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Faculty of Graduate Studies

In Vitro Regeneration of selected local Tomato Cultivars in Palestine

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Dedication

This thesis is dedicated to my father, mother, brother, sisters and my friends; for their endless love, support and encouragement

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I would like to sincerely thank my supervisor, Dr. Hassan Abu-Qaoud for his guidance and support throughout this study, and especially for his confidence in me.

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I have to thank my parents for their love, support, and help throughout my life. Thank you both for giving me strength, throughout this study. To all my family, thank you for your encouragement and support.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان

***In Vitro* Regeneration of Selected Local Tomato Cultivars in Palestine**

أقر بأن ما اشتملت عليه هذه الرسالة هي نتاج جهدي الخاص ، بإستثناء ما تمت الإشارة إليه حيثما ورد ، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى .

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:**

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Table of Contents

List of Tables

List of Figure

In Vitro Regeneration of Selected Local Tomato Cultivars in Palestine

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Abstract

A study was conducted to investigate the effect of different growth factors on shoot multiplication and regeneration on some tomato cultivars planted in Palestine. Cultivar 593 and Baladi landrace were involved in these experiments with four explants (hypocotyl, cotyledon, stem, and leaf). The effect of different levels and combinations of auxin Naphthalene acetic acid (NAA) and cytokinin Benzyl adenine (BA), Kinetin (Kin) and N-1,2,3-Thiadiazol-5-yl-N'-phenylurea (TDZ) on shoot multiplication and regeneration was examined in this study. Shoot tips from established seedlings were used as source materials for shoot multiplication. The shoot tips were cultured on Murashige and Skoog media (MS medium) with (2.2, 4.4 μM) BA, (9.2, 18.4 μM) Kinetin combined with 0.0, 2.7 μM NAA. The higher shoot number (8.4) was obtained with MS medium supplied with 18.4 μM Kinetin. Low shoots number were observed on MS medium supplied with 18.4 μM Kinetin and 2.7 μM NAA in Baladi cultivar. Direct regeneration with four different explants was obtained on MS basal medium supplemented with TDZ at (0.0, 1.0, 2.0 and 4.0 μM) and NAA at (0.0, 2.7 and 5.4 μM), as well as BA at (0.0 or 2.2 μM) and Kinetin at (0.0, 2.3 μM) in two separated experiments. The highest shoot percentage (62.25%) was obtained with Kinetin and BA at (2.3 and 2.2 μM) respectively. However, when NAA and TDZ were combined, 46.9% shoot

regeneration was achieved with 2.7 and 4.0 µM, respectively. Very low shoot regeneration was observed with all NAA levels combined with 1.0 and 2.0 µM TDZ. High rooting percentage (100%) was obtained when NAA was added to the media at (2.7 and 5.4 µM).

Chapter One

Introduction

1.1 Background

Tomato *(Lycopersicon esculentum* Mill) is the most cultivated vegetable crop in the world, where it is planted in almost 4 million hectares worldwide (Chaudhry *et al*., 2007). In 2009, tomato production was 152 million tons (FAO, 2009). Tomato is a diploid plant (2n=24) belongs to *Solanaceae* family, originated in South America.

Tomato production is affected by various abiotic stresses such as high temperature; drought, and salinity. Tomato is also vulnerable to frequent biotic stresses such as pathogens and insect attacks. During the last two decades many biotechnological approaches were focused on tomato crop improvement (Mandal and Sheeja, 2003). Different biotechnological applications of *in vitro* culture have been used in tomato. The applications aimed at improving production and tolerating pest and diseases (Moghaieb *et al*., 2004), as well as genetic transformation (Ling *et al*., 1998).

In vitro plant regenerations were found to depend on many factors as: genotype, composition of the basic medium, concentration and type of growth regulators, explant type, gelling agent, temperature, light intensity and quality, photoperiod, cultivation vessels and vessel covers (Reed, 1999; Sheeja *et al*., 2004; Bhatia *et al.*, 2004).

For tomato regeneration, wide variety of plant growth regulators were used at varying concentrations. The concentration of growth

regulators employed is depended on the cultivar being cultured and the growth regulators being used. Various hormonal combinations were used to induce callus and regeneration like (BAP and IAA) and (IAA and Kin) (Chen *et al*., 1999). Therefore, The aim of this study was mainly to study the effect of different growth regulator on tomato cultivars grown in Palestine in order to establish an *in vitro* multiplication and regeneration systems of tomato.

1.2 Objectives:

1. To establish an *in vitro* culture system of local tomato cultivars.

2. To Study the effect of plant growth regulators (NAA, Kinetin, and BA) on shoot multiplication of local tomato cultivars.

3. To establish an efficient shoot and root regeneration system of the local tomato cultivars using different explant types and different plant growth regulators (PGR) combinations.

Chapter Two

Literature Review

2.1 Plant tissue culture

Plant tissue culture is a technique of culturing plant cells, tissues and organs on synthetic media under aseptic environment and controlled conditions of light, temperature, and humidity. The development of plant tissue culture as a fundamental science was closely linked with the discovery and characterization of plant hormones, and has facilitated the understanding of plant growth and development. Furthermore, the ability to grow plant cells and tissues in culture and to control their development forms the basis of many practical applications in agriculture, horticulture industrial chemistry and is a prerequisite for plant genetic engineering (Evans *et al*., 2003). Tissue culture techniques for plant micropropagation, genetic transformation, biotech assisted selection, mutagenesis, etc., depends on two fundamental morphogenesis processes: organogenesis where in shoot buds are organized by concerted meristemic activity of a number of cell and somatic embryogenesis where usually single cell or small cluster of cells undergo differentiation to produce somatic embryo similar to zygotic embryos (Gonzalez-Olemedo *et al.,* 2005).

History of plant tissue culture is a record of systematic efforts by botanists to culture excised plant tissues and organs explants to understand their growth and development under controlled conditions (Monnier, 1995).

In 1902 a German botanist, Gottlieb Haberlandt developed the concept of *in vitro* cell culture and he was the first person to culture

different cells, Although, Haberlandt failed to achieve the division of free cells for various reasons, his detailed paper stimulated several workers to pursue this line of investigation (Bhojwani and Razdan, 2004). After the 1920s, the discovery and development of tissue culture techniques continues.

The two important discoveries made in the mid-1930s which gave a big push to the development of plant tissue culture technique were: (a) identification of auxin as a natural growth regulator, and (b) recognition of the importance of B-vitamins in plant growth. (Bhojwani and Razdan, 1996). Jones, (1994) designed a micro-culture method for growing single cells in conditioned medium (medium in which tissue has been grown for some time). The advantage of this technique was that it allowed continuous observation of the cultured cells. Using this technique but replacing the conditioned medium by a fresh medium, enriched with coconut milk, Vasil and Hildebrandt (1965) raised whole plants starting from single cells of tobacco.

Until the mid-1970s hormonal manipulation in the culture medium remained the main approach to achieve plant regeneration from cultured cells and it proved very successful with many species.

During the 1990s, continued expansion in the application of *in vitro* technologies to a larger number of plant species was observed. Tissue culture techniques are being used with all types of plants, including cereals and grasses (Vasil and Vasil, 1994), legumes (Davey *et al.,* 1994),

vegetable crops (Reynolds, 1994), and other root and tuber crops (Krikorian, 1994), plantation crops (Zimmerman and Swartz, 1994), and ornamentals (Debergh, 1994).

2.2: Tomato: Classification, Origin and Taxonomy

Tomato belongs to the family Solanaceae (also known as night shade family), genus *Solanum*, sub family Solanoideae and tribe solaneae (Taylor, 1986). The genus includes a small collection of cultivated and wild species like *S. Lycopersicon* Mill, wild species like, *S. peruvianum, S. hirsutm, S. glandulosam, S. pimpinellifolium, S. cheesemannii.* Tournefort (1694) was the first to name cultivated tomatoes as *Lycopersicon* ("wolf peach" in Greek) (Luckwill, 1943).

Tomato is one of the three most important horticultural crop worldwide (Dorais *et al.*, 2001), the tomato is a perennial but usually grown as an annual plant. Tomato plant can reach up to 3 meters. The stem is somewhat weak and often requires staking or support such as a tomato cage. Branching at the base is monopodia, becomes sympodial higher up. The tomato leaves are 10 to 30 cm long and unevenly imparipinnate compound with variously indented or lobed margins (Tindall, 1983). Both the stems and the leaves are slightly rough and fuzzy. The inflorescence of tomato contains small yellow flowers, each with five pointed lobes on the corolla. The tomato fruit is a fleshy berry, green when unripe and becomes deep red and shiny when ripe. The tomato cultivars differs in fruit size, shape and color. There are also yellow, orange, green and brown varieties

of fruits. The shape can varies from small cherry tomatoes, pear shaped tomatoes to large irregular shaped beef tomatoes. The shape, size and color of tomato decide their market value. Number of processed items prepared on large scale for consumption as well as for export using different varieties of tomatoes.

Tomato is originally from Central and South America (Angole, 2010) and was brought way back to Europe by Christopher Columbus on his second voyage in 1498. The crop probably from Peru-Ecuador area, where it was distributed to many parts of tropical America as a weed, but become domesticated first in Mexico (Tindall, 1993).Tomatoes arrived in West Africa through Portuguese traders, or were brought across the continent from Egypt and then Sudan (Tindall, 1986).

Tomato is rich in vitamins A and C which is a remedy to night blindness, energy values, iron and dietary fiber (Goode, 1989). Also tomato is known as a health stimulating fruit because of the antioxidant properties of their main compounds. The most important antioxidants in tomatoes are carotenes (Clinton, 1998). Among the carotenes, lycopene approximately (20-25mg/100g) of fruit weight and its content varies significantly depending on ripening, variety and environment (Brandt *et al*., 2006). A group of biochemicals in red tomatoes are found to have antioxidant properties which reduces several cancers (Rick, 1980; Giovannucci, 1999).

Previously, tomatoes were grown only during favorable season, but nowadays tomato is grown round the year. Because of its economic

importance area under cultivation is increasing every year; worldwide tomato production in 2005 totaled 29.9 million metric tons and production for the 2005/2006 season in Europe totaled 10.6 million metric tons (FAO, 2006). Yields in Asia are highest in the east where the climate is mainly temperate and sub-temperate.

Tomato (*Lycopersicon esculentum* Mill) is a very important vegetable crop in Palestine. Due to its long production period (9 – 11 month), many farmers depend on as a main crop. In the Palestine it is estimated that 27763 dunums are cultivated with tomato which produce about 212148 tons of tomato (FAO, 2009).

2.3: Tomato Tissue Culture

Many studies were conducted on tomato to establish an efficient system of shoot multiplication and regeneration with growth regulators.

Regeneration on MS medium containing 5.0 mgL^{-1} BAP, using shoot tips as explants. Tomato micro plants growth under less than 10.0 g L^{-1} of sucrose or reduced nutrient salt level in MS medium resulted in reduced micro-plant height and root. As reported by (Schnapp and Preece, 1986). Chandel and Katiyar (2000) finds a media in plant regeneration for tomato using 2,4-D, Kinetin, BAP, NAA and IAA with leaf discs (0.5 cm^2) and shoot explants (1.0 cm). MS medium with 1.5 mgL^{-1} BAP and 1.5 mgL^{-1} IAA was found to be most responsive for organogenesis from 8-12 week old callus cultures from leaf explants. Maximum callus induction frequency

with leaf disc was 97.5% and from shoot explants was 75% with kinetin and NAA and 97.5% and 88.57% with BAP and IAA, respectively.

Venkatachalam *et al*., (2000) recorded that the highest callus induction in tomato from hypocotyls, on MS medium supplemented with NAA (1.0 mgL^{-1}) kinetin and BAP each (0.1 mgL^{-1}). BAP was found the most effective plant growth regulator and multiple shoot induction occurred in these tissues. Rooting of the regenerated shoots readily induced on half-strength MS medium supplemented with IBA (0.1 -0.5 mgL^{-1}).

In 2006, Plana *et al.* reveal that direct regeneration using proximal part of hypocotyl and the radical cultured in tomato on medium consisting of MS salts with 4.0 mgL^{-1} thiamine, 100 mgL^{-1} myo-inositol and 3% sucrose. 60% explants adventitious shoot formation was observed within 4 weeks.

Chaudhry and Malhotra, (2001) determined callogenesis in two cultivars of tomato (Nagina and Feston) on MS medium with 0.5 mgL^{-1} NAA and 2.0 mgL^{-1} BAP. Maximum regeneration was obtained on MS medium with 0.5 mgL^{-1} IAA and 4.0 mgL^{-1} of BAP in Feston using hypocotyls (56%) and leaf disc (42%). However in 2004 Chaudhry reported the regeneration using hypocotyl and leaf discs from 3-week-old *in vitro* seedlings of tomato cultivar Roma. Maximum callus induction 82.5% from hypocotyl and 57% from leaf discs was reported with 2.0 mgL^{-1} of IAA, 2.0 mgL^{-1} of BAP, 2.0 mgL^{-1} of NAA and 4.0 mgL^{-1} of kinetin. Maximum 45.8% and 30.8% regeneration was observed from hypocotyl

and leaf discs respectively on MS medium supplemented with IAA (2.0 mgL^{-1}), BAP (5.0 mgL^{-1}), NAA (2.0 mgL^{-1}) and kinetin (4.0 mgL^{-1}), Chaudhry *et al*., 2004). (Jabeen *et al*., 2005) reported the Effects of genotype and explants type on *in vitro* shoot regeneration in tomato. *In vitro* regeneration frequency of hypocotyls, leaf disc and shoot tip of five tomato cultivars was investigated and maximum regeneration was reported with regeneration medium supplemented with 1.0 mg L^{-1} zeatin and 0.1 mg L^{-1} IAA. The highest regeneration capacity was observed in cultivar Rio Grande (80% by using shoot tip, 64.5% by using hypocotyls and 56% by using leaf disc) from all types of explants.

Botau *et al*., (2002) found that callus was induced and regeneration observed in tomato cultivars (Unirea and Ace Royal) on MS media supplemented with 1.0 mgL^{-1} NAA, 1.0 mgL^{-1} BAP, and 2.0 mgL^{-1} 2,4-D using cotyledons and hypocotyls as an explant. Callus regeneration, multiplication and rooting percentage were higher in Unirea than in Ace Royal.

In another study, Gubis *et al*., (2004) stated that the highest regeneration with zeatin and IAA in tomato was obtained using hypocotyls and cotyledons of 21 days old aseptically grown seedlings.

Costa *et al*., (2000) reported regeneration from cotyledon explants of tomato (*L. esculentum* Mill.) in four cultivars (Santa Clara, 'Firme' mutant, 'IPA-5' and 'IPA-6') of 8 days old *in vitro* grown seedlings. Higher regeneration frequencies and a greater number of elongated shoots were

obtained with IAA and kinetin. Rooting of shoots was positively influenced, both in the presence and absence of time tin.

Plevnes *et al*., (2006) used MS media supplemented with 2.0 mgL^{-1} of IAA and 1.0 mgL^{-1} of BAP for callus induction in cotyledons of tomato cultivar 'Maskotka' and the wild form of *L. peruvianum*.

Regeneration in tomato variety (Justar and Nemador) from leaf and cotyledon explants. Seedlings (3 weeks old) were used and the highest regeneration was observed on MS basal medium supplemented with 1.0 mgL^{-1} zeatin/ 1.0 mgL^{-1} IAA and 2.0 mgL^{-1} of BAP and rooted with 0.1 mgL^{-1} of IAA. Leaf explants showed the most important organogenesis capacity in comparison to cotyledon explants. (Majoul *et al*., 2007).

Harish *et al.*, (2010) studied the efficient in vitro callus induction and regeneration for six different tomato cultivars in India, this study was done as a pre-requisite for genetic manipulation studies to enrich vitamin E in tomato, which ongoing research in the group. The effect of hormones (NAA, IAA), in combinations with (BAP, Kin) at varying concentration were investigated on callus induction frequency. The highest frequency was up to 90% for all the cultivars in 0.5 mgL^{-1} NAA + 2.0 mgL^{-1} of BAP and regeneration was significantly higher with 3.0 mgL^{-1} BAP for all the cultivars.

Chaudhry *et al.*, (2010) reported the callus induction and regeneration in tomato var. Moneymaker. Hypocotyl and leaf disc tomato

explants were culture on MS medium supplemented with IAA, NAA, BAP, and Kin. Leaf disc showed 81.3% but hypocotyl showed 69.2%.

Rashid and Bal, (2010) studied the effect of hormones on direct shoot regeneration in hypocotyl explants of two genotypes of tomato (Punjab Upma and IPA-3), the explants were cultured on MS medium supplemented with different concentrations and combinations of hormones (Kinetin 0.5 mg L^{-1}) and (BAP 0.5 mgL^{-1}).Shoot regeneration percent in 'Punjab Upma' and 'IPA-3' percent was recorded to be highest (86.02) and (82.57) respectively.

Ishag *et al.*, (2009) used Shoot tip and cotyledon explants isolated from 8-10 day old seedlings were aseptically cultured on (MS) medium supplemented with various concentrations and combinations of growth regulators (BA, Kin and 2ip) at various concentrations (0.5 – 5.0 mgL^{-1}) individually or in combination with naphthalene acetic acid (NAA; 0.5 – 1.0 mgL^{-1}) were tested. They found that shoot tip was more efficient than cotyledon explants, and Kin proved to be more effective than BA. But the efficiency of both BA and Kin for multiple shoot induction was negatively affected when combined with NAA.

Rapid regeneration and transformation systems of tomato cultivar have been developed using hypocotyl with a part of cotyledon, after removal of primary meristem, as an explants. The explants were cultured on ten different combinations of hormones and vitamins (1.0 mgL^{-1} BAP, 1.0 mgL^{-1} Zeatin ripozide, 5.0 mgL^{-1} AgNO3 and Nitch and Nitch vitamin).

In addition, transformation system has been established with two different methods, *Agrobacterium* mediated transformation and Biolistic gun as reported by Ghada *et al.*, (2008).

Devi *et al.*, (2008) reported the effect of growth regulators (3.0 mgL^{-1} of BAP, and 2.5 mgL^{-1} of IAA) on *in vitro* morphogenic response of tomato, which was optimum for callus induction, plant regeneration and number of shoots per explants. At higher and lower levels of hormones, a considerable decline was recorded in percent callus induction, plant regeneration and number of shoots per explants.

Chaudhry *et al.*, (2007) recorded that the regeneration capacity is significantly influenced by cultivar and explant type, when optimize a reproducible protocol for callus induction and regeneration of three tomato cultivars and also to select the cultivar which better perform under *in vitro* conditions for further experimentation.

Khan *et al.*, (2006) working with different tomato cultivars (Moneymaker, Packet, Nagina and Aroma) recorded that depending on the cultivar used, shoot regeneration varied on RMOT medium (MS salts supplemented with 1.0 mgL^{-1} Zeatin and 1.0 mgL^{-1} IAA), by regenerated maximum number of shoots from tomato leaves C*v*. Moneymaker, Packet, Nagina and Aroma.

Sheeja *et al*., (2004) reported appropriate levels of gelling agents, agar, phytohormones, carbon sources, glucose and sucrose, adjuvants like

folic acid, biotin and coconut water on regeneration. Use of young hypocotyl enhanced plantlet regeneration and length of plantlets in tomato. N6 medium with 2.0 mgL^{-1} BAP and 0.5 mgL^{-1} kinetin produced maximum shootlets within 20 days, while MS medium produced taller whole plantlets.

Chapter Three

Materials and Methods

3.1: Plant material

Seeds of two tomato cultivars (Baladi and 593) obtained from commercial nursery (Al-Juneidi Modern Nursery) were used in this study.

3.2: Seed sterilization

Seeds of both cultivars were surface sterilized using 20% of Chlorox (sodium hypochlorite) for 15 minutes containing 0.1% (v/v) Tween 20 as wetting agent, then were rinsed three time with sterile distilled water for five minutes every time.

3.3: Medium preparation

Tomato seeds were transferred aseptically to MS medium supplemented with 30 gml^{-1} of sucrose and 0.01 gml^{-1} of myo-inistol, in addition of 8.0 gm of Agar**.** The pH was adjusted to (5.6), and then the medium was sterilized in the autoclave at 121^0C for 21 minutes.

3.4: Establishment of sterile plant material

Tomato seeds were cultured in test tubes containing 10.0 ml of MS basal media. Two seed were planted in each test tube (Baladi and 593). Test tube were transferred to incubator and kept for (15-20) days at 22^0C under 16 h day light of photoperiod illumination of 40 μmol m^{-2} s^{-1} and 8 h dark period.

3.5: Explant preparation

The *in vitro* grown seedlings were used as a source of four types of explants (hypocotyl, cotyledon, stem, and leaf). Explants were excised after 15 – 20 days of germination.

For regeneration experiments, explants were taken from the seedling cultures. The fourth explants were ready to use in regeneration media containing MS basal media supplied with different levels of different growth regulators TDZ, NAA, Kinetin, and BA.

3.6: Multiplication experiment

In this experiment shoots tips of about 2.0 cm length were cut from the establishment seedlings and transferred into hormonal combination with particular media. The cultures were incubated in a growth chamber at 22 ± 1°C for two weeks with 16 h of photoperiod illumination of 40 μmol m^{-2} s^{-1} supplied from cool white fluorescent.

3.6.1: Effect of NAA and Kinetin on shoot multiplication

Shoot explants were excised and cultured on MS (Murashige and Skoog, 1962) medium supplemented with different concentration of Kinetin (9.2, 18.4 μM) in combination with auxin NAA (0.0, 2.7 μM) (Table 3.1).

3.6.2: Effect of NAA and BA on shoot multiplication

Shoot explants were excised and cultured on MS medium supplement with different concentrations of benzyl amino-purine (BA) at (2.2, 4.4 µM) in combination with auxin NAA (0.0, 2.7 µM) (Table 3.2). (Murashige and Skoog, 1962).

After four weeks of incubation, the numbers of shoots were recorded. Optimal hormonal combination was determined based on the result of these variables.

Table 3.1: Concentration of PGRs used for tomato shoots multiplication.

Treatment	Concentration of PGRs (µM)	
	NAA	**Kinetin**
1	0.0	9.2
2	0.0	18.4
3	2.7	9.2
4	2.7	18.4

Table 3.2: Concentration of PGRs used for tomato shoots multiplication

Treatment	Concentration of PGRs (µM)	
	NAA	**BA**
1	0.0	2.2
2	0.0	4.4
3	2.7	2.2
4	2.7	4.4

3.7: Regeneration experiment

In this experiment four different explants from the *in vitro* established plant were used (hypocotyl, cotyledon, stem, and leaf). Each explant was cut into two pieces and cultured into 9 cm diameter petridishes (4 segments for each plate).

3.7.1: Effect of NAA and TDZ on shoot and root regeneration

MS media supplement with two different hormone levels of both auxin (NAA) at (0.0, 2.7, 5.4 µM) levels, and cytokinin (Thidiazuron) (TDZ) at (0.0, 1.0, 2.0, 4.0 µM) levels were used in this experiment (Table 3.3).

3.7.2: Effect of BA and Kinetin on shoot and root regeneration

Explants were cultured on medium supplemented with two cytokinins BA at (0.0, 2.2 µM) and Kinetin at (0.0, 2.3 µM) as shown in (table 3.4).

All cultured plates were incubated under complete dark conditions for 3 weeks, and then transferred in the incubator at 22^{o}C ± 1 and 16 h daylight. After one month each plate was tested for the shoot regeneration and root development.

Table 3.3: Concentration of PGRs used for tomato shoots and roots regeneration.

Treatment	Concentration of PGRs (µM)	
	NAA	TDZ
1	0.0	0.0
2	0.0	1.0
3	0.0	2.0
4	0.0	4.0
5	2.7	0.0
6	2.7	1.0
7	2.7	2.0
8	2.7	4.0
9	5.4	0.0
10	5.4	1.0
11	5.4	2.0
12	5.4	4.0

Table 3.4: Concentration of PGRs used for tomato shoots and roots regeneration.

Treatment	Concentration of PGRs (µM)	
	BA	Kinetin
1	0.0	2.3
2	2.2	0.0
3	2.2	2.3

3.8: Statistical analysis

The treatments in both multiplication and regeneration experiments were considered as factorial treatment design, each combination of the growth regulators in each experiment was considered as a treatment, the treatments were arranged in a completely randomized design with 4.0 replicates in regeneration experiments and 10.0 replicates in multiplication per treatments. Collected variables were summarized and analyzed in one way analysis of variance (ANOVA) using SAS software (SAS, 1990). Comparative analysis were conducted for the significant results using LSD at 0.05 probability.

Chapter Four

Results

4.1: *In vitro* seed germination

After 3 weeks, all seeds germinated successfully onto the basal medium (fig 4. 1). They continued to grow on this media for (3-4) weeks. No contamination was observed.

Fig 4.1 Growing tomato seeds on MS basal media

4.2: Multiplication experiment.

4.2.1: Effect of NAA and kinetin on shoot multiplication.

4.2.1.1: Baladi landrace

The effect of different levels of NAA and Kinetin on the number of adventitious shoot produced of tomato Baladi landrace is shown in table (4.1). Kinetin at different concentrations induced more shoots per explant;

the highest shoot number (8.4) was achieved on medium containing 18.4 µM Kinetin only (Fig 4.2). More shoots were produced when higher levels of kinetin were used. However, when kinetin was combined with 2.7 µM NAA, the shoot number was significantly decreased (3.6).

Fig 4.2 Multiplied shoots obtained on MS media containing 18.4 µM Kinetin.

Table 4.1: The effect of different levels of NAA and Kinetin on shoot number in Baladi landrace.

NAA	Kinetin	Shoot number
0.0	**9.2**	6.0 a
	18.4	8.4 a
2.7	**9.2**	4.6 b
	18.4	3.6 b

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.2.1.2: Cultivar 593

The effect of different levels of NAA and Kinetin on the number of adventitious shoots of tomato '593' is shown in table (4.2). There was no significant interaction between Kinetin and NAA on shoot number; therefore, the effect of each hormone was present separately. When kinetin was used, no significant effect on shoot number was observed (Table 4.2), however, there was a significant difference with NAA, and the highest number of shoots (7.6) was obtained when NAA was not included (Fig 4.3).

Fig 4.3 Shoot multiplication on cultivar 593

Table 4.2: Effect of NAA and Kinetin on shoot multiplication in 593 cultivar.

Concentration of PGRs (µM)	Level	Shoot number
Kinetin	**18.4**	5.68 a
	9.2	6.5 a
NAA	**0.0**	7.6 a
	2.7	4.65 b

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.2.2: Effect of NAA and BA on shoot multiplication

4.2.2.1: Baladi landrace

The effect of different levels of NAA and BA on the number of adventitious shoot produced of tomato 'Baladi' is shown in table (4.3). Both BA and NAA exhibited a significant effect on the shoot number with significant interaction. The highest shoot number (7.6) was obtained on medium containing 2.2 µM BA (Fig 4.4). But this did not differ significantly from medium containing 4.4 µM BA.

The lowest shoot number (3.7) was obtained with medium containing 4.4 and 5.4 µM of BA and NAA respectively.

Fig 4.4 Shoot multiplication obtained on MS media containing 2.2 µM BA.

Table 4.3: The effect of different levels of NAA and BA on shoot number in Baladi landrace.

NAA	BA	Shoot number
0.0	**2.2**	7.6 a
	4.4	5.0 a
2.7	**2.2**	3.9 b
	4.4	3.7 b

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.2.2.2: Cultivar 593

No significant interaction was detected on adventitious shoot formation between BA and NAA; therefore, each factor was presented separately, (Table 4.4). The result showed that at higher concentration of BA and NAA (4.4 and 2.7 µM), respectively, the number of shoots were decreased (4.2, 3.8). The shoot multiplication number was high when 2.2 µM of BA was used (5.7, 5.8) (Fig 4.5).

Fig 4.5 Shoot multiplication on cultivar 593

Table 4.4: Effect of NAA and BA on shoot multiplication in 593 cultivar

Concentration of PGRs (µM)	Level	Shoot number
BA	**2.2**	5.7 a
	4.4	4.2 b
NAA	**0.0**	5.8 a
	2.7	3.8 b

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3: Regeneration experiment.

4.3.1: Experiment one

4.3.1.1: Effect of NAA and TDZ on Shoot Regeneration

4.3.1.1.1: Baladi landrace.

Significant interaction between NAA and TDZ was observed on shoot percentage, the higher shoot percentage and average shoot number were obtained with the higher level of TDZ (4.0 µM) (Table 4.5). Maximum shooting percent (39.3%) was obtained at 2.7 µM and4.0 µM of NAA and TDZ respectively (Fig 4.6). No regeneration was observed at these hormonal combinations (0.0, 0.0). (2.7, 0.0). (2.7, 1.0). (5.4, 0.0). (5.4, 1.0). (5.4, 2.0) µM of NAA and TDZ respectively.

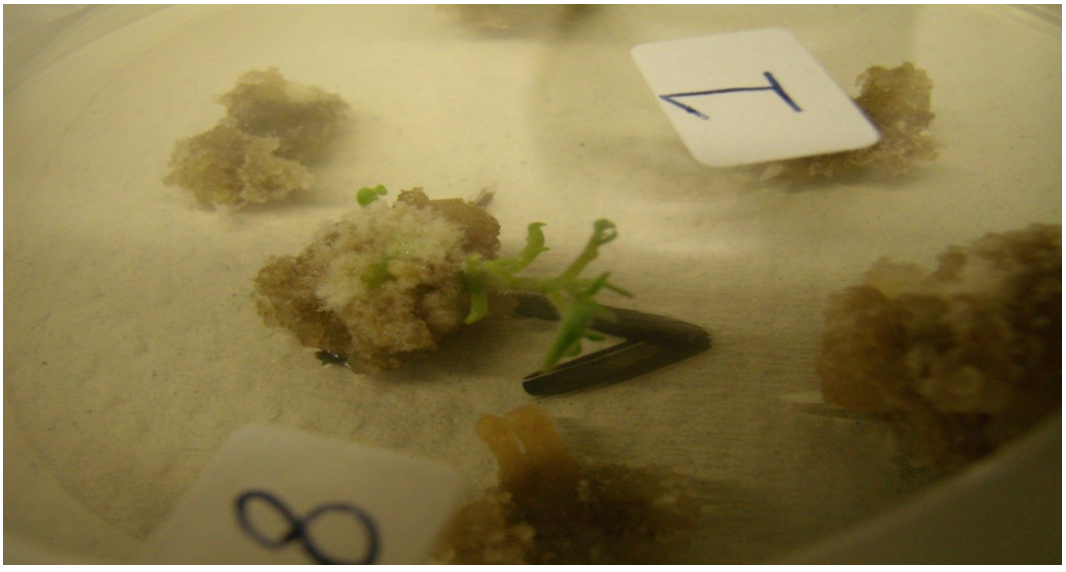

Fig 4.6 Shoot regeneration % on Baladi landrace

Table 4.5: Effect of NAA and TDZ on shoot formation in Baladi landrace

NAA	TDZ	Shoot %	Average number of shoots / reg. explant
0.0	**0.0**	0.0 b	0.0 b
	1.0	3.8 b	1.5 b
	2.0	1.7 b	2.0 b
	4.0	28.7 a	4.43 a
2.7	**0.0**	0.0 b	0.0 b
	1.0	0.0 b	0.0 b
	2.0	3.3 b	0.0 b
	4.0	39.3 a	4.38 a
5.4	**0.0**	0.0 b	0.0 b
	1.0	0.0 b	0.0 b
	2.0	0.0 b	0.0 b
	4.0	16.9 a	3.0 a

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.1.1.2: Cultivar 593

The effect of different levels of NAA and TDZ on shoot percentage and average number of shoot in '593' is shown in table (4.6). A significant interaction between the two growth regulators was observed. The highest shoot percent (46.9%) was obtained with (2.7, 4.0 µM) of NAA and TDZ respectively (Fig 4.7). However the average shoot number was high (5) at level (0.0, 4.0 µM) of both NAA and TDZ respectively. No regeneration was observed with (0.0, 0.0). (5.4, 0.0). (5.4, 2.0) µM of NAA and TDZ respectively.

Fig 4.7 Shoot regeneration % obtained on MS media containing 2.7 µM of NAA and 4.0 µM TDZ

Table 4.6: Effect of NAA and TDZ on shoot formation in cultivar 593

NAA	TDZ	Shoot %	Average number of shoots / reg. explant
0.0	**0.0**	0.0 c	0.0 d
	1.0	1.4 c	1.0 cd
	2.0	21.7 b	4.75 a
	4.0	7.5 bc	5.0 a
2.7	**0.0**	5.4 bc	1.0 cd
	1.0	2.1 bc	3.0 abc
	2.0	2.8 bc	2.0 bcd
	4.0	46.9 a	3.59 ab
5.4	**0.0**	0.0 c	0.0 d
	1.0	9.6 bc	3.5 ab
	2.0	0.0 c	0.0 d
	4.0	5.6 bc	1.0 cd

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.1.2: Effect of NAA and TDZ on root regeneration.

4.3.1.2.1: Baladi landrace

The effect of four explant types and hormonal combinations on adventitious root formation in Baladi landrace is shown in table (4.7). There was a signification interaction among the different variables, the higher root percentage 100% was obtained with both stem and leaf explants cultured on media contains (5.4 and 2.7 µM) of NAA (Fig 4.8).

High root regeneration was also obtained in cotyledon explant with (2.7 and 5.4 µM) NAA levels. Other combination gave lower root percentage ranged from 43% to 71.7%, however, no root formation was observed with higher TDZ level except with hypocotyl explant. The average numbers of root possess the same trend.

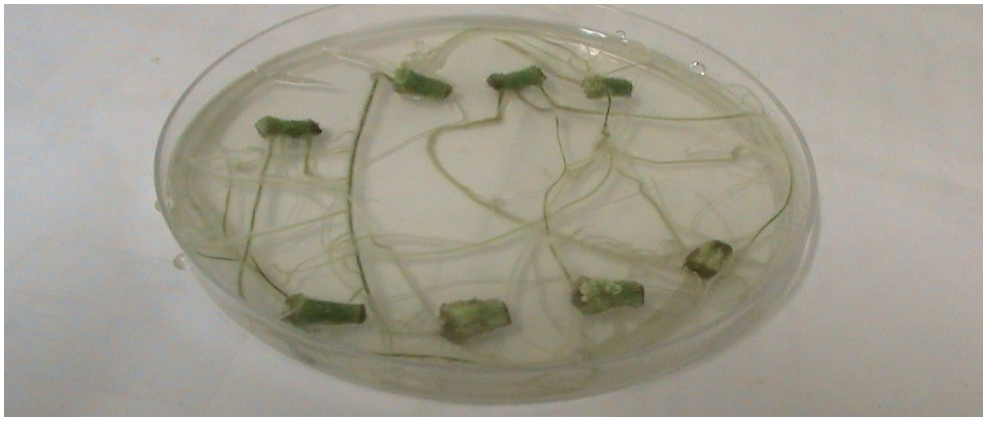

Fig 4.8 Root percentage obtained with stem explant cultured on media contains (5.4) µM of NAA.

Table 4.7: Effect of NAA and TDZ on root formation in Baladi landrace

Explant	NAA	TDZ	Root %	Average number of roots
Hypocotyl	0.0	0.0	0.0 c	0.0 c
		1.0	45 b	3.7 bc
		2.0	45 b	3.7 bc
		4.0	43.3 b	4.0 bc
	2.7	0.0	62.2 b	8.3 a
		1.0	11.1 c	1.3 c
		2.0	8.3 c	0.33 c
		4.0	0.0 c	0.0 c
	5.4	0.0	0.0 c	0.0 c
		1.0	5.6 c	0.67 c
		2.0	11 c	0.67 c
		4.0	0.0 c	0.0 c
Cotyledon	0.0	0.0	0.0 c	0.0 c
		1.0	20 c	2.0 bc
		2.0	0.0 c	0.0 c
		4.0	0.0 c	0.0 c
	2.7	0.0	94 a	8.67 ab
		1.0	43.3 b	3.0 bc
		2.0	0.0 c	0.0 c
		4.0	71.7 b	5.67 ab
	5.4	0.0	93.3 a	10.0 a
		1.0	16.7 c	1.67 c
		2.0	11 c	0.33 c
		4.0	27.8 c	1.33 c
Stem	0.0	0.0	26.7 c	0.67 c
		1.0	6.7 c	0.33 c
		2.0	0.0 c	0.0 c
		4.0	0.0 c	0.0 c
	2.7	0.0	30 c	3.33 bc
		1.0	0.0 c	0.0 c
		2.0	8.3 c	0.67 c
		4.0	5.6 c	0.17 c
	5.4	0.0	100 a	10.0 a
		1.0	0.0 c	0.0 c
		2.0	22.2 c	0.67 c
		4.0	0 c	0.0 c

Leaf	**0.0**	**0.0**	20 c	1.0 c
		1.0	0.0 c	0.0 c
		2.0	5.6 c	0.67 c
		4.0	25 c	1.80 bc
	2.7	**0.0**	100 a	10.0 a
		1.0	0.0 c	0.0 c
		2.0	0.0 c	0.0 c
		4.0	0.0 c	0.0 c
	5.4	**0.0**	17.8 c	3.67 bc
		1.0	16.7 c	1.33 c
		2.0	11 c	0.67 c
		4.0	11 c	0.33 c

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.1.2.2: Cultivar 593

Similar effect in the two cultivars was observed with root formation. The effect of both explant type and hormonal combinations on adventitious root formation of cultivar 593 is shown in table (4.8). There was a signification interaction among the different variables, the higher root percentage (100%) was obtained with stem, cotyledon and leaf explants cultured on medium contains (2.7 µM) of NAA (Table 4.8) (Fig 4.9). Other combinations gave lower root percentage ranged from 21% to 55%, similar to Baladi landrace, no root formation was observed with higher TDZ levels. Similar trend was observed with the average number of root.

Fig 4.9 Root percentage obtained with stem explant cultured on media contains (2.7) µM of NAA.

Table 4.8: Effect of NAA and TDZ on root formation in cultivar 593.

Explant	NAA	TDZ	Root %	Average number of roots
Hypocotyl	0.0	0.0	38.7 b	2.7 bc
		1.0	0.0 c	0.0 c
		2.0	0.0 c	0.0 c
		4.0	22.2 b	0.67 c
	2.7	0.0	78.3 ab	10.7 b
		1.0	6.7 bc	0.67 c
		2.0	8.3 bc	0.33 c
		4.0	0.0 c	0.0 c
	5.4	0.0	44.4 b	5.0 bc
		1.0	13.3 bc	1.0 bc
		2.0	8.3 bc	0.33 c
		4.0	33.3 b	0.67 c
Cotyledon	0.0	0.0	0.0 c	0.0 c
		1.0	28.9 b	1.0 bc
		2.0	0.0 c	0.0 c
		4.0	0.0 c	0.0 c
	2.7	0.0	100 a	20.0 a
		1.0	6.7 bc	0.33 c
		2.0	0.0 c	0.0 c
		4.0	0.0 c	0.0 c
	5.4	0.0	100 a	10.0 b
		1.0	13.3 bc	1.0 bc
		2.0	9.5 bc	1.0 bc
		4.0	55.6 b	1.33 bc
Stem	0.0	0.0	20 bc	0.67 c
		1.0	31.7 b	2.0 bc
		2.0	0.0 c	0.0 c
		4.0	25 b	1.33 bc
	2.7	0.0	88.9 a	19.3 a
		1.0	8.3 bc	1.33 bc
		2.0	6.7 bc	0.33 c
		4.0	0.0 c	0.0 c
	5.4	0.0	11.1 bc	0.33 c
		1.0	0.0 c	0.0 c
		2.0	33.3 b	1.0 bc
		4.0	22.2 b	1.0 bc

Leaf	**0.0**	**0.0**	0.0 c	0.0 c
		1.0	36.7 b	2.33 bc
		2.0	0.0 c	0.0 c
		4.0	11.1 bc	0.33 c
	2.7	**0.0**	100 a	20.0 a
		1.0	0.0 c	0.0 c
		2.0	0.0 c	0.0 c
		4.0	21.7 b	1.0 bc
	5.4	**0.0**	6.7 bc	1.0 bc
		1.0	26.7 bc	2.33 bc
		2.0	8.3 bc	0.67 c
		4.0	55.6 b	1.33 bc

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.1.3: Effect of different type of explants on adventitious shoot and root formation in cultivar 593.

Both stem and hypocotyl explants gave significant higher shoot percentage, the highest shoot percentage was observed on stem explant (15.66 %) (Table 4.9, Fig 4.10). The higher average number of shoot was obtained with leaf explant (6.39), however, cotyledon explant possess a non-significant maximum root percent (25.61%). On the other hand, no significant difference was observed among the four explants on the average root number.

Fig 4.10 Shoot % on stem explant

Table 4.9: The effect of different type of explants on shoots and roots regeneration in cultivar 593

Explant	Shoot %	Average number of shoots	Root %	Average number of roots
Hypocotyl	11.22 a	0.805 b	21.12	1.84
Cotyledon	2.92 b	0.22 b	25.61	2.89
Stem	15.66 a	0.95 b	20.60	2.27
Leaf	3.40 b	6.39 a	22.20	2.42

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.2: Experiment two

4.3.2.1: Effect of BA and Kinetin on shoots and roots regeneration.

4.3.2.1.1: Baladi landrace

Shoot regeneration percentage was (33.4%) when obtained in a combination of both BA and Kinetin were combined (Table 4.10, Fig. 4.11). This percent was significantly higher than that obtained with other hormones. Similar trend was observed with the average number of shoots. However, for root regeneration the higher percentage was observed when Kinetin was used alone (48.7). Regarding the average number of root there was no significant difference among the different treatments.

Fig 4.11 Shoot regeneration for stem explant in Baladi landrace

Table 4.10: Effect of BA and Kinetin on shoot and root regeneration in Baladi landrace.

Plant growth regulators	Shoot %	Average number of shoots	Root %	Average number of roots
1) 2.3 Kinetin	2.4 b	0.15	48.7 a	3.9
2) 2.2 BA	0.0 b	0.0	34.0 a	4.2
3)2.2 BA and 2.3 Kinetin	33.4 a	1.5	13.4 b	1.2

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.2.1.2: Cultivar 593

The effect of two levels of BA and Kinetin on shoot regeneration percent and average number of regenerated shoots and roots is shown in table 4.11. Maximum significant shoot percentage and average number of shoot (62.25, 3.66) were observed when BA and Kinetin were combined together at (2.2 and 2.3 µM), respectively (Fig 4.12). Regarding to root regeneration there were no significant difference among the different treatments.

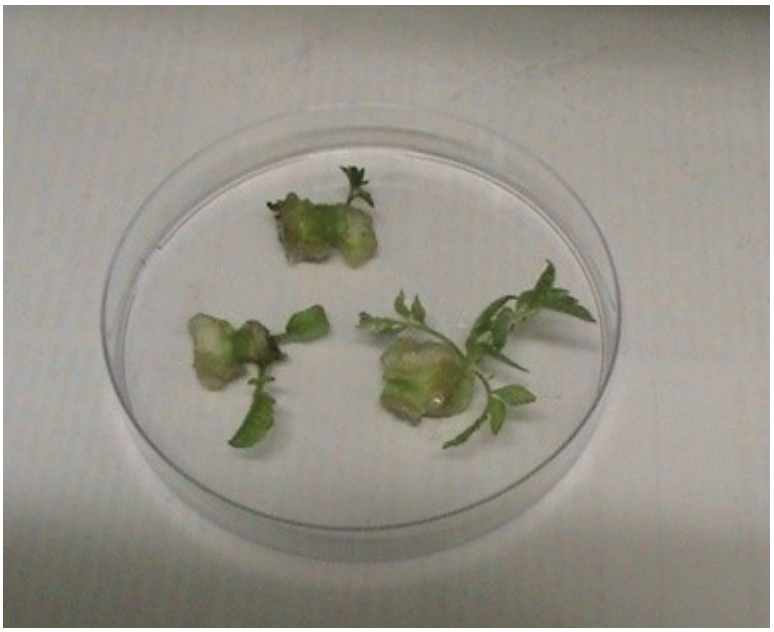

Fig 4.12 Shoot regeneration from stem explant (cultivar 395)

Table 4.11: Effect of BA and Kinetin on shoot and root regeneration in cultivar 593.

Plant growth regulators	Shoot %	Average number of shoots	Root %	Average number of roots
1) 2.3 Kinetin	6.65 b	0.30 b	33.0	3.3
2) 2.2 BA	8.35 b	0.25 b	32.5	2.4
3) 2.2 BA and 2.3 Kinetin	62.25 a	3.66 a	23.4	1.96

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.2.2: Effect of explants on shoots and roots regeneration.

4.3.2.2.1: Baladi landrace

The effect of different explants (hypocotyl and stem) on shoot and root regeneration is shown in table (4.12). In spite of the low regeneration percentages observed, stem explant exhibited significantly higher shoot percent (13.7) than the hypocotyl explant (Table 4.12, Fig. 4.13). However, regarding root percentage and average number of roots, a significant differences were observed between the two explants.

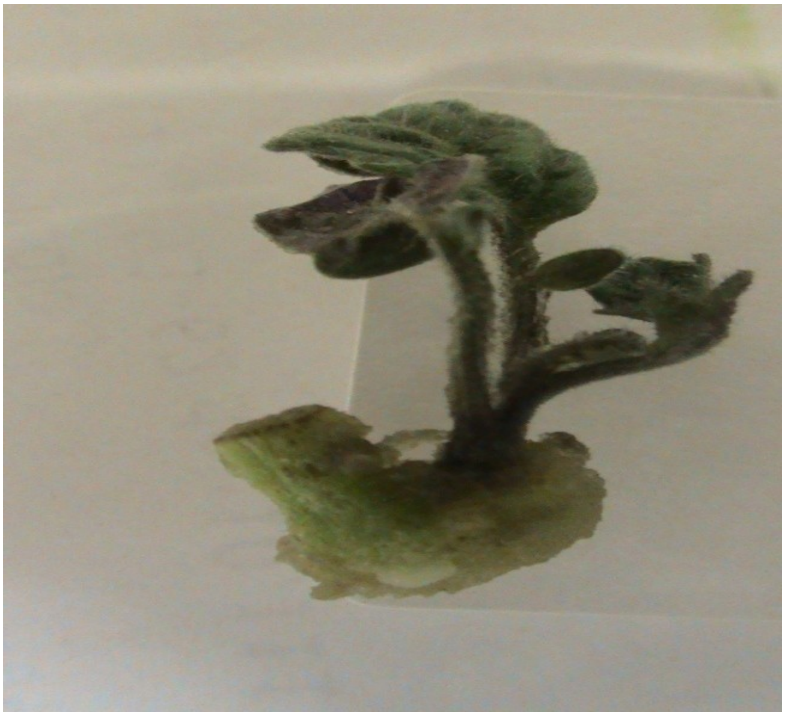

Fig 4.13 Shoot % on stem explant in Baladi landrace

Table 4.12: The effects of different type of explants on shoots and roots regeneration

Explant type	Shoot %	Average number of shoots	Root %	Average number of roots
1. Hypocotyl	3.6 b	0.21 b	65.8 a	6.36
2. Stem	13.7 a	0.59 a	4.40 b	0.59

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

4.3.2.2.2: Cultivar 593

The effect of different explants (hypocotyl and stem) on adventitious shoot and root development is shown in table (4.13). There were significant differences in shoot percentages and average number of shoots among the different treatments, the higher shoot percentage (35.1%) was observed in stem (Fig 4.14). A significant difference was observed between the two explants regarding root regeneration.

Fig 4.14 Shoot % on stem explant in cultivar 593

Table 4.13: The effects of different type of explants on shoots and roots regeneration

Explant type	Shoot %	Average number of shoots	Root %	Average number of roots
1. Hypocotyl	19.8 b	1.19 b	56.6 a	5.0 a
2. Stem	35.1 a	1.88 a	4.70 b	0.31 b

Number followed by the same letter or letters are not significantly different at 5% level according to least significant difference (**LSD**).

Chapter Five

Discussion

The study reported for the first time an *in vitro* study of local tomato landraces in Palestine. landraces are diverse genetically. This diversity provided protection against pests, diseases, and abiotic stresses. Landraces genetic resources are commonly maintained *in situ* conservation (Ford and Jackson, 1986). The replacement of local landraces with improved varieties of narrow genetic base, in turn, may lead to genetic vulnerability, which could lead to the susceptibility of most of the cultivated varieties to biotic (diseases and insect pests) and abiotic (adverse climatic changes) stresses due to similarities in their genotypes (Singh, 2000).

Natural populations may suffer from natural calamities but they are still genetically more flexible to adapt themselves or to evolve with the calamities while commercial cultivars are genetically uniform that their population is inflexible enough to do so (Boef *et al*., 1996). The regeneration and multiplication of landraces reported in this study, is an important step toward the improvement of these landraces through biotechnology technique.

In our experiment, Chlorox was used at 20% (v/v) to sterilize the seeds. This level was sufficient to prevent contamination without any effect on seed germination. Disinfestations of seeds by using Chlorox have already been proved to be essential in tomato tissue culture and *in vitro* seed germination (Chaudhry *et al*., 2007).

Multiple shoots regeneration of tomato cultivar 593 and Baladi landrace were initiated from the shoot tip explants after (3-4) weeks of

culture. The frequency of shoot number was influenced by both the type and concentration of hormones used. Micro-propagation has become a reliable and routine approach for large-scale rapid plant multiplication (Akbas *et al*., 2009).

The dose of cytokinin is known to be critical in multiple shoots induction (Abdellatef and Khalafalla, 2007). Therefore, the effect of BA, Kinetin and NAA on shoot multiplication was studied. Kinetin was more effective than BA for multiple shoot production.

Similar results was reported by Ishag *et al.,* (2009) who proved that Kinetin at different concentrations can produce more multiple shoot when compared with BA and 2ip. Gubis *et al*., (2004) reported that the frequency of adventitious shoot regeneration depends on the type of explant and both the type and concentration of growth regulators.

Kinetin in combination with auxin (NAA) did not show any positive response on shoot number and development; however, when it was used alone it was more effective. Results showed that the use of only BA on medium proved to be more beneficial than combination with auxin for shoot number.

In this study, we demonstrated the effect of different cultivars, explants, and hormones on shoot and root regeneration. Chaudhry *et al.*, (2007) reported that the regeneration capacity is significantly influenced by cultivar and explant type**.**

In this study, we compared the shoot and root regeneration of different explants (hypocotyl, cotyledon, stem, and leaf). Stem induced more shoot regeneration followed by hypocotyl, leaf, and cotyledon. Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyls or cotyledon (Brichkova *et al.,* 2002, Raziuddin *et al*., 2004). Similar result was also reported by Jabeen *et al*., (2005) who stated that the higher shoot regeneration were obtained from shoot tip followed by hypocotyl and leaf disc.

These results are supported by the results of Faria and Illg, (1996) in which they reported, a maximum shoot formation was obtained from the tomato hypocotyls. Similar results was reported by Chaudhary *et al*., (2004) who found that hypocotyl appeared to be better explant source as compared to leaf disc.

Among the various explants used, hypocotyl was best in terms of average number of shoot, which could probably be attributed to the age compared to other explant leaf and stem. Younger explants showed better callus induction and organogenetic response, the importance of hypocotyl derived callus in terms of plantlet regeneration consistent with the earlier findings of Locy, (1981).

Other studies were inconsistent to our finding in which cotyledons were superior to other source of explants, including hypocotyls, stems and leaves as an explant for shoot organogenesis in tomato (Hamza and Chupeau, 1993; VanRoekel *et al.,* 1993; Ling *et al.,* 1998). Similar results

was reported by Nogueira *et al.*, (2001) who observed high regeneration frequency with cotyledon explants of two tomato genotypes (Santa Clara or its natural mutant Firme), respectively.

In this study we investigated the effect of NAA and TDZ on shoot and root formation, higher shoot percentage was obtained when TDZ was used at higher level (4.0 μM) combined with different concentration of NAA. Chaudhary *et al*., (2004) reported that 45.8% and 30.8% shoot regeneration from hypocotyls and leaf discs respectively were obtained by using relatively higher level of both auxin and cytokinin (2.0, 4.0, 5.0) mg L^{-1}.

Chaudhary *et al*., (2007) reported that auxins and cytokinins are involved in cell division and elongation, while cytokinin helps in the process of differentiation, therefore, the appropriate concentration of these growth hormones is necessary for cell division and differentiation. In this study most of the effect on shoot regeneration was due to cytokinin (TDZ).

In another study, shoot regeneration was reported from hypocotyls (Gubis *et al*., 2003; Jabeen *et al*., 2005).Chaudhry *et al*., (2007) reported that MS medium containing 1.5 mgL^{-1} 2ip and 0.5 mgL^{-1} IAA exhibited the highest shoot regeneration because of the presence of cytokinin.

These results are supported by (Gubis *et al.,* 2004) reported shoot regeneration from hypocotyls on (MS) medium supplemented with 1.0 mgL^{-1} of zeatin and 0.1 mgL^{-1} of IAA. Similarly Shivakumar *et al.,* (2007)

developed a regeneration protocol for tomato cultivars (ArkaSaurabh and ArkaVikas), using cotyledon and hypocotyls explants. Optimum regenerative response for all genotypes was obtained on MS medium supplemented with 2.0 mgL^{-1} BAP and 0.1mg L^{-1} IAA. Devi *et al.*, (2008) reported, no shoot formation occurred on media containing 0.5 mgL^{-1} of IAA only.

The presence of high cytokinin with low or equal amount of auxin was confirmed by Gubis *et al*., (2003). Whereas, Raj *et al*., (2005) also used low levels of auxin and cytokinin for regeneration of leaf explants of the Pusa Ruby tomato (0.1 mgL^{-1} of IAA and 0.1 mgL^{-1} of zeatin).

In this study root percentage was higher when the dose of auxin (NAA) was increased. This result was similar to Plevnes *et al*., (2006) who found that the largest number of roots in both species examined were obtained on media containing NAA and BAP in 1:1 or 2:1 ratios and on media containing only NAA (1.0 or 2.0 mgL^{-1}).

Relations were observed between the regeneration of roots and the ratio between auxin and cytokinin (Brown and Charlwood 1990). A similar type of comparison for hormone and root percentage was reported by Devi *et al*., (2008), who found that that the best rooting was obtained in half-strength medium supplemented with 0.2 mgL^{-1} IBA. Higher rooting percentage was observed in tomato shoot explants cultured on media with zeatin alone or with auxin (Harish *et al*., 2010). Ouyang *et al.*, (2003) observed that the high rooting % was obtained onto media supplied with

(0.5 and 1.0 mgL^{-1}) level of auxin; he suggested that tomato possesses high levels of endogenous auxins.

In this experiment, the effect of different levels and combinations of BA and Kinetin on shoot and root regeneration was investigated. The highest shoot percentage was obtained when both cytokinins were used at (2.2 and 2.3 μM), respectively. Our results supported the findings of other authors (Ichimura and Oda, 1995; Nogueria *et al*., 2001) who found that the most efficient medium for *in vitro* regeneration of tomato was MS medium supplemented with cytokinin only.

A similar type of hormone was used by Rashid and Bal, (2010) who observed that the optimal medium for plant regeneration was MS supplemented with 0.5 mgL^{-1} BAP and 0.5 mgL^{-1} Kinetin in two tomato genotypes (Punjab Upma and IPA-3). Brichkova *et al*., (2002) observed that the presence of two cytokinin (BAP at 5.0 mgL^{-1} and Zeatin at 1.0 mgL^{-1}) contributed better to plant regeneration.

In our study, BA with cultivar 593 was better than Kinetin. This result was also reported by Huetteman and Preece, (1993) who found that the presence ofBA in the culture medium has long been reported to promote shoot organogenesis in a large number of plant species. BA or zeatin alone induced shoot formation from leaf callus (Kartha *et al*., 1976). Zeatin and BA were also found superior to kinetin for shoot formation from tomato leaf explants (Dhruva *et al.,* 1978).

Chapter Six

Conclusions and Recommendations

6.1 Conclusions

The results of the present investigation demonstrated that the high variability in the growth and regeneration at tomato cultivars (Baladi and 593), where Kinetin at 18.4 μM was the highest for shoot multiplication from shoot tip in Baladi landrace, while BA at 2.2 with Kinetin at 2.3 μM was the highest for shoot regeneration in cultivar 593. Stem explants were good source than hypocotyl, cotyledon, and leaf section for shoot regeneration. The establishment of an *in vitro* multiplication and regeneration protocol for Palestinian tomato landraces could be effectively used for the improvement of these landraces through biotechnology technique.

6.2 Recommendation

The result of this study is considered as initial findings on the multiplication and regeneration of local tomato varieties in Palestine. More investigations are needed to improve the multiplication and regeneration of tomato cultivar. *Ex vitro* studies are also needed to evaluate the growth and performance of the regenerated plants.

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جامعه النجاح الوطنية

كلية الدراسات العليا

تمايز وتضاعف أصناف محليه من البندورة بواسطه زراعه الأنسجه في فلسطين

اعداد

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اشراف

ا . حسان ابو قاعود

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في الانتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

2013

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الملخص

تم دراسة تأثير عوامل مختلفة على نمو، مضاعفه، وتمايز البندورة داخل الأنابيب . استخدم في التجربة صنف (93ز) وأيضا واحد من السلالات المحلية بلدي ، بأخذ أجزاء مختلفة من النبات (ypocotyl, cotyledon, stem, and leaf). تم دراسة تأثير تراكيز مختلفة من الاكسينات حامض النفثالين (NAA ، وأيضا هرمونات مختلفة من السيتوكينين بنزيل أدينين(BA ، كاينتين(Kin) ، والثايدازارون (TDZ) على المضاعفة والتمايز . تم استخدام سويقات منبته داخل الأنابيب في تجربه المضاعفة . تم زراعة السويقات المنبتة في وسط موراشيج و سكوج (MS) مضاف لها تراكيز مختلفة من أل (BA) 2.2 و 4.4 مايكرومولر، (inetin) و تراكيز 9.2 و 8.4 مايكرومولر، وأيضا تم اضافه (NAA) بتراكيز 0.0 و 2.7 مايكرومولر . تم الحصول على أكبر عدد من السيقان (4.) باستخدام وسط S] مضاف له 8.4 مايكرومولر من (in). تم ملاحظه اقل ع د من السيقان المضاعفة في سلاله البلدي المزروعة في (MS) مضاف لها 18.4 مايكرومولر من (in) و 7. مايكرومولر من (NAA). أما بالنسبة للتمايز فتم استخدام أربع أجزاء مختلفة من النبات وتسجيل النتائج باستخدام (MS) مضاف لها ربع تراكيز من TDZ (ا ، ، ! ، 4 مايكرومولر). وثلاث تراكيز من NAA (ا ، 2.7 ، 5.4 مايكرومولر ، وأيضا تركيزين من BA (ا ، 2.2 مايكرومولر) وتركيزين من Kin (ا ، 2.3 مايكرومولر) في تجربتين منفصلتين . تم الحصول على أعلى نسبه تمايز للسيقان (2.5 %) عند استخدام 2.3 مايكرومولر من Kin و 2.2 مايكرومولر من BA ، ولكن كانت أعلى نسبه تمايز للسيقان (6.91 %) عند استخدام NAA (2.7 مايكرومولر) بإضافة (4 مايكرومولر) من DZ . تم ملاحظه اقل نسبه من السيقان المتمايزه عند استخدام

NAA في جميع التراكيز بإضافة (، 2 مايكرومولر) من DZ . تم الحصول على أعلى نسبه تجذير (0 ٪) عند اضافه NAA إلى الوسط (7.! ، 5.4 مايكرومولر).