyledon, leaf, node, inter node. Explants cultured on MS basal medium supplemented with auxins (NAA and 2,4-D) and cytokinins (Kinetin and BAP) alone or in different combinations, but not in hormone free medium (Table 4.1).

Callus response and callusing index were markedly affected by the types of explant and growth regulators used. Different concentrations of auxins and cytokinins either singly or in combinations had a distinct effect on callus induction and shoot formation from the types of explants.

The highest number of adventitious shoot regeneration (100.00 %) was produced from node explant in MS medium supplemented with 2 mg/l BAP+1 mg/l NAA and 0.5 mg/l BAP separately (Table 4.1). Subsequently, MS medium supplemented with 2mg/l Kinetin produced 80.00 % of adventitious shoots from node explant). The lower number of shoot regeneration was observed in MS medium supplemented with 3 mg/l BAP, 2 mg/l BAP + 0.5 mg/l NAA separately (50.00%).

The highest number of callus production was provided from hypocotyl explant in MS medium supplemented with 2mg/l Kinetin (83.33 %). Subsequently, callus producing conditions are; MS medium supplemented with 2 mg/l BAP+1 mg/l NAA (66.66 % callus), and MS medium supplemented with 2 mg/l BAP + 0.5 mg/l NAA (25.00 % callus) from hypocotyl. The lowest number of callus production was provided from MS medium supplemented with 0.5 mg/l BAP (20.00 % callus) using hypocotyl as an explant.   In node explants the highest number of callus was provided from MS medium supplemented with 3 mg/l BAP, 1 mg/l 2,4-D separately (50.00% callus). Subsequently, 40.00 % callus production was provided from node in MS medium supplemented with 2mg/l Kinetin. And the lowest number of callus in node explant was produced in 2 mg/l BAP + 0.5 mg/l NAA (20.00% callus). In leaf explant callus was produced in 2 mg/l Kinetin (75.00 % callus), and in 0.5 mg/l BAP (50.00 % callus) supplemented MS medium. (Table 4.1).

Table 4. 1. The Effects of different PGR concentration and different explant types on shoot and callus formation.

PGR concentration Mg/L	Hypocotyl explant		Leaf explant		Cotyledon explant		Node explant		Inter node explant	
	callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %
2 kinetin	83.33	0.00	75.00	0.00	16.66	0.00	40.00	80.00	50.00	0.00
2BAP +0.5NAA	25.00	0.00	0.00	0.00	0.00	0.00	20.00	50.00	N	N
2 BAP+1NAA	66.66	0.00	0.00	0.00	0.00	0.00	0.00	100.00	50.00	0.00
3 BAP	0.00	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00
1 2,4-D	0.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00
0.5 BAP	20.00	0.00	50.00	0.00	N	N	0.00	100.00	42.85	0.00

N = this type of explant not used in the tests, 0.00 = callus or shoot not produced

Callus formation and shoot production in explant types were affected by different PGR concentration (Figure 4. 2).
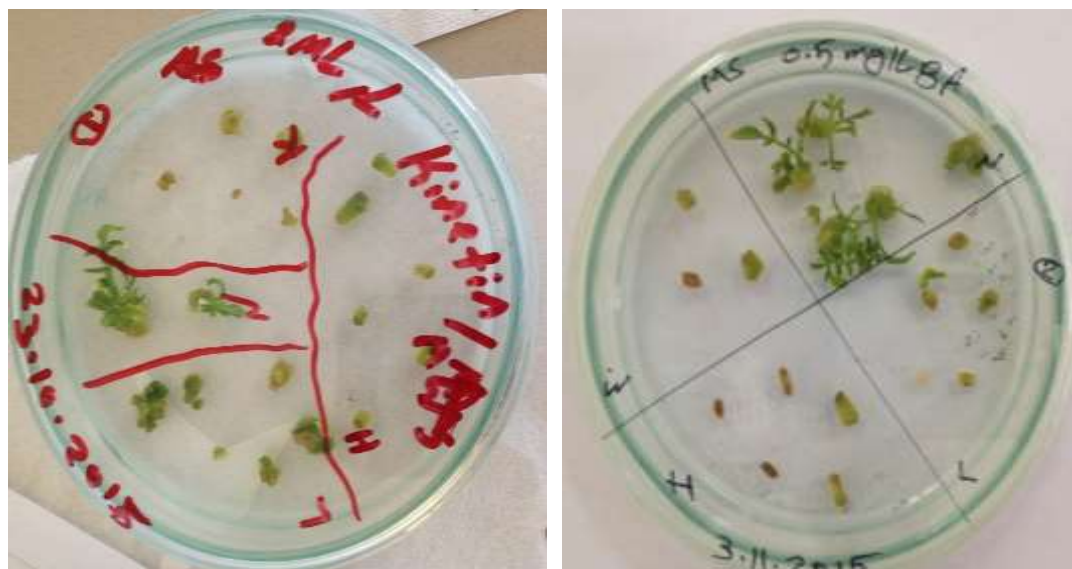

Figure 4. 2. Shoot and callus formation in kinetin and BAP hormones.

Different concentration of PGRs affect shoot germination in the different explant type. As shown in Table 4.2: In shoot tip (ST) explant, the highest number of shoot formation was provided in MS medium supplemented with 4 mg/l NAA + 2 mg/l BAP, 0.5 mg/l 2,4-D + 0.8 mg/l BAP (100.00% adventitious shoot regeneration); subsequently, 2 mg/l 2,4-D supplemented MS medium (88.88 %). The lowest number of adventitious shoot formation was in MS medium supplemented with 2 mg/l IAA, 4 mg/l NAA, 4 mg/l BAP, 4 mg/l Kinetin (75.00 % adventitious shoot regeneration). But in node explant the highest number of shoot formation was observed in MS medium supplemented with 2mg/l IAA, 4 mg/l NAA, 4 mg/l BAP, 4 mg/l Kinetin (90.00% adventitious shoot regeneration), Subsequently, 2 mg/l 2,4-D (85.00 %), 0.5 mg/l 2,4-D + 0.8 mg/l BAP (66.66 %), 4 mg/l NAA + 2 mg/l BAP (37.5 %), 0.5 mg/l IAA (25.00 %). In the hypocotyl explant only 0.5mg/l 2,4-D + 0.8mg/l BAP supported MS medium produced adventitious shoot (08.33 %). But in leaf explant shoots were not produced.

Table 4. 2. The Effect of different PGR concentration and different explant on shoot formation.

PGR Concentration Mg/L	ST explant	node explant	leaf explant	hypocotyl explant
	Shoot formation %	shoot formation %	shoot formation %	Shoot formation %
2 2,4-D	88.88	85.00	0.00	0.00
4 NAA + 2 BAP	100.00	37.50	0.00	0.00
0.5 2,4-D + 0.8 BAP	100.00	66.66	0.00	8.33
2 IAA , 4 NAA 4 BAP, 4Kinetin	75.00	90.00	0.00	0.00
0.5 IAA	0.00	25.00	0.00	0.00

ST = Shoot tip, 0.00 = shoot formation not occurred

It means that shoot formation occurred in ST and node explant more than other explants Such as a leaf or hypocotyl explants (Figure 4.3).
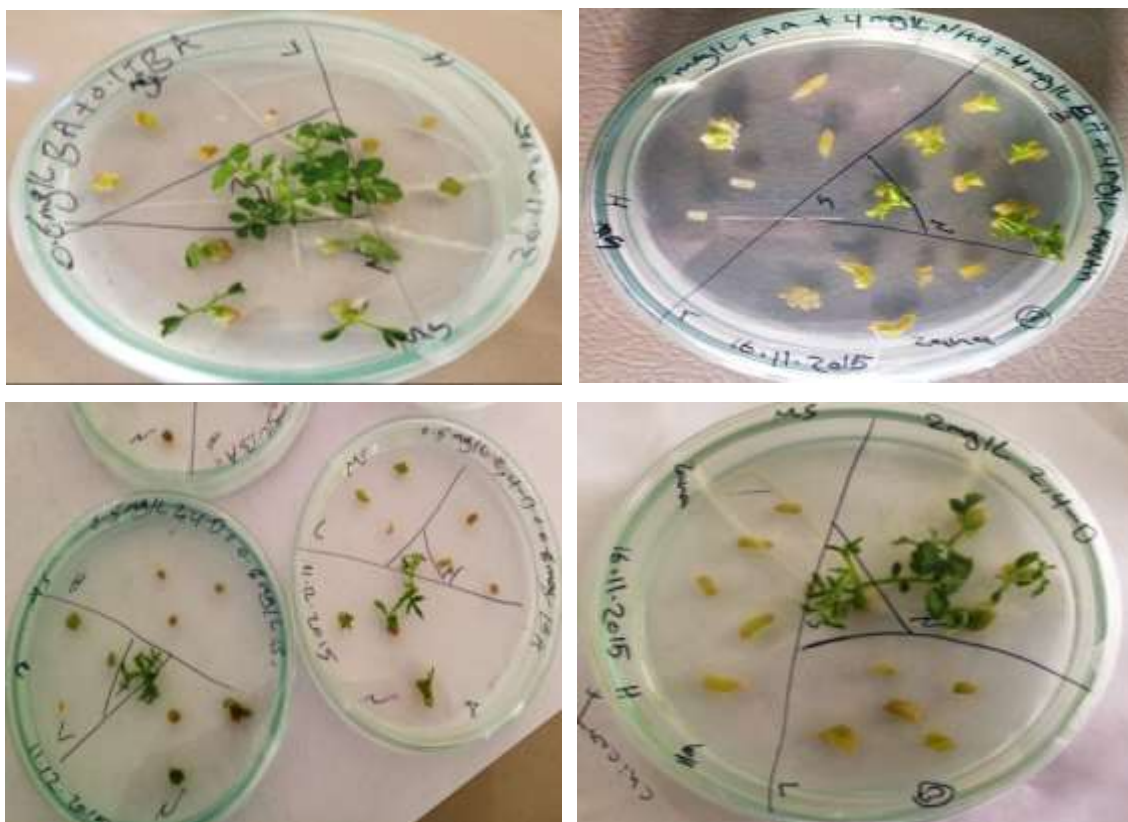
Figure 4. 3. The Effects of different hormone concentration and explant types on shoot formation.

Node explant was applied for sub culturing in BAP and combination with 2,4-D once for direct organogenesis (Figure 4.4). The results are listed in the following: The highest number of shoot formation was in 0.5 mg/l BAP it was (90.00%), subsequently, 0.80 mg/l BAP+0.50 mg/l 2,4-D (66.66 %), followed by 2 mg/l BAP (50.00 %), 3 mg/l BAP (33.33 %).
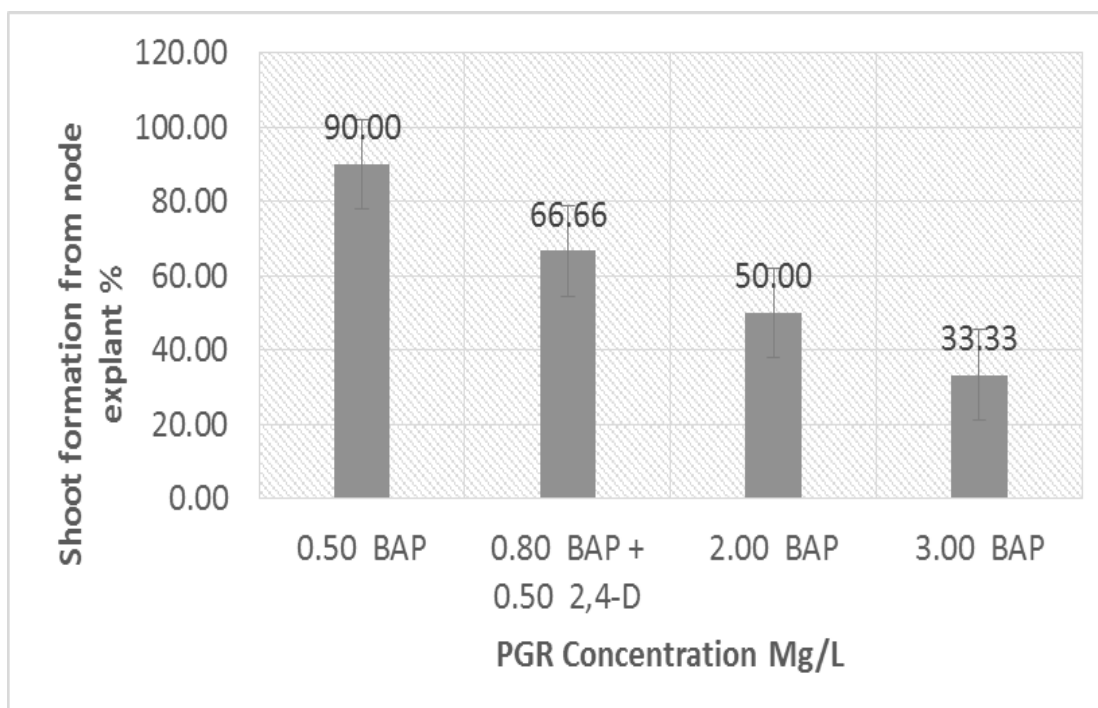
Figure 4. 4. The Effects of BAP on shoot formation from node explant.

4.3. Callus response (indirect organogenesis)

Different explants were cultured for indirect organogenesis, like leaf, node and hypocotyl explant. In different PGRs concentration, callus were produced in various amounts (Table 4.3). In leaf explant, the highest number of callus formation was produced in MS medium supplemented with 2mg/l Kinetin (72.00%), subsequently, 0.5 mg/l 2,4-D (66.66%). 1 mg/l 2,4-D (60.00 %), 2 mg/l IAA+ 4 mg/l NAA+ 4 mg/l BAP+ 4 mg/l Kinetin (40.00 %), followed by 2 mg/l 2,4-D (30.00 %), The lower number of callus was provided from MS medium supplemented with 0.25 mg/l 2,4-D (20.00 %) and lowest 0.5 mg/l BAP (10.00 %). In hypocotyl explant the results were listed. MS medium supplemented with 0.5 mg/l 2,4-D (83.33 % callus formation), 2 mg/l Kinetin (75.00%), 1mg/l 2,4-D (55.00 %), 2 mg/l 2,4-D (40.00 %), 0.25 mg/l 2,4-D (15.00 %), 2 mg/l IAA+ 4 mg/l NAA+ 4 mg/l BAP+ 4 mg/l Kinetin (10.00 %). In node explant the results were listed. The highest number of callus has been observed in MS medium supplemented with 1 mg/l 2,4-D (50.00 % callus formation), subsequently, 2 mg/l kinetin (40.00%), 2 mg/l 2,4-D (33.33 %), 2 mg/l IAA+ 4 mg/l NAA+ 4 mg/l BAP+ 4 mg/l Kinetin, 0.5 BAP (10.00 %) (Table 4.3).

Table 4. 3. Different PGR concentration and explant effect callus formation.

PGR Concentration Mg/L	leaf explant	node explant	hypocotyl explant
	callus formation %	callus formation %	callus formation %
0.25 2,4-D	20.00	0.00	15.00
0.5 2,4-D	66.66	0.00	83.33
1 2,4-D	60.00	50.00	55.00
2 2,4-D	30.00	33.33	40.00
0.5 BAP	10.00	10.00	0.00
2 Kinetin	72.00	40.00	75.00
2 IAA , 4 NAA 4 BAP , 4 Kinetin	40.00	10.00	10.00

0.00 = callus not produced in this explant.

In indirect organogenesis, callus was produced from explants (Figure 4.5). Then the shoots was initiated from calli by different culturing conditions to regenerate plantlets.
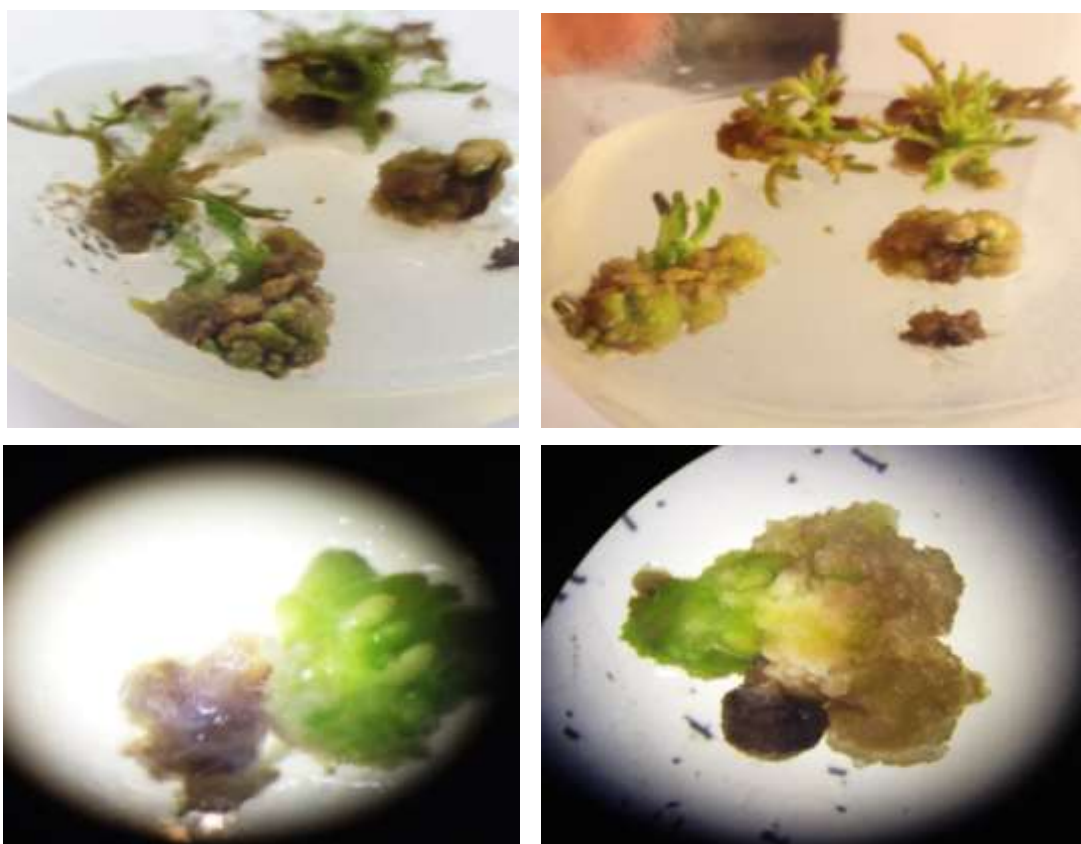

Figure 4. 5. The Effects of different PGRs on callus formation.

After producing callus from different explant in indirect organogenesis, it was applied to produce adventitious shoot and root for subculturing in different 2,4-D concentrations (Figure 4.6). Callus was initiated and grown with different concentration and combinations of PGRs. The results were listed in the following. The highest number of callus formation was in MS medium supplemented with 0.50 mg/l 2,4-D it was (75.00 % callus formation), subsequently, 1mg/l 2,4-D it was (66.66 %), 2mg/l 2,4-D (35.00 %), 0.25mg/l 2,4-D (18.00 %) (Figure 4.6).
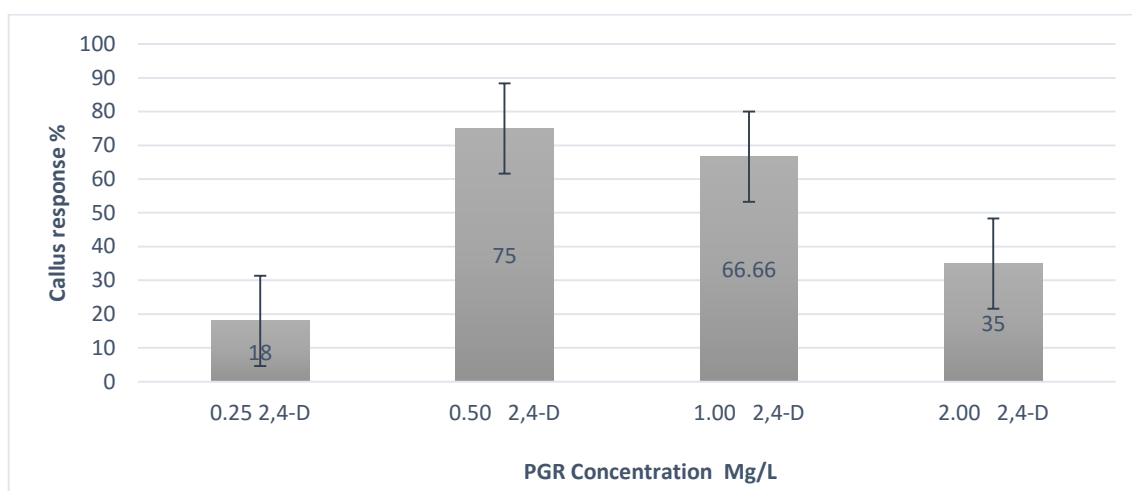
Figure 4. 6. The Effects of 2, 4-D on callus formation.

Different types of explant were applied in different PGRs concentrations and combinations. After 10 -12 days, shoots and roots were produced together. The results are listed in the following Table 4.4. In the ST explant, the highest number of shoot formation was produced in MS medium supplemented with 0.6 mg/l BAP +0.5 mg/l IBA (100.00 %), subsequently, 0.6 mg/l BAP+ 0.1 mg/l IBA (85.00 %), 1 mg/l BAP + 0.5 mg/l IBA (80.00%), 0.3 mg/l BAP + 0.5 mg/l IBA (75.00%). But the highest number of root formation was produced in MS medium supplemented with 1 mg/l BAP + 0.5 mg/l IBA (100.00%), subsequently, 0.6 mg/l BAP+ 0.1 mg/l IBA, 0.3 mg/l BAP + 0.5 mg/l IBA (50.00 %), in 0.6 mg/l BAP + 0.5 mg/l IBA roots were not produced. In hypocotyl explant neither shoots produced nor roots. In node explant the highest number of shoot formation was produced in MS medium supplemented with 0.6 mg/l BAP + 0.5 mg/l IBA (100.00 %), subsequently, 0.6 mg/l BAP+ 0.1 mg/l IBA, 0.3 mg/l BAP + 0.5 mg/l IBA

(75.00 %), 1 mg/l BAP + 0.5 mg/l IBA (50.00%). In leaf explant shoots were not produced. But root was produced in MS medium supplemented with 1 mg/l BAP + 0.5 mg/l IBA (25.00%) (Table 4.4).

Table 4. 4. The Effects of different PGR and PGR concentration and explants on shoot and root formation.

PGR concentration Mg/L	ST explant		hypocotyl explant		node explant		leaf explant	
	shoot %	root %	shoot %	root %	shoot %	root %	shoot %	root %
1 BAP + 0.5 IBA	80.00	100.00	0.00	0.00	50.00	25.00	0.00	25.00
0.6 BAP+ 0.1IBA	85.00	50.00	0.00	0.00	75.00	0.00	0.00	0.00
0.6 BAP + 0.5 IBA	100.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
0.3 BAP + 0.5 IBA	75.00	50.00	0.00	0.00	75.00	50.00	0.00	0.00

ST = Shoot tip, 0.00 = shoots or roots not produced.

In some explants shoots and roots were produced together (Figure 4.7). PGRs types and concentrations took a role to motivate different explant to form shoots and roots.
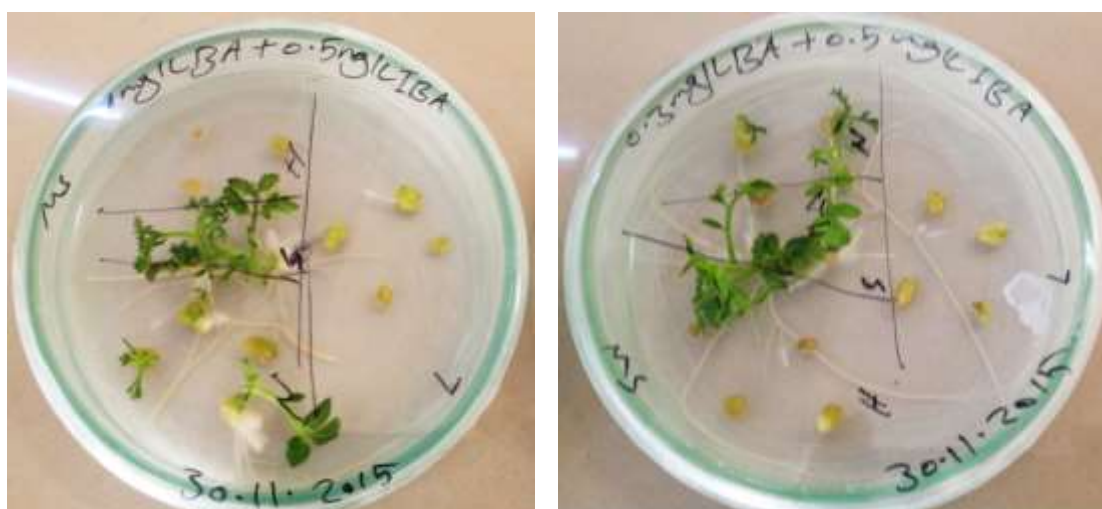

Figure 4. 7. The Effects of PGR type and concentration on shoot and root formation.

After producing shoots from direct (direct shoots from explant) and indirect (callus) organogenesis, all shoots were transferred to rooting media to produce new plantlets. The results are listed in the following Table 4.5. In ST explant, the highest number of root produced in MS medium supplemented with 0.5 mg/l IBA + 0.5 mg/l IAA (100.00 %), subsequently, 0.5 mg/l IAA (50.00 %). In 1 mg/l IAA, 1 mg/l NAA, 1 mg/l IBA+2 mg/l NAA supplemented MS medium root was not produced. In node explant, the highest number of root formation was indicated in MS medium supplemented with 0.5 mg/l IBA + 0.5 mg/l IAA (100.00%), subsequently, 1 mg/l IBA+2 mg/l NAA (62.50 % adventitious root formation), 0.5 mg/l IAA (50.00 %), 1 mg/l IAA (40.00 %), 1mg/l NAA (22.00 %). Leaf explant in MS medium supplemented with 1mg/l IBA+2 mg/l NAA produced 25.00 % of adventitious root.

Table 4.5. The Effects of different PGR concentration and explants on root formation.

PGR concentration Mg/L	ST explant	node explant	leaf explant
	Root formation %	Root formation %	Root formation %
0.5 IAA	50.00	50.00	N
1 IAA	0.00	40.00	N
1 NAA	0.00	22.00	N
0.5 IBA + 0.5 IAA	100.00	100.00	N
1 IBA + 2 NAA	0.00	62.50	25.00

N = this type of explant not used in the concentration, 0.00 = roots were not produced

All grown shoots from direct and indirect organogenesis were transferred to different glass bottles (Figure 4.8). That plantlets were grow well and root formation was occurred.

Figure 4. 8. Root formation in different hormone concentrations.

4.4. Acclimatization

After shoot and root regeneration from different explants, plantlets were transferred into the pots (Figure 4.9) containing 1/3 sand and 2/3 soil and covered by polyethylene to remain the moisture in the high level for acclimatization to the different field condition. After two-three weeks plantlets were acclimatized to the field conditions, and 60.00 % of plants were remain alive and grown ambient natural conditions.
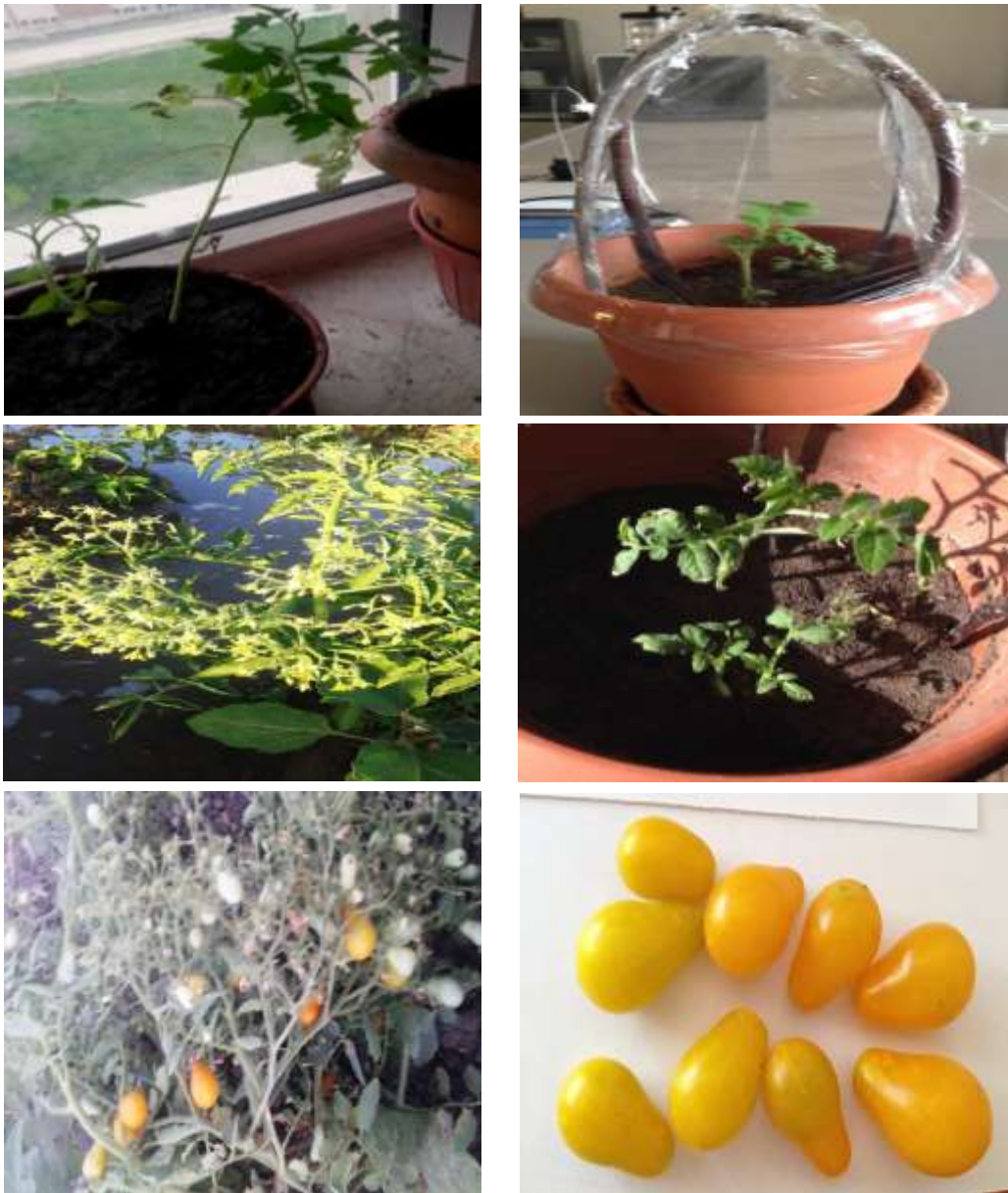

Figure 4. 9. Acclimatization of Plantlets in natural environment.

5. DISCUSSION AND CONCLUSION

Yellow tomato plants with different types of explants were used in the organogenesis, as shoot tip, node, inter node, hypocotyl, cotyledon, leaf. Seeds were cultured in tow type of media, PGRs free MS and white media, in white medium seeds were germinated earlier than MS Medium. The germination ratio in white medium (83.33 percent), more than the germination ratio in MS Medium which it was (67.6 percent), of the seeds germinated. So, the color of seedlings in MS Medium was darker than in white medium. It was because, the composition of macro, micronutrients, vitamins and organics were high and suitable for the bulk of the plant species, in Murashige and Skoog media. But, the micro and macro nutrients, vitamins in Murashige and Skoog media more concentrate than in white medium. It could be inhibited seed germination. Thus, MS Media widely used to explant culture more than other hormones.

Node explant applied for subculturing in BAP and in combination with 2,4-D once for direct organogenesis (figure 4.4). The results were that: The highest number of shoot formation was in 0.5mg/l BAP it was (90.00 % shoots). Similar results have also been reported (Roy, 2016). The node explants cultured on MS supplemented with different concentrations of BAP, KN and NAA alone or in various combinations for multiple shoot regeneration. The best shoot induction rate was observed in nodal segment explant and it was 88% at with BAP in MS medium. It is concluded that BAP combination is more effective on shoot proliferation from nodal explant. Ling et al., 2013 reported that the regeneration was observed from all types of explants but the best response was from shoot tip and nodal explants. The reason of such a result might be due to the meristematic region on shoot tip and nodal segment.

The lowest number of shoot formation from the node has been observed in 3mg/l BAP it was (33.33 % shoots). The increase in concentration of BAP alone from 0.5 to 2.0 mg/l in the MS medium enhanced the shoot proliferation. The concentrations had a less significant effect on the multiple shoot formation in comparison to the combine effect of BAP and NAA. MS medium supplemented with only NAA was also not effective for shoot development in comparison to different concentrations of BAP alone as well as different combination treatment of BAP and NAA. (Pradhan et al., 2013). Medium

supplemented with BA resulted in development of well-differentiated shoots from the axillary bud. The length of the shoot decreased with increasing concentration of BA (Sujatha et al., 2005).

Different concentrations of hormones affected shoot germination in the different explant type. As shown in Table 4.2. In shoot tip explant, the highest number of shoot formation has been observed in 4mg/l NAA + 2mg/l BAP, 0.5mg/l 2,4-D + 0.8mg/l BAP it was (100.00 %). The data is higher than the results reported by Roy, 2016. Shoot tip explants was cultured on MS medium supplemented with different concentrations of BAP, KN and NAA alone or in various combinations for multiple shoot regeneration. The best shoot induction was observed in shoot tip explant (83%) in MS medium supplemented with BAP. The reason of the result is supposed to be the physiological properties of shoot formation of explant types are differences in types of plant. The highest number of callus in Hypocotyl explant was provided from MS medium supplemented with 2mg/l KN 83.33%). Near the results have also been reported by Khaliluev et al., 2014. That, the frequency of callus formation exceeded 95% during the cultivation of hypocotyl segments of the studied tomato genotypes in most variants of culture mediums containing plant growth regulators.

Callus response and callusing index were markedly affected by both the types of explant and PGRs used. Different concentrations of auxins and cytokinins either alone or in combinations had a discrete effect on callus induction. *In vitro* morphogenetic responses of cultured plants are affected by different components of the culture media and therefore, it is important to evaluate their effects on plant callus induction and regeneration (Gubis et al., 2004; Osman et al., 2010).

Explants from several pieces of large intact plants can be employed to form callus. The most successful explants are often young tissues of one or a few cell types. Pith cells of young stem are usually a dependable source of explant material (Mineo, 1990).

In cotyledon explant, the highest callus induction was found in MS medium supplemented with 2mg/l KN (16.66 % callus). Similar results have been reported by Kumari et al., 2008. Cotyledon explants showed best callus induction. Individual treatment of KN and BA showed low response for callus induction.

In the shoot tip explant, the highest number of root formation was observed in MS medium supplemented with 1mg/l BAP + 0.5mg/l IBA (100.00 %). Similar results have also been reported by Roy, 2016. The developed and elongated shoots were excised and implanted in the rooting medium containing half strength MS with different concentrations and combinations of IBA, IAA and NAA. The best effect was obtained in half-strength MS supplemented with IBA. In this combination, it was noted that 90% shoots were rooted. Purkayastha et al., (2008) reported that IBA was more effective for *in vitro* rooting of *Andrographis paniculata*. They observed that MS supplemented with IBA was suitable combination for best rooting.

In leaf explant the highest number of callus formation has been observed in MS medium supplemented with 2mg/l KN (72.00%). Different effects have been reported by Ling et al., 2013. Among the PGRs tested, only IBA treatments were successful in expressing different degrees of callusing whereas KN failed to stimulate any callus formation from the leaf explants. MS medium supplemented with IBA showed the optimal callus induction from the leaf explants. The highest percentage of callus formation from the leaf explants ware 72.34% in the MS medium supplemented with IBA. It was because the morphogenic callus formation depends also on the type of explant and on the stage of development. At the same time, its origin, physiological age, and size are all important as well. It is believed that young, poorly differentiated tissues containing an increasing number of competent cells have a morphogenetic ability (Chandel and Katiyar, 2000; Bhatia et al., 2004).

All shoots were transferred to rooting media to producing new plantlets. In node explant, the highest number of root formation was in MS medium supplemented with 0.5mg/l IBA + 0.5mg/l IAA (100.00%). Similar effects have also been reported by Osman et al., 2010. The induction of roots on regenerated shoots is essential for successful establishment of the plantlet on the soil. 100% rooting was obtained in all hormones supplemented half strength MS medium. The best tomato rooting medium turned out to be half strength MS supplemented with any of the synthetic hormones.

The auxins facilitate the cell division and root differentiation. The cytokinins induce cell division and specialization. Auxins stimulates special pumps in the cell membrane of target cells to release H ions into the cell wall, resulting in a pH drop to approximately 5.0 in the cell wall. Enzymes that are pH-dependent then break down

important structural bonds between cellulose microfibrils causing an increase in cell wall plasticity. As the cell wall becomes more plastic, water is able to flow in and the cell enlarges. Auxin also may have an effect on transcription of nuclear DNA that can contribute to cell enlargement. It is well established that in stems, leaf-veins, petioles and coleoptiles the auxin always moves in a predominantly polar direction, i.e. from apex to base. This polarity has an internal, and not an external, cause, and is responsible for many morphological effects. (Thimann, 1939).

Cytokinins work synergistically with auxin in the control of tissue and organ differentiation. Cytokinins are produced in root tips and may be transported in the xylem toward the shoot. Conclusions about the biological functions of cytokinins have mainly been derived from studies on the consequences of exogenous cytokinin application or endogenously enhanced cytokinin levels. Exogenous applications of cytokinins induce cell division in tissue culture in the presence of auxin. This also occurs endogenously in crown gall tumors on plants. The presence of cytokinins in tissues with actively dividing cells (e.g., fruits, shoot tips) indicates that cytokinins may naturally perform this function in the plant (Davies, 2010).

The average ratio of auxin and cytokinin promotes callus induction, while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration. Both cell division and cell expansion occur in actively dividing tissue, therefore the cytokinin and auxin balance plays a function in the overall development of plant tissue. The single factor determining organ initiation was the comparative quantities of auxins and cytokinins. When tissues *in vitro* did not seem to require an exogenous supply of auxin and cytokinin, it may be due to the sufficient endogenous levels of hormones existed in the culture system for organogenesis (Ling et al., 2013).

After two-three weeks of acclimatization some of the plantlets were acclimated and grown. 60.00 % of plants were remain alive and raised. Near results also has been reported by Aggarwal et al., 2012.

With the present study a protocol was firstly reported for *in vitro* regeneration of yellow tomato. The effects of medium, explant and PGRs were reported. The plant was acclimated and hardened in field and natural environment. For further study a method for large scale production can be developed and a mass production of plant might be achieved.

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GEVİŞLETİLMİŞ TÜRKÇE ÖZET

Tezin Adı: *Lycopersicon esculentum* Mill. (Sarı Domates) Bitkisinin Doku Kültüründe Mikroüretimi

Yüksek Lisans Öğrencisi: Zana Abdalrahman Jawad

Danışman: Prof. Dr. Musa TÜRKER

Bu çalışmada Kuzey Irak'ta ve Türkiye'de nadir olarak yetişen ve soyu tükenme tehlikesi altında olan Sarı Domates bitkisinin Doku Kültüründe yetiştirilmesi ile ilgili bir protokol geliştirilmeye çalışılmıştır. Besi ortamı, eksplant ve Bitki Büyüme Düzenleyicilerinin (BBD) bitkinin laboratuvar ortamında geliştirilmesi üzerine etkileri araştırılmıştır.

Domates, insan beslenmesinde önemli yeri olan bir sebzedir. Açık alanda ve seralarda kültürü yapılmaktadır. Sarı meyveler genelde küre şeklinde ve altın sarısı renktedir. İnce ve etli bir kabuğa sahiptir ve yüksek miktarda su içermektedir. Hafif asidik ve lezzetli bir aroması vardır. İç kısmımda yenebilir çekirdek bulunur, bu da güvenilir bir bitki olduğunu göstermektedir. Büyük bir sarı domates yaklaşık yarım kilogram kadar ağır olabilir.

Açık alanda yaz aylarında sıcak bölgelerde seralarda yıl boyunca üretilir Sarı domates *Lycopersicon esculentum* Mill. bilimsel adıyla bilinen domatesin bir çeşididir. Solanaceae familyası üyesidir. Dik, yayılıcı ve otsu bir sebzedir. Yiyecek olarak sebze amacıyla kullanılırlar. İlk kullanılışı taze yenmek şeklinde sandviç ve salata yapımında önemli yeri vardır. Kırmızı domates yerine tüm yemek tariflerde taze sıcak ya da soğuk olarak salça reçel ve ketçap yapımında kullanılır. Aynı zamanda çorba yapımında kullanılır. Sitrus türleriyle, deniz ürünleriyle, kalamar, kızartma ve ızgara etlerle, zeytinyağı ve sirkeyle salatalarda, yumurta, krema, avokado, soğan, fındık ve naneyle kullanılır.

Ekvator, Peru ve Şilini Andean tepelerine özgüdür ancak kesin yeri tanılanamamıştır. Sarı domates en erken evcilleştirilmiş/kültürü yapılmış bir domates çeşididir. Başta Meksika yerlileri olan aztekler tarafından kültürleri yapılmıştır.

Genel domates büyüklüğüne uygun orta büyüklükte, fazla iri ve küçük olmayan meyve yapısına sahiptir. Ama kültürde istisnai olarak iri ve küçük boyutta (şeri domates)

üretimi gerçekleştirilmiştir. Lezzetlidir, hafif tatlıdır, olgunlaşmadan önce şekil olarak altın çileğe benzetilir. Bu nedenle bazı Avrupa ülkelerinde aynı ad ile anılır. Salatalar için idealdir.

Tohum çimlenmesi

Bitki tohumları Kuzey Irak bölgesinden temin edilmiştir. Yüzüncü Yıl Üniversitesi, Moleküler Biyoloji ve Genetik Bölümü, Bitki Biyoteknolojisi Laboratuvarında çimlendirilmiş, çiçek ve meyve döneminde Biyoloji Bölümü, Botanik ABD öğretim üyeleri tarafından *Lycopersicon esculentum* Mill. Olarak teşhisi yapılmıştır. Bazı kaynaklarda *Solanum lycopersicum* L. sinonium ismi kullanılmaktadır.

Deneysel çalışmalarda tohumlar MS (Murashige and Skoog 1962) basal besi ortamı (MS 0), ve white basal besi ortamında (WM 0) Bitki Büyüme Düzenleyicileri (BBD) kullanılmaksızın steril ortamda çimlendirilmiştir. Çimlenme deneylerinde en az üç tekrar olmak üzere onlarca tohum çimlendirilerek yüzde çimlenme hesabı yapılmıştır. Buna göre WM 0 ortamında çimlenme yüzdesi % 83.3 iken bu oran MS 0 ortamında 67.6 dır (Şekil 4.1).
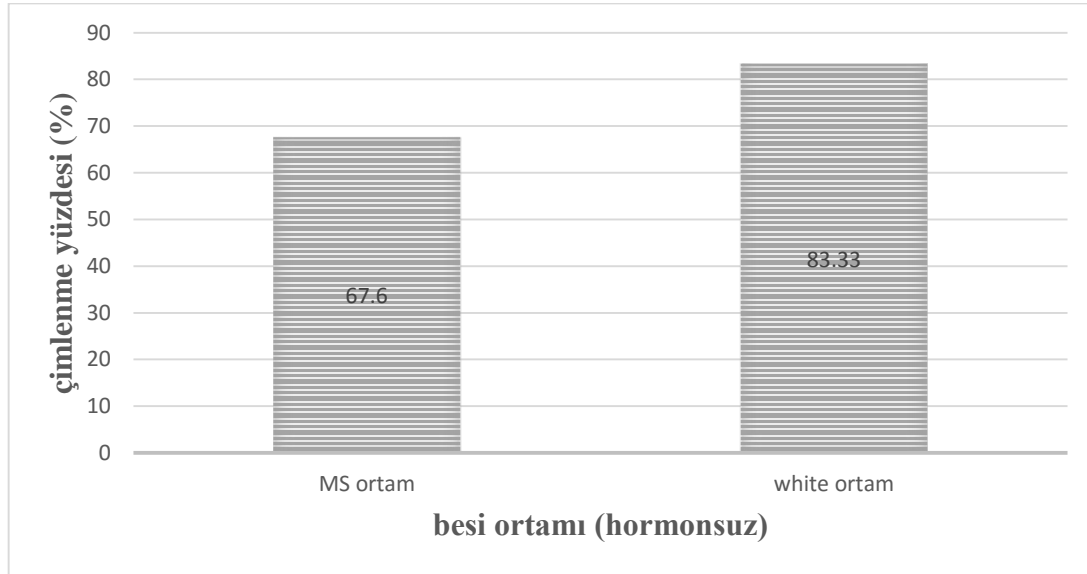
Şekil 4.1 Sarı Domatesin hormonsuz MS ve White ortamlarında çimlenme yüzdesi

Sürgün Gelişimi (direkt organogenez)

BBD den ari yani hormonsuz ortamda çimlendirilen fidelerden elde edilen hipokotil, yaprak ayası, kotiledon, nod ve internod eksplantları farklı konsantrasyon ve kombinasyonlarda Kinetin (K), Benzil Amino Pürüin (BAP), Naftalen Asetik Asit (NAA) ve 2,4-Dikloro Fenoksi Asetik Asit (2,4-D) ilave edilmiş ortamlara ekilmiş ve Tablo 4.3 te belirtilen sonuçlar elde edilmiştir. Buna göre; en yüksek sürgün gelişimi % 100 başarı ile nod eksplantlarının ekildiği ve 2 mg/l BAP+1mg/l NAA, 0.5 mg/l BAP BBD'ler ile takviye edilmiş, MS ortamlarından elde edilmiştir. Daha sonra %80 başarı ile hipokotil eksplantından, 2 mg/l Kinetin ile desteklenmiş MS ortamından elde edilmiştir.

BAP ve NAA ile desteklenmiş ortamlarda %50 oranında sürgün gelişimi gözlenmiştir. En yüksek kallus gelişimi hipokotil eksplantından 2 mg/l Kinetin ile desteklenmiş ortamdan %66.66 oranında elde edilmiştir. 2mg/l BAP + 0.5mg/l NAA destekli ortamda %25 oranında kallus elde edilmiştir. 0.5mg/l BAP destekli ortamda %20 oranında kallus elde edilmiştir.

Nod eksplantında ise en yüksek kallus 3mg/l BAP, 1mg/l 2,4-D destekli ortamdan %50 oranında elde edilmiştir. 2mg/l Kinetin destekli ortamda %40, 0.5mg/l NAA destekli ortamda %20 oranında kallus elde edilmiştir.

Tablo 4.3. Farklı hormon konsantrasyon ve kombinasyonlarının farklı eksplantlar üzerinde sürgün ve kallus oluşumuna etkileri

Hormon Konsantrasyonu Mg/L	Hipokotil		Yaprak		Kotiledon		Nod		Internod	
	kallus %	sürgün %	kallus %	sürgün %	kallus %	sürgün %	kallus %	sürgün %	kallus %	sürgün %
2 kinetin	83.33	0.00	75.00	0.00	16.66	0.00	40.00	80.00	50.00	0.00
2 BAP +0.5NAA	25.00	0.00	0.00	0.00	0.00	0.00	20.00	50.00	N	N
2 BAP+1NAA	66.66	0.00	0.00	0.00	0.00	0.00	0.00	100.00	50.00	0.00
3 BAP	0.00	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00
1 2,4-D	0.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00
0.5 BAP	20.00	0.00	50.00	0.00	N	N	0.00	100.00	42.85	0.00

Kallus oluşumu (indirekt organogenez)

Eksplantlardan doğrudan sürgün veya kök elde edilirse bu oluşum direkt organogenez olarak adlandırılır. Eğer önce kallus oluşup, kallustan kök veya sürgün gelişirse bu oluşuma da indirekt organogenez denir.

İndirekt organogenez çalışmaları Tablo 4.5'te verilmiştir. Buna göre en yüksek kallus gelişimi %72 ile yaprak eksplantında 2mg/l kinetinle desteklenmiş ortamdan elde edilmiştir. Daha sonra sırasıyla %66.66 ile 0.5mg/l 2,4-D, %60 ile 1mg/l 2,4-D, %40 ile 2mg/l IAA+ 4mg/l NAA+ 4mg/l BAP+ 4mg/l kinetin ile desteklenmiş ortamlardan elde edilmiştir.

Hipokotil ekspantında ise en yüksek kallus oranı %83 ile 0.5mg/l 2,4-D, %75 ile 2mg/l kinetin, %55 ile 1mg/l 2,4-D ve %40 ile 2mg/l 2,4-D destekli ortamlardan elde edilmiştir.

Tablo 4.5 Farklı hormon konsantrasyon ve kombinasyonlarının farklı eksplantlarda kallus oluşumuna olan etkileri

BBD konsantrasyon Mg/L	yaprak	nod	hipokotil
	kallus cevabı %	kallus cevabı %	kallus cevabı %
0.25 2,4-D	20.00	0.00	15.00
0.5 2,4-D	66.66	0.00	83.33
1 2,4-D	60.00	50.00	55.00
2 2,4-D	30.00	33.33	40.00
0.5 BAP	10.00	10.00	0.00
2 Kinetin	72.00	40.00	75.00
2 IAA , 4 NAA 4 BAP , 4 Kinetin	40.00	10.00	10.00

Direk ve indirekt sürgün gelişimlerinden sonra sürgünler köklendirme ortamlarına transfer edilerek fideler elde edilmiştir (Tablo 4.7).

Hipokotil eksplantından en yüksek kök gelişimi %100 oranında 0.5mg/l IBA + 0.5mg/l IAA ile desteklenmiş ortamdan elde dilmiştir. Daha sonra %50 ile 0.5mg/l IAA destekli ortamdan elde edilmiştir. IBA ve NAA destekli ortamlardan kök elde edilememiştir.

Nod eksplantından en yüksek kök gelişimi %100 oranında 0.5mg/l IBA + 0.5mg/l IAA destekli ortamdan elde edilmiştir. Daha sonra %62.5 oranında 1mg/l IBA+2mg/l NAA, %50 oranında 0.5mg/l IAA, %40 oranında 1mg/l IAA, %22 oranında 1mg/l NAA elde edilmiştir. En düşük kök eldesi, yaprak eksplantında %25 ile 1mg/l IBA+2mg/l NAA desteli ortamdan elde edilmiştir.

Tablo 4.7. Farklı hormon konsantrasyonlarının kök gelişimi üzerine etkileri

BBD konsantrasyonu Mg/L	hipokotil eksplantı	nod eksplantı	yaprak eksplantı
	köklenme %	köklenme %	köklenme %
0.5 IAA	50.00	50.00	N
1 IAA	0.00	40.00	N
1 NAA	0.00	22.00	N
0.5 IBA + 0.5 IAA	100.00	100.00	N
1 IBA + 2 NAA	0.00	62.50	25.00

Kallus elde edildikten sonra yapılan alt kültürlerde yeniden kallus rejenerasyonu kültürün devamlılığı için önemlidir. Buna göre Şekil 4.7 de ifade edildiği şekli ile alt kültürlerde en yüksek düzeyde kallus gelişimi %75 oranında 0.50 mg/l 2,4-D desteklenmiş ortamdan elde edilmiştir.
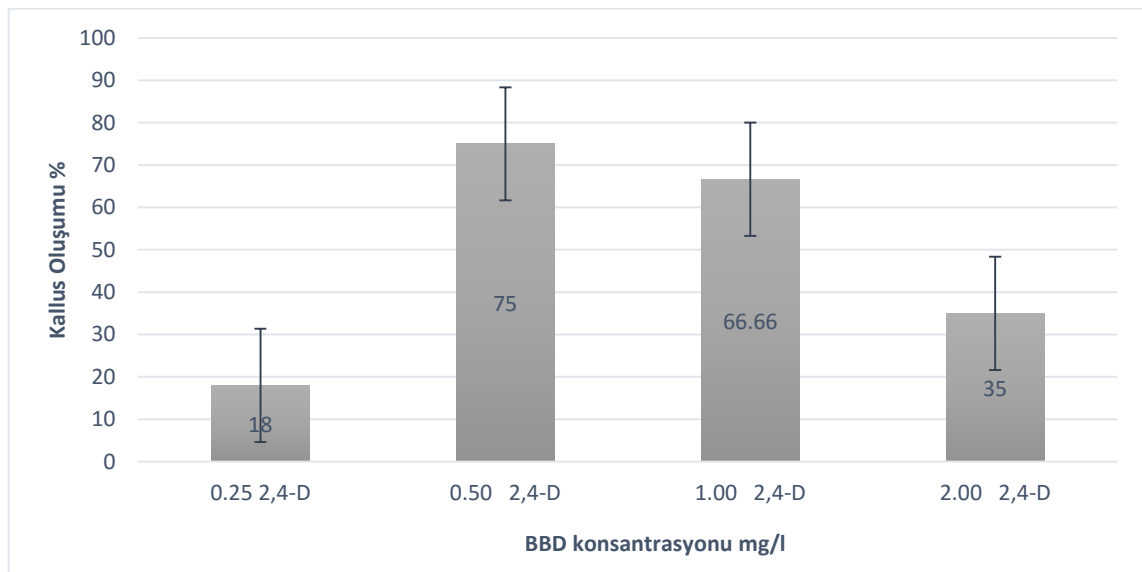
Şekil 4.7 2,4-D homonunun farklı konsantrasyonlarda kallus oluşumu üzerine etkileri

Elde edilen sürgünler köklendirme ortamlarına transfer edilerek köklendirilmiş ve fideler elde edilmiştir. Fideler steril ortamda geliştirilerek toprak içeren saksılara aktarılmış ve steril olmayan dış ortama alıştırılmıştır. Saksılarda domates rejenere edilmiştir.

Sürgün eldesi en başarılı şekilde direkt organogenez yönteminde nod eksplantında, 0.5 mg/l BAP and 2 mg/l BAP+1 mg/l NAA destekli farklı iki ortamda %100 oranında elde edilmiştir. İndirekt organogenez yönteminde hipokotil eksplantından 2 mg/l Kinetin eklenmiş MS ortamında %83.33 oranıda kallus ve kallustan sürgün elde edilmiştir. Gövde ucu ve nod eksplantlarından elde edilen sürgünler 0.5 mg/l IBA + 0.5 mg/l IAA desteklenmiş MS ortamında %100 kök üretmiştir.

CURRICULUM VITAE

The Author was born in Dukan of Sulaimanya, Northern Iraq. He completed primary and secondary education in Hjiawa Town. He graduated from Horticalture Department, College of Agriculture, University of Sulaimanya- Iiraq in 2010.

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