An-Najah National University

Faculty of Graduate Studies

In Vitro Regeneration of Local Chickpea Varieties in Palestine

By

Rozan Irfan Al-Tanbouz

Supervisor

Dr. Hassan Abu-Qaoud

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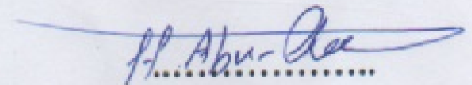
Dr. Hassan Abu Qaoud	(Supervisor)	
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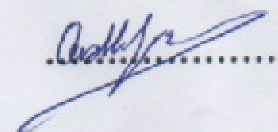
Dr. Heba Al-Fares	(Internal Examiner)	

Dedication

This thesis is dedicated to my parents, sisters and brother who have given me the opportunity of an education from the best institutions and support throughout my life and to my aunts and friends with love and respect.

Acknowledgment

It is with immense gratitude that I Acknowledge the support and help from my Dr H. Abu-Qaoud for his guidance, caring, patience and his advises for achieving this thesis. In addition I would like to thank Dr. Munqez Shtaya who provided me with chickpea seeds.

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الإقرار

أنا الموقع أدناه مقدم الرساله التي تحمل عنوان

In Vitro Regeneration of Local Chickpea Varieties in Palestine

أقر بأن ما شملت عليه هذه الدراسة إنما هو انتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرساله ككل أو جزء منها لم يقدم من قبل لنيل أيه درجه أو بحث علمي أو بحثي لدى أي مؤسسه تعليميه أو بحثيه.

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.

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Abstract

A study was conducted to investigate the effect of different factors on shoot multiplication and regeneration of chickpea (*Cicer arietinum* L.) Three varieties (HUDAS, FLIP03-147c and FLIP05-100C) and two Palestinian landraces 'Ein Al Bayda' and 'Baladi' were used in this study. The effect of different levels and combinations of the auxin 1-Naphthaleneacetic acid (NAA) and the cytokinins Benzyl adenine (BA), Kinetin (Kin) and N-1, 2, 3-Thiadiazol-5-yl-N'-phenylurea (TDZ) on shoot multiplication and regeneration was examined in thisstudy. Shoots tip from *in vitro* established seedlings were used as a source material for shoot multiplication. The shoot tips were cultured on MS medium supplied with 0.0, 2.2 and 4.4 µM BA, or 0.0, 9.2 and 18.4 µM Kinetin, both cytokinins were combined with 0.0 or 2.7 µM NAA. The highest shoot number (8.0) was obtained on media without hormone or media supplied with 2.2 µM BA in the Baladi landrace. However, Low shoots number wereobserved on MS medium supplied with 9.2 or 18.4 µM Kin and 2.7 µM NAA in both landraces. For shoot regeneration stem and leaf explants of the studied cultivars and landraces were cultured on MS media supplied with different combinations of TDZ, Kinetin and NAA. The highest shoot percentage (62.0%) and number of shoots (3.0) was obtained with leaf explant using

2.0 μM TDZ with ‘FLIP05-100C’, stem explants exhibited no shoot regeneration in all five chickpea varieties. Very low shoot regeneration was observed with all NAA level combined with TDZ. However, regeneration percentage was highly reduced with higher cytokinin levels. No shoot regeneration was observed with any Kinetin level.

Chapter One

Introduction

1.1 Background

Chickpea (*Cicer arietinum* L.) is one of the most important leguminous food crops in the world, the total cultivated area is nearly 10 million hectares, the area distributed across the Americans, Mediterranean basin, east Africa, the Middle East, Asia and Australia (Jayashree *et al.,* 2005). India is the largest producer of chickpea contributing to 65% of world's production (FAO, 2008).

The productivity of chickpea has not improved considerably over the years (Singh and Kataria, 2012). Many factors are responsible for the productivity of chickpea, which include excessive vegetative growth, narrow genetic basis, old cultivated genotypes and poor harvest index. (Hassan and Khan, 1991). The production of this crop is highly influenced by biotic stresses like Ascochyta blight, Fusarium, and pod borer (Hossain, 2009), as well as abiotic stresses such as salinity, drought and cold (Kiran *et al.,* 2005). Cultivated chickpea has limited sources of genetic variability, however, species of *Cicer* have many economically important traits like resistance to diseases and pests (Gopalakrishnan *et al.,* 2005). Conventional breeding methods for stress resistance are often costly and time consuming, limited to lack of proper genes in the gene pool and intraspecific cross incompatibility.

In recent years, there has been a great interest in biotechnological methods among breeders including *in vitro* culture, which can intensify the breeding process (Gamborg, 2002). Modern biotechnology has provided

new opportunities including tissue culture, genetic engineering, and genetic transformation to enhance the germplasm for crops (Sharma and Ortiz, 2000).

Genetically modified chickpea was already been produced by *Agrobacterium tumefactions* mediated transformation with the genes npII, GUS, bar, X-amylase, CryIAc (Polowick *et al.,* 2004; Sanyal *et al.,* 2005), however, the transformation frequency was low in chickpea (Senthil *et al.,* 2004). *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. *In vitro* techniques are important tools for modern plant improvement programs (Moghaleb *et al.,* 1999), in addition to introduce new traits into selected plants, and to develop suitable cultivars in a minimal time (Taji *et al.,* 2002). Many of the economically important plants productivity through genetic transformation and other cellular techniques have been improved regarding yield and productivity; many legumes have generally proved notoriously recalcitrant due to the lack of reliable *In vitro* regeneration system due to difficulties of regeneration from callus (Barna and Wakhlu, 1993; Khawar *et al.,* 2004). The regeneration protocols are not repeatable because of complete regeneration to variety of factors such as genotype, growth regulator, explants, physical factors like temperature, humidity, and photoperiod (Reed, 1999). Regeneration in chickpea *via* direct shoot induction and somatic embryogenesis has been reported from various

explants (Sagare *et al.,* 1993; Islam*et al.,* 1995; Barna and Wakhlu*,* 1995; Murthy *et al.,* 1996; Vani and Reddy, 1996; Batra *et al.,* 2002 and Kiran *et al*., 2005). Efficient regeneration protocol in chickpea and development of highly reproducible is still awaited.

Previous studies suggest that chickpea culture response is genotypically oriented, chickpea cultivars regenerated under similar environmental conditions exhibit variable regeneration frequency (Aasim *et al*., 2013). The aim of this study is to investigate the direct effect of various concentrations of TDZ (Thidiazuron), NAA (1-Naphthaleneacetic acid), BAP (6-Benzylaminopurine) and Kinetin on shoot regeneration directly from leaves and stem of local chickpea varieties.

1.2 Objectives

The study aims at

1. Studying the effect of plant growth regulators (NAA, Kinetin, and BA) on shoot multiplication of local chickpea landraces.
2. Studying the effect of plant growth regulators (NAA, TDZ, and Kinetin) on adventitious shoot regeneration of several chickpea varieties including local landraces.
3. Studying the regeneration ability of different explants from local chickpea landraces.

Chapter Two

Literature Review

2.1 Plant tissue culture

Tissue culture is the aseptic culture of cells, tissues, organs, or whole plants under controlled nutritional and environmental conditions (Thrope, 2007). The first reports regarding tissue culture date back to the beginning of the 20th century when Gottlieb Haberlandt (Haberlandt, 1902) developed experiments to maintain mesophyll cells in culture. Haberlandt succeeded in maintaining isolated leaf cells alive for extended periods.

The earliest nutrient media used for growing plant tissues *in vitro* were based on the nutrient formulations for whole plants; but Knop's solution and that of Uspenski and Uspenskia were used the most, and provided less than 200 mgl^{-1} of total salts (White, 1963).

In 1962, Murashige and Skoog developed a new medium today known as (MS) media. The concentration of some salts was 25 times than of Knop's solution. In particular, the levels of $NO3^-$ and $NH4^+$ were very high and the arrays of micronutrients were increased. MS formulation allowed for a further increase in the number of plant species that could be cultured, many of them using only a defined medium consisting of macro- and micronutrients, a carbon source, reduced N, B vitamins, and growth regulators (Gamborg *et al.,* 1976). The MS salt formulation is now the most widely used nutrient medium in plant tissue culture.

The first true plant tissue cultures were obtained by Gautheret (Gautheret, 1934**)** from cambial tissue of *Acer pseudoplatanus*. He also

achieved success with similar explants of *Ulmus campestre, Robinia pseudoacacia*, and *Salix capraea* using agar-solidified medium of Knop's solution, glucose and cysteine hydrochloride.

Embryo culture also had its beginning early in the first decade of the last century with barley embryos (Monnier, 1995).This was followed by the successful rescue of embryos from nonviable seeds of a cross between *Linum perenne* and *Linum austriacum* (Laibach, 1929), and for full embryo development in some early-ripening species of fruit trees (Tuky, 1934), thus providing one of the earliest applications of *in vitro* culture.

Micropropagation may be utilized in production of virus – free planting material, cryopreservation of endangered and elite woody species (Mohan and Hoggman, 2007). Cytokinins generally promote cell division and induce shoot formation and axillary shoot proliferation. High cytokinin to auxin ratio promotes shoot proliferation while high auxin to cytokinins ratio results in root formation (Aloni *et al.,* 2006). Gibberellins are used for enhanced growth and to promote cell elongation.BA, TDZ and Kinetin are generally used cytokininsfor *in vitro*regeneration, singly or in combination with an auxins in legumes (Aasim *et al*., 2010). TDZ has been widely used to promote shoot regeneration in many plant species with a significant effect over other cytokinins (Fraguas *et al.,* 2009).

Plant growth regulators can induce what is known as hyperhydricity in tissue cultures of several species (Ziv, 1991; Fraguas *et al.,* 2004; Toth *et al.,* 2004).Hyperhydricity formerly called "vitrification", but it has become

a term used to characterize cryopreserved tissues and it is another physiological effect very common in plant tissue culture (Gaspar *et al.,* 1985; Kevers *et al.,* 2004). Hyperhydricity is also linked to shoot-tip necrosis, a physiological disorder showed by *in vitro*plants as aconsequence of high relative humidity, transpiration rate, and Calcium availability in the medium and plant growth regulators (Bairu *et al.,* 2009).

A plant tissue is considered to be "introduced and established" to the *in vitro* culture when explants are not only free from superficial or visible contaminants, which interfere with the morphogenic response, but also when it shows a morphogenic response (Christensen *et al.,* 2008). This morphogenic response is characterized by multiplication and/or differentiation of the plant tissues such as shoots, roots, leaves or production of calli (Noshad *et al.,* 2009).

During the 1990s, continued expansion in the application of *in vitro* technologies to an increasing 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), potato (Jones, 1994) and other root and tuber crops (Krikorian, 1994), plantation crops (Zimmerman and Swartz*,* 1994), and ornamentals (Debergh*,* 1994). Tissue culture techniques for plant micropropagation, genetic transformation, biotech assisted selection, mutagenesis, etc., rest 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).

Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material (Ahmadi *et al.,* 2010). For these, "cleanup" techniques to eliminate plant pathogenic organisms have been developed, such as meristem cultures or explant disinfection treatments through chemical or physical methods (Chatenet *et al.,* 2001). Cell and tissue *in vitro* culture is a useful tool for the induction of somaclonal variation (Sengar*et al.,* 2010). Tissue culture protocols can be used for preservation of vegetative tissues when the targets for conservation are clones instead of seeds, to keep the genetic background of a crop and to avoid the loss of the conserved patrimony due to natural disasters, whether biotic or a biotic stress (Tyagi *et al.,* 2007).

2.2 Chickpea (Classification, taxonomy and importance).

Chickpea (*Cicer arietinum* L. (2n=2x=16) is one of the oldest (earlier than 9500 BC) and widely cultivated pulse crops in over 50 countries of the world. Chickpea is a member of the West Asian Leolithic crop assemblage, associated with the origin of agriculture in the Fertile Crescent some 10,000 years ago (Upadhyaya *et al.,* 2011).

Legumes (Fabaceae) constitute the third largest family of flowering plants comprising more than 650 genera and 18000 species (Zhu *et al.,* 2005). Chickpea (*Cicer arietinum* L.) is the only cultivated species of the genus *Cicer* which include 43 species (Donmez,2011).

It is a cool season legume crop has been thought to originate in south-eastern Turkey, from where it has spread to other countries of the world. It was first grown in turkey around 7,500 B.C. *Cicer arietinum* has been domesticated from *C. reticulatum Ladizinsky*, a closely related wild species (Tuker, 2009). Chickpea belongs to genus *Cicer*, tribe *Cicereae*, family Fabaceae (Donmez, 2011). All other annual and perennial *Cicer* spp. are genetically isolated in the tertiary gene pool and equidistant from the domestic species as per amplified fragment length polymorphism (AFLP) diversity analyses (Nguyen *et al.,* 2004). The key to chickpea domestication was the change from a winter habit with an autumn sowing to a spring habit, which avoided or reduced the threat of lethal infestation of the endemic ascochyta pathogen complex (Abbo *et al.,* 2003).

Chickpea stems are branched, erect or spreading, sometimes shrubby much branched, 0.2-1.0 m tall, glandular pubescent, olive, dark green or bluish green in color (Corby, 1981). Root system is robust, up to 2m deep, but the major portion up to 60cm. Leaves imparipinnate, glandular-pubescent with 3-8 pairs of leaflets and a top leaflet (rachis ending in a leaflet); leaflets ovate to elliptic, 0.6-2.0cm long, 0.3-1.4cm wide; margin serrate, apex acuminate to aristate, base cuneate; stipules 2-5 toothed or

absent (Cubero, 1987). Its life span is 2-3 months and on maturity bears fruit which is called pod with 2-3 seeds per pod. The mature pod along with the plant is harvested and thrashed to collect the seeds, the pods split vertically which is the characteristics of all pulses (Cubero, 1975).

Chickpea is an important source of protein in the diets of the poor and particularly important in vegetarian diets, it provides many amino acids but it is typically low in methionine and histamine, it is not considered to be a complete source of amino acids (Upadhhyaya *et al.,* 2008). According to the international Crops Research Institute for the Semi-Arid Tropics (ICRISAT) chickpea seeds contain an average of 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugar), 5% fat, 6% crude fiber and 3% ash. High mineral content has been reported for phosphorus (340mg/100g), calcium (190mg/100g), magnesium (140mg/100g), iron (7mg/100g) and zinc (3mg/100g). Recent studies have also shown that they can assist in lowering of cholesterol in the bloodstream (Murty *et al.,* 2010).

Chickpeas are commonly used as medicine due to the presence of glandular secretions. Glandular secretion of the leaves, stems & pods consists of malice and oxalic acids giving a sour taste (Oudhia, 2003). Medical applications include use for aphrodisiac bronchitis, cataract, cutamenia, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke and warts (Kumar *et al*., 2006).

Moreover, the leaves are used to cure chronic bronchitis and the seeds are considered as ant bilious, used as tonic, stimulant and aphrodisiac acid is also supposed to lower the blood cholesterol level (Brenes *et al.*, 2008).

Chickpea is produced all over the world covering more than 10 million hectors of cultivated land (Kottapalli *et al.,* 2009). In 2004, global chickpea production was about 8.6 million metric tons, second only to dry beans among edible pluses (Smith and Jimmerson, 2005). Chickpea are produced in over 45 different countries all over the world. West and Central Asia accounted for 14% and 12% of world chickpea area and production, respectively, during 1996–2005(Yadav *et al.*, 2007).

During 2006-2007, worldwide production of chickpea was 8.24 million tons from an area of 9.4 million hectares and average production of 0.77 t ha^{-1}.While, contribution of Asia was 7.36 million tons (89.4%) (FAO, 2008). Despite its economic importance and strong national and international breeding programs, the productivity of chickpea has not improved considerably over the years (Hossain, 2009).

2.3 Chickpea tissue culture

Legumes are considered as the second most important source of food after cereals for human and animal consumption (Smith and jmmerson, 2005). Therefore, the improvement techniques of legumes through tissue culture are important.

Medium supplemented with BA and NAA induced callus from the cut end of the stem explants in contact with the medium, the regenerated shoots produced roots on medium supplemented with IBA; this technique could be standardized to improve regeneration system (Sheila*et al*., 1992).

Many studies were conducted on chickpeas to establish an efficient system of shoot multiplication and regeneration.

Rekha and Thiruvengadam. (2009) studied shoot regeneration from the cotyledon, internodes and axillary buds of chickpea. The maximum number of multiple shoot and longest shoot were obtained on MS medium containing 1.0 mgl^{-1} BAP in cotyledon node and 1.5 mgl^{-1} in axillary buds.

Krishna and Joshi. (2008) found the best media for shoot multiplication from the node and callus cultures have been identified as MS + NAA 0.5 + BAP 0.5 mgl^{-1} and MS + BAP 2.0 mgl^{-1}.

Shaheena *et al*., (2012) found efficient protocol for direct in vitro multiple shoot induction and plantlet regeneration achieved from shoot tip explants of *Cicer arietinum* L. Multiple shoots proliferation was best observed on 3.0 mgl^{-1} TDZ from the shoot tip explants within three weeks of culture.

MS media with B5 vitamins supplemented with 5.0µM benzyl amino purine (BAP) has been found to be a highly effective medium for multiple shoot formation from intact seedlings (Polisetty *et al*., 1997).

Shagufta *et al*., (2007) found the multiple shoots of chickpea were shifted to full and half strength MS. Medium supplemented with different concentrations of NAA and IBA for induction of roots while the half strength media with 1.0 µM NAA gave excellent response of root induction.

Saleem *et al*., (2010) study the effect of indigenous chickpea (*Cicer arietinum* L.) cultivars, KK-1 and Hassan-2K , Shoot initiation was best achieved on lower concentration (3.0 and 5.0 µM) while high concentration of BAP delayed shoot initiation in explants of both cultivars. BAP at 3.0 and 5.0 µM produced maximum number of shoots (88 and 89%) in both cultivars.

Somatic embryogenesis was induced in both direct and indirect pathways from immature cotyledons and young leaf explants of chickpea (*Cicer arietinum* L.) on MS medium with various concentrations of auxins and cytokinins. NAA and 2,4-D were used in the range of 1.0-16.0 mgl-^{1}. NAA 10.0-14.0 mg L^{-1} induced direct embryo formation on cut edges of immature cotyledons. (Naz*et al*., 2008)

Ali and Bano. (2008) investigates the effect of Kinetin and Abscisic acid (ABA) on leaf and nodule senescence in chickpea (*Cicer arietinum* L.) CV. ABA at 10.0-5.0 µM was effective in enhancing leaf and nodule senescence resulting in significant decrease in plant growth and yield.

A higher frequency of adventitious shoots buds/multiple shoots formation was observed in TDZ containing medium. TDZ induces high frequency of somatic embryogenesis or adventitious bud formation either alone or incombination with other growth regulators IBA, NAA and Kinetin (Anwar*et al*., 2008).

Vani and Reddy. (1996) study the epicotyls explants rise to multiple shoots when cultured on B5 medium containing BA (1.0 mgl^{-1}) and Kin (1.0 mgl^{-1}) + IAA (0.5mgl^{-1}).

Kumar *et al*., (1994) found that 2,4-D (1.0-1.25 mgl^{-1}) could induce embryogenic response only in 20 per cent for leaf explants in the chickpea cultivar C235. Whereas, using of kinetin (0.25 mgl^{-1}) in combination with 2,4-D could bring out embryogenic response in about 90% explants. The well-formed embryos germinated into plantlets on basal B5 medium. The same group also gave regeneration protocol, using 0.25 mgl^{-1} 2,4-D picloram each 0.1mgl^{-1} BAP in the variety BG 256 for maturation of embryos modified B5 medium with 2.0 mgl^{-1}.

Immature leaflet explants of chickpea (p-256) develop callus on MS medium supplemented with 1.25 µM 2,4-D and 10.0 µM BAP and calli were regenerated on MS+ 5.0 µM BAP + 10.0 µM IBA. (Rizvi and Singh, 2000).

Maximum 26 shoots explant were induced from single cotyledon with half embryonic axis in MS medium supplemented with 1.6 mgl^{-1} BA,

0.04 mgl-$^{-1}$ NAA and shoots elongatedwhen 0.2 mgl^{-1} IAA was added (Chakraborti *et al*., 2006).

Isolation and regeneration of chickpea protoplasts from hypocotyl derived protoplasts cultured on V47 medium supplemented with NAA (1.5 mgl^{-1}) and BAP (0.5 mgl^{-1}) were able to produce microcalli, but microcalli failed to undergo differentiation and organogenesis to produce plantlets (Sagare and Krishnamurthy, 1991).

Polowick *et al*., (2004) used rooting medium consisting of B5 basal salts and vitamins supplemented with 1.0 µM NAA for rooting the chickpea shoots and subsequently shoots with short roots which were transferred to magenta vessels containing B5 salts and vitamins and0.7% agar until root system was well established in 1 - 3 weeks while those with roots longer than 3.0 cm were transferred directly to soil.

Richa and Singh. (2002) developed efficient plant regeneration protocol via somatic embryogenesis from embryonic axes on MS medium supplemented with 1.25 mgl^{-1} and 0.25 mgl^{-1} kinetin. Differentiation of masses callus with globular structured embryos was observed during dark incubation.

Jayanand *et al*., (2003) used explants derived from the axillary meristems from the cotyledon nodes of *in vitro*–germinated seedlings of chickpea. TDZ and lower concentration of 2-iP and kinetin in the

elongation of medium resulted in faster and enhanced frequency of elongated shoots.

Chapter Three

Materials and Methods

3.1: Plant material

Seeds of three varieties (HUDAS, FLIP03-147C and FLIP05-100C) provided from ICARDA and two landraces ('Ein Al- Bayda' and 'Baladi') provided from local collection (Faculty of Agriculture, An-Najah Nat. University)

3.2: Seed disinfestations

Seeds were sterilized for 15 minutes in a 40 % Chlorax solution (5.25% sodium hypochlorite) containing 0.1% (v/v) tween 20 as wetting agent. Seeds were rinsed three times with sterile distilled water for 5 minutes to remove the traces of the bleach.

3.3: Medium preparation

Chickpea seeds were transferred aseptically to MS medium supplemented with 30 gml^{-1} sucrose, 0,1 gml^{-1} myinistol and solidified with 8.0 gm agar. The pH of the medium was adjusted to 5.6 with 0.1M KOH or 0.1M NaOH before solidifying with agar. The solidified medium was sterilized by autoclaving at 121°C for 21 minutes.

3.4: Establishment of the plant material

After disinfestations, seeds of chickpea were planted in test tubes each containing 10 ml of MS based medium, one seed per test tub. The tubes 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.

3.5: Explant preparation

The *in vitro* grown seedlings were used as a source of two types (stem and leaf) explant; the explants were excised after 14 days of germination. For regeneration experiments, explants were taken from the seedling cultures.

3.6: Shoot multiplication experiment

In this experiment shoots tip of about 2.0 cm length were cut from the establishedseedlings transferred into the hormonal combination media and incubated under 22 ± 1°C 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 BA on shoot multiplication

Shoot explants were excised and cultured on MS (Murashige and skoog, 1962) medium supplement with different concentrations of Benzyl Amino Purine BAP (0.0, 2.2 and 4.4 μM) in combination or alone withtheauxin 1-Naphthaleneacetic acid NAA at 0.0 and 2.7 μM (Table 3.1).

3.6.2: Effect of NAA and Kinetin on shoot multiplication

Shoot explants were excised and cultured on MS medium supplement with different concentrations of Kinetin at (0.0, 9.2 and 18.4 μM) in combination or alone with the auxin 1- Naphthaleneacetic acid

NAA at 0.0 and 2.7 µM (Table 3.2). pH was adjusted to 5.56 prior to autoclave. Cultures were incubated at similar conditions. Data were recorded after 3 weeks of culture.

Table 3.1: Concentration of PGRs used for chickpea shoot multiplication

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

Table 3.2: Concentration of PGRs used for chickpea shoot multiplication

Treatment	**PGRsconcentration (µM)**	
	NAA	**KIN**
1	0.0	9.2
2	0.0	18.4
3	2.7	9.2
4	2.7	18.4

3.7: Regeneration experiment

In this experiment two different explants from *in vitro* established seedlings were used (stem and leaf). Each explant was cut into two pieces and cultured on 9.0 cm diameter petri dishes (4 segments for each plate) containing 25.0 ml of MS media.

3.7.1: Effect of NAA and TDZ on shoot regeneration

3.7.1.1: Experiment One

MS media supplement with two different PGR levelsNAA at 0.0, 2.7 μM, and Thidiazuron (TDZ) at 0.0, 1 and 2 μM was used in this experiment (Table 3.3). pH was adjusted to 5.56 prior to autoclave. The cultures were incubated under dark condition for 2 weeks; then they were transferred under similar condition of the germination experiment. After 3 weeks every petri was tested for shoot regeneration.

3.7.1.2 Experiment two

In this experiment, higher levels of TDZ (0.0, 3 and 4 μM) combined with 0.0, 2.7 μM of NAA were used (Table 3.4). pH was adjusted to 5.56 prior to autoclave.

3.7.2: Effect of NAA and Kinetin on shoot regeneration

Explants were cultured on medium supplemented with different PGR levels of kinetin (0.0, 2.3 and 4.6 μM) applied alone or combined with

NAA at 0.0, 2.7 µM (Table 3.5). PH was adjusted to 5.56 prior to autoclave. All cultured were incubated under dark condition for 2 weeks; then they transferred in the incubator under 22 ± 1°C with 16 h day light. After 3 weeks every petri was tested for shoot regeneration.

Table 3.3: Concentration of PGRs used for chickpea shoot regeneration (Experiment One)

Treatment	PGRs concentration (µM)	
	NAA	TDZ
1	0.0	0.0
2	0.0	1.0
3	0.0	2.0
4	2.7	0.0
5	2.7	1.0
6	2.7	2.0

Table 3.4: Concentration of PGRs used for chickpea shoot regeneration (Experiment two)

Treatment	PGRs concentration (µM)	
	NAA	TDZ
1	0.0	0.0
2	0.0	3.0
3	0.0	4.0
4	2.7	0.0
5	2.7	3.0
6	2.7	4.0

Table 3.5: Concentration of PGRs used for chickpeashoot regeneration

Treatment	PGRs concentration (µM)	
	NAA	**KIN**
1	0.0	0.0
2	0.0	2.3
3	0.0	4.6
4	2.7	0.0
5	2.7	2.3
6	2.7	4.6

3.8: Statistical analysis:

The treatments in multiplication and regeneration experiments were considered as factorial treatment design, each combination of the growth regulators in each experiment were considered as a treatment, the treatments were arranged in completely randomized design with 10 replicates in multiplication and 3 replicates in regeneration experiments. Collected variables were summarized and analyzed in one way analysis of variance (ANOVA) using SAS software (SAS, 1990). Comparative analysis was conducted for the significant result using LSD at 0.05 probability level.

Chapter Four

Results

4.1: Seed plantation

After 2 weeks, seeds of different varieties and landraces germinated successfully onto the basal medium (Fig 4.1 A,B), however 'Baladi' exhibit the highest growth onto MS medium comparing with FLIP05-100c, Ein Al-Bayd landrace, FLIP03-147c, and 'Hudas' (Table 4.1). No contamination was observed in any of the treatments. There was significant differences in seed germination percentages among the 5 investigated chickpea types. The highest germination % was observed for Baladi landrace 75% followed by Ein Al - Bayda landrace, HUDAS, and FLIP05-100c 43%, 30%, and 30% respectively and least recorded FLIP03-147c with 25%.

Table 4.1: Germination % of chickpea seeds on MS- media

CV	Germination %
1	25
2	30
3	30
4	75
5	43

1= FLIP03-147c variety, 2= 'HUDAS' variety, 3= FLIP05-1ooc variety, 4= Baladi landrace, 5= Ein Al -Bayda landrace

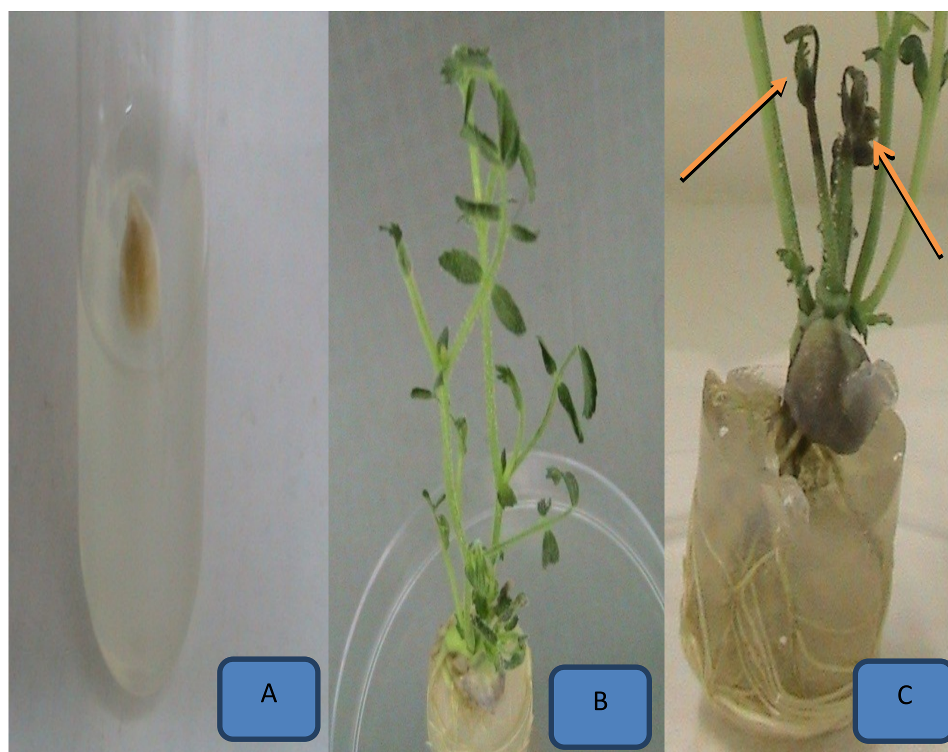

Fig (4.1) Growing chickpea seed on MS basal media: A) Chickpea seed on MS media, B) Chickpea seed on MS media after 14 days, C) The variety (FLIP03-147c) on MS media where browning of tissues appear as a result of phenol compounds.

4.2: Shoot multiplication experiment

4.2.1: The effect of different levels of BA and NAA on shoot multiplication

4.2.1.1: Baladi landrace

The effect of both NAA and BA on shoot number of 'Baladi' is presented in (Table 4.2). No significant interaction was observed between NAA and BA; therefore, the result of each factor was presented separately. The highest shoot number (8.0) was obtained at media without hormone and when BA was used at 2.2 µM (Fig 4.2 A), whereas lower shoot number

per plant (1.3 and 3.5) were observed in explants treated with 2.7 µM NAA and 4.4 µM BA (Fig 4.2 B). All of shoot explantswere axillary shoot.

4.2.1.2: Ein Al- Bayda landrace

The effect of both BA and NAA on shoot number of 'Ein Al-Bayda'is presented in (Table 4.3). The statistical analysis reveal no significant interaction between NAA and BA on shoot multiplication, therefore, the result of each factor was presented separately. The highest shoot number (5.95) was obtained at media without hormones and when BA was used at 2.2 µM (Fig 4.2 C), whereas lower shoot number per plant (2.1, 3.8) were observed in explants treated with 2.7 µM NAA and 4.4 µM BA (Fig 4.2 D).

Table 4.2: The effect of different concentration of BA, NAA on the number of shoots multiplication of chickpea Baladi local landrace.

Hormone(µM)	Level	No of shoot
NAA	0.0	8.0 a
	2.7	1.3 b
BA	2.2	5.8 a
	4.4	3.5 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Table 4.3: The effect of different concentration of BA, NAA on the number of shoots multiplication of chickpea Ein Al- Bayda local landrace.

Hormone (µM)	Level	No of shoots
NAA	0.0	5.950 a
	2.7	2.100 b
BA	2.2	4.250 a
	4.4	3.800 a

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Fig (4.2): Shoot multiplication of chickpea cultivars: A) Shoot multiplication ofBaladi local landrace on (2.2µM) BA with stunted growth, B) Shoot multiplication of Baladi local landrace on (4.4 µM), C) Shoot multiplication of Ein Al-Bayda locallandrace on (2.2µM), D)Shoot multiplication of Ein Al-Bayda local landrace on (4.4 µM).

4.2.2: The effect of different levels of Kinetin and NAA on shoot multiplication

4.2.2.1: Baladi landrace

Data on shootmultiplication of 'Baladi' from shoottip cultured on MS – medium with both NAA and Kin is presented in (Table 4.4). No significant interaction was observed between NAA and Kin, therefore, the result of each factor was presented separately. The highest shoot number (5.31) was obtained at media without hormones (Fig 4.3 A). However When NAA was used at 2.7 µM no shoots were produced.Lower number of shoots were observed (2.31, 2.85) with (9.2, 18.4) µM Kin.

4.2.2.2: Ein AL- Bayda landrace

The effect of both NAA and Kin on shoot number of ' Ein Al- Bayda' is presented in (Table 4.5). There was no significant interaction effect between NAA and Kin on shoot number, therefore, the result of each factor was presented separately. The highest shoot number (4.45) was obtained at media without hormone (Fig 4.3 B). However, when NAA was used at 2.7 µM no shoots were produced, lower number of shoots were observed (2.75-1.70) with 9.2, 18.4 µM Kin.

Table 4.4: The effect of different concentration of Kin, NAA on the number of shoots multiplication of chickpea Baladi local landrace

Hormone (µM)	Level	No of shoots
NAA	0.0	5.31 a
	2.7	0.0 b
Kin	9.2	2.31 a
	18.4	2.85 a

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Table 4.5: The effect of different concentration of Kin, NAA on the number of shoots multiplication of chickpea Ein AL- Bayda local landrace

Hormone (µM)	Level	No of shoots
NAA	0.0	4.45 a
	2.7	0.0 b
Kin	9.2	2.75 a
	18.4	1.70 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

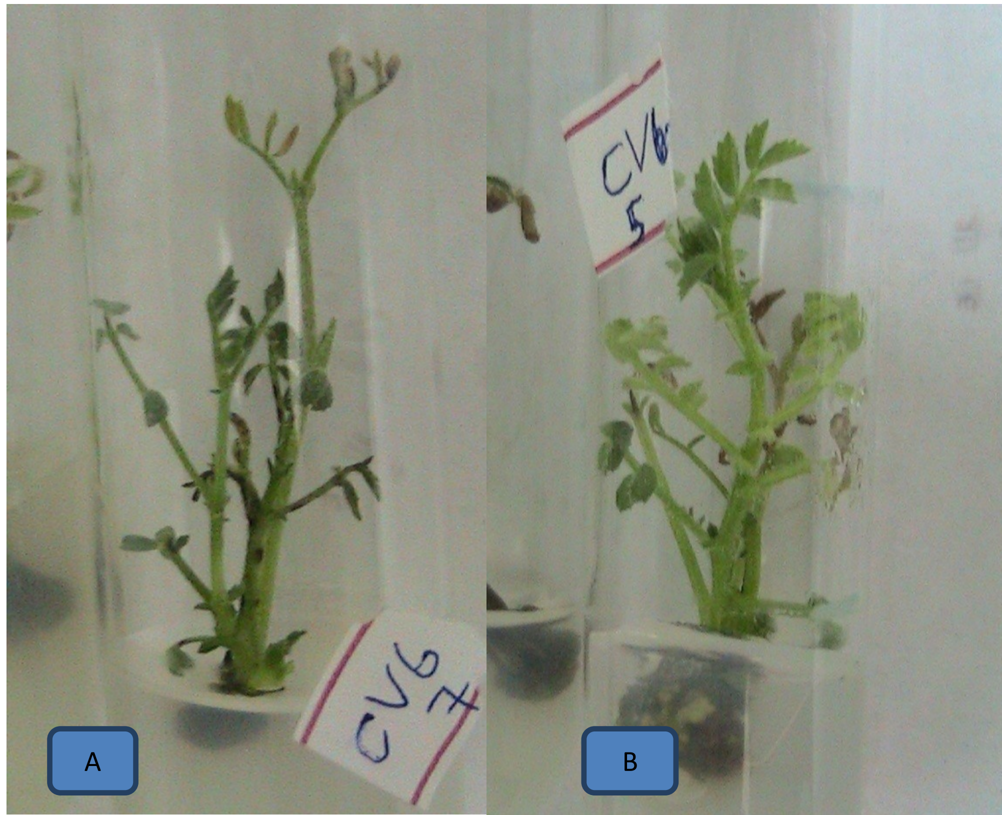

Fig (4.3): Shoot multiplication of chickpea landraces: A) shoot multiplication of Baladi local landrace, B) shoot multiplication of Ein Al-Bayda local landrace.

4.3: Regeneration experiment

4.3.1: Experiment one

4.3.1.1: Effect of explants, TDZ and NAA on shoot regeneration

4.3.1.1.1: Ein Al-Bayda landrace

Two types of explants (stem and leaf) were used in regeneration experiments with two cytokinin (TDZ and Kinetin) and auxin (NAA). The effect of explants and growth regulator on shoot percent and average number of shoot is presented in (Table 4.6, 4.7, 4.8). No significant interaction among the studied factors (explants and hormone) on shoot

regeneration was observed, therefore each factor was presented separately in different tables.

Table 4.6 shows, the effect of explants on shoot regeneration. Stem explants exhibited no regeneration; however leaf explants showed (43%) shoot regeneration, the same trend was observed with shoot number.

No significant effect on shoot percentage and average number of shoots observed when NAA was used, the same trend was observed with shoot number (Table 4.7). However, no significant effect of TDZ on shoot regeneration was observed in (Table 4.8). The highest shoot regeneration (29.2%) was recorded onto medium supplied 2.0 µM TDZ, medium without TDZ exhibited lower regeneration % as well shoot number (Fig 4.4).

Table4.6: The effect of explants on shoot % and average number of shoots of Ein Al-Bayda landrace

Explant	Shoot %	Av. Shoot no
Stem	0.0 b	0.0 b
Leaf	43.0 a	2.0 a

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Table4.7: The effect of NAA level on shoot % and average number of shoots of Ein Al-Bayda landrace

NAA level (µM)	Shoot %	Av.Shoot no
0.0	21.3 a	1.0 a
2.7	21.6 a	1.1 a

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Table 4.8: The effect of TDZ level on shoot % and average number of shoots of Ein Al-Bayda landrace

TDZ level (µM)	Shoot %	Av. Shoot no
0.0	12.5 a	0.5 a
1.0	22.9 a	1.25 a
2.0	29.2 a	1.33 a

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Fig (4.4) Shoot regeneration of Ein Al-Bayda local landrace on 2.0 µM TDZ with stunted growth.

4.3.1.1.2: FLIP03-174C variety

The effect of explants and growth regulator on shoot percentage and average number of shootsis presented in (Table 4.9). Significant interaction between the studied factors (explants and hormone) on shoot regeneration was observed. Stem explants exhibited no regeneration; however leaf explants showed (33.3%) of shoot regeneration at 1.0 µM TDZ. Medium with 2.7 µM NAA exhibited lower regeneration %, no significant interaction in shoot number was observed.

Table 4.9: The effect of explants, NAA and TDZ on shoot percent and average number of shoots of FLIP03-147C variety

Explant	NAA (µM)	TDZ(µM)	Shoot%	Av.Shoot no
Stem	0.0	0.0	0.0 c	0.0
Stem	2.7	0.0	0.0 c	0.0
Stem	0.0	1.0	0.0 c	0.0
Stem	2.7	1.0	0.0 c	0.0
Stem	0.0	2.0	0.0c	0.0
Stem	2.7	2.0	0.0 c	0.0
Leaf	0.0	0.0	0.0 c	0.0
Leaf	2.7	0.0	16.7 b	0.28
Leaf	0.0	1.0	33.3 a	0.14
Leaf	2.7	1.0	0.0 c	0.0
Leaf	0.0	2.0	16.7 b	0.14
Leaf	2.7	2.0	0.0 c	0.0
Sig interaction			Sig	NS

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

4.3.1.1.3: HUDAS variety

The effect of explants and growth regulator on shoot percentage and average number of shoots is presented in (Table 4.10, 4.11). Significant interaction among the studied factors (explants and NAA) on shoot regeneration was observed, however, TDZ showed no significant interaction so it was presented separately (Table 4.11). Both NAA and explants exhibited a significant effect on shoot regeneration (Table 4.10). Stem explants exhibited no regeneration, however leaf explants showed (33.0%) of shoot regeneration, the same trend was observed with shoot number. The effect of TDZ on shoot regeneration was shown in (Table 4.11). The highest shoot percentage (14.6 %) was recorded onto medium supplied with1.0 µM TDZ (Fig 4.5), medium without TDZ exhibited lower regeneration % as well with shoot number.

Fig (4.5) Shoot regeneration of HUDAS variety on 1.0 µM TDZ

Table4.10: The effect of NAA level and explants on shoot % and average number of shoots of HUDAS variety

	Explant			
NAA(µM)	Leaf		Stem	
	Shoot%	Av.Shoot No	Shoot%	Av.Shoot no
0.0	33.3 a	1.3 a	0.0 b	0.0 b
2.7	5.5 b	0.22 b	0.0 b	0.0 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Table4. 11: The effect of TDZ level on shoot % and average number of shoots of HUDASvariety

TDZ(µM)	Shoot%	Av.Shoot no
0.0	4.2	0.16
1.0	14.6	0.58
2.0	10.4	0.42
	NS	NS

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

4.3.1.1.4: Baladi landrace

The effect of explants and growth regulator on shoot percentage and average number of shoots is presented in (Table 4.12). Significant interaction among the studied factors (explants and hormones) on shoot regeneration was observed. The highest shoot percentage (50.0%) was obtained from leaf explants on medium containing 1.0 and 2.0 µM TDZ without NAA (Fig 4.6), when NAA was used at 2.7 µM no regeneration was observed. On the other hand, no shoot regeneration was achieved using stem explant.

Fig (4.6) Shoot regeneration of Baladi local landrace on 1.0 µM TDZ

Table 4.12: The effect of explants, NAA and TDZ on shoot % and average number of shoots of Baladi landrace

Explant	NAA(µM)	TDZ(µM)	Shoot %	AV. Shoot no
Stem	0.0	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	0.0 b	0.0 b
	2.7	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	0.0 b	0.0 b
Leaf	0.0	0.0	0.0 b	0.0 b
		1.0	50.0 a	2.5 a
		2.0	50.0 a	2.0 a
	2.7	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	0.0 b	0.0 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

4.3.1.1.5: FLIP05-100C variety

The effect of explants and growth regulators on shoot percentage and average number of shoot is presented in (Table 4.13). Significant interaction among the studied factors (explants and hormone) on shoot regeneration was observed. However, the highest shoot percentage (62.0%) was achieved from leaf explant growing on medium containing 2 µM TDZ without NAA (Fig 4.7), at 1.0 µM TDZ shoot number was reduced (42.0%). No shoot regeneration was obtained from stem explant. The medium contains 2.7 µM NAA, gave lower shoot number.

Fig (4.7) Shoot regeneration of FLIP05-100C variety on 2.0 µM TDZ

Table4.13: The effect of explants, NAA and TDZ on shoot % and average number of shoots of FLIP05-100c variety

Explant	NAA(µM)	TDZ(µM)	Shoot %	AV.Shoot no
Stem	0.0	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	0.0 b	0.0 b
	2.7	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	0.0 b	0.0 b
Leaf	0.0	0.0	0.0 b	0.0 b
		1.0	42.0 a	1.6 b
		2.0	62.0 a	3.0 a
	2.7	0.0	0.0 b	0.0 b
		1.0	0.0 b	0.0 b
		2.0	8.3 b	0.33 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

4.3.2: Experiment two

4.3.2.1: Effect of explants, TDZ and NAA on shoot regeneration

When higher level of TDZ was used, the regeneration was reduced as presented in (Table 4.14, 4.15), no regeneration was obtained with FLIP05-100C variety, however, very low regeneration % was obtained in FLIP05-100C variety, Baladi landrace and Ein Al-Bayda landrace.

4.3.2.1.1Baladi landrace

The effect of explants and growth regulator on shoot percentage and average number of shootsis presented in (Table 4.14).Regeneration was only obtained with leaf explants.No significant interaction among the studied factors (explants and hormones) on shoot regeneration was observed. Only 2.0% of regeneration was obtained with 3.0 μM TDZ with average shoot number of 0.25 (Fig 4.8).

Fig (4.8) Shoot regeneration of Baladi local landrace on 3.0 μM TDZ due to vitrification.

Table 4.14: The effect of explants, NAA and TDZ on shoot % and average number of shoots of Baladi landrace

Explant	NAA(µM)	TDZ(µM)	Shoot %	AV. Shoot no
Stem	0.0	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b
	2.7	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b
Leaf	0.0	0.0	0.0 b	0.0 b
		3.0	2.0 a	0.25 a
		4.0	0.0 b	0.0 b
	2.7	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

3.2.2.1.2: Ein Al-Bayda landrace

The effect of explants and growth regulator on shoot percentage and average number of shoot is shown in (Table 4.15). Regeneration was only obtained with leaf explants. No significant interaction between among the studied factors (explants and hormones) on shoot regeneration was observed. Only 4.0 % of regeneration was obtained with 4.0 μM TDZ with average shoot number 1.7 (Fig 4.9). The regeneration was obtained in leaf explants.

Fig (4.9) Shoot regeneration of Ein Al- Bayda local landrace on 4.0 μM TDZ

Table 4.15: The effect of explants, NAA and TDZ on shoot % and average number of shoots of Ein Al- Bayda landrace

Explant	NAA(µM)	TDZ(µM)	Shoot %	AV. Shoot no
Stem	0.0	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b
	2.7	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b
Leaf	0.0	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0b
		4.0	4.0 a	1.7 a
	2.7	0.0	0.0 b	0.0 b
		3.0	0.0 b	0.0 b
		4.0	0.0 b	0.0 b

Number followed by the same letter or letters are not significantly differ at 5% level according to (LSD Multiple Range test) (DMRT)

Chapter Five

Discussion

The study reported for the first time an *in vitro* study of local chickpea 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, genetic vulnerability may be defined as the susceptibility of most of the cultivated varieties of a crop species 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 study, seeds of all chickpea cultivars were treated with 40% Chlorox to optimize the level of Chlorox suitable for *in vitro* germination, this level prevented contamination, however, seed germination percentage was not the same in all varieties and landraces (Chaudhry *et al*., 2007). FLIP03-147C chickpea exhibited low growth after the initiation of germination, phenol metabolites was exudates from the seeds, this could explain the low

germination % associated with this variety (Fig 4.1C) (Fernandez-Orozco *et al*., 2009).

Sterilization is an important step, which affects growth and regeneration, Chlorox (Sodium hypochlorite) used as a surface sterilization agent, played an important role in germination of seeds (Chaudhry*et al*., 2007).

Phenolic are secondary metabolites that modulate plant development (Arnaldos *et al*., 2001) and plant reaction against biotic and a biotic stresses (Kefeli*et al*., 2003; Conceicao *et al*., 2006; Fan *et al*., 2006).

Oxidized phenolic compounds inhibit enzymatic activity and darken the culture medium, subsequently the explants brown or blacken and die (Laukkanen *et al*., 1999; Arnaldos *et al*., 2001). Several authors have suggested solutions to minimize the lethal browning or blackening of explants caused by phenolic compounds in plant tissue culture. Among these protocols are treating explants with ascorbic acid (Arditti and Ernst, 1993) adding a polyphenol adsorbent, such as activated charcoal (Arditti and Ernst, 1993), or antioxidants, such as cysteine (Sanyal *et al*., 2005), ascorbic acid (Arditti and Ernst, 1993), PVP (Lainé and David, 1994)or silver nitrate (Sanyal *et al*., 2005). Lopez-Amoros *et al*., (2006) indicated that chickpeas contains different concentrations of hydroxybenzonic phenol compounds, protocatechuic, p-hydroxybenzoic, vanillic acid trans-ferulic acid, cis and trans p-coumaric acid, however during the germination, the amount of phenol compounds was increased.

In this study, the number of multiplied shoots was not high, plants belong to the leguminosae are difficult to regenerate through*in vitro* propagation (Huda *et al*., 2000). The rate of shoot multiplication depends on the number of nodal cuttings that can be excised from the shoot at the end of each passage (Veraplakorn *et al*., 2012). Multiple shoot formation *via* organogenesis could occur directly from the explants or indirectly from the dedifferentiated callus. Both approaches are controlled by plant hormones and other factors added to the medium (Tang and Chen, 2011).

The importance of BA in multiple shoot formation in chickpea and other legumes is widely reported (Barik *et al*., 2004; Odutayo *et al*., 2005). Similar to the findings of Shagufta *et al*., (2007), the result of the present study indicate that BA level in the media is an important factor influencing the shoot. The highest shoot number was obtained when BA was used at 2.2 µM whereas lower shoot number per plant were observed in explants treated with 4.4 µM BA.

Sujatha *et al*., (2007) studied the effect of BA and explants on shoot multiplication in chickpea. BA at 4.44 µM was found to be more effective in inducing multiple shoots in three explant source, Cotyledon, shoot tip and nodes; they observed that cotyledon node produced the maximum number of shoots compared to shoot tip and nodal explants. Our result disagreed with Elke and Hess, (1994) who demonstrated that shoot number per explant increases with increasing BAP (6-Benzyl-Amino-Purine), Franklin *et al*., (1998) obtained maximum shoot number (49) on 3.0 mgl^{-1}

BAP with seedling explants (cotyledon node and shoot tip). In contrast, in this study, BA at 2.2 μM resulted in the highest shoot number, whereas with the higher level (4.4 μM) lower shoot number per plant was observed.

On the other hand, our result agree with Veraplakorn *et al*., (2012), who reported higher shoot number (6.4) in Cavalcade cowpea produced in MS medium supplemented with 1.0 mgl^{-1} BA, however, when BA concentration was increase the shoot formation decline, increasing both NAA and BA concentration tend to reduce shoot number.

Reduction in the number of shoots with increasing concentration of BAP was reported by Rehman *et al*., (2004), similar to our study higher concentrations of BAP reduced the shoot number in both chickpea Baladi and Ein Al- Bayda landraces.

Multiple shoot formation from shoot apices was obtained on MS medium supplemented with 20 μM BA + 0.1 μM NAA in pea (Griga *et al*., 1986). In both Baladiand Ein Al- Bayda landraces the addition of NAA at 2.7 μM reduces shoot production; similar finding in chickpea was reported by (Aasim *et al*., 2011).

BAis the most commonly used cytokinin followed by kinetin in plant tissue culture. In this study, the shoot number wasreduced when 9.2, 18.4 μM Kinetin was used, hence it is proved that the concentration of BAP can increase up to certain limit otherwise, it causes undesirable effects. Sawardekar. (2007) indicate that BAP produced limited number of multiple

shoots; he suggest that BAP can produce more than 12 shoots but BAP at higher concentration cause the swelling of explants and shoots become watery. MSmedia with B5 vitamins supplemented with 5.0 µM BAP were found to be highly effective in medium for multiple shoot formation (Polisetty *et al*., 1997).

Multiple shoots have been induced from shoot tips cultured on MS medium supplemented with 2.0 mgl^{-1} NAA and IBA 2.0 mgl^{-1} (Chandra *et al*., 1993) stunted shoots were obtained with 2.2 µM BA experiment in Baladi chickpea (Fig 4.2 A). The occurrence of stunted shoots on unconditioned explants cultured on medium containing BA with NAA might be due to inhibition of active cell division in these explants (Aasim *et al*., 2009a). Abdelwahd *et al*., (2008) reported high shoot induction (26.0) obtained on shoot elongation medium consisting of MS medium supplemented with 6.0 µM 2-ip and 3.0 µM kin for 10 days.

In our study the highest number of shoots was obtained when 0.0 µM NAA in both chickpea 'Baladi' and 'EIN Al -Bayad'. Shoots transferred to plant growth regulator free medium have potential to synthesize and maintain desired endogenous levels of gibberellins and other auxins, MS basal medium without the addition of plant growth regulators resulted in a large number of shoot production (Anwar *et al*., 2010a).

In our study direct regeneration from axillary buds was obtained.Similar results were reportedfrom other herbaceous legumes such as grasspea (Barik *et al*., 2007). Plant multiplied by forced axillary

branching may be due to cytokinin responsibility (Bhowjwani and Razdan, 1996).

The presence of cytokinin to stimulate cell division is required to propagate the embryonic stage and to form new multiple shoots, these compounds used for shoot proliferation by release of axillary buds from apical dominance (Khalisi, 2009). TDZ is an expensive GR that is not economic and results in abnormal shoots, so BAP could be used as efficient GR with economic cost in multiple shoot production (Shalini *et al*., 2001). It was reported that BA proved to be an ideal hormone for shoot multiplication of shoot tip culture in legumes (Sounder *et al*., 1989).

In this study, variation in shoot regeneration among explants used was clear, this result was consistent in all varieties and landraces. Leaf explants showed higher regeneration than stem. Morphogenetic responses of plant tissue culture are controlled by the optimal selection of the explants (Mohamed *et al*., 1992). In chickpea explants such as, cotyledon, epicotyls, hypocotyl, immature leaflets, leaves, internodes and root tips have been tried to induce callus (Vani and Reddy, 1993). The findings of this study agreed with Hofmann *et al*., (2004) who reported that leaf explants is a good source for shoot induction through callus in chickpea. However, in other studies no shoot or callus regeneration was achieved with both leaf or stem explant in lentil (Khawar *et al*., 2004). On the other hand, Aasim *et al*., (2011) reported successful shoot regeneration from mature embryo and embryonic axis explants of chickpea.

The higher shoot regeneration ability from leaf explantcompared to other explants could be due to meristem activity (Hinchee *et al*., 1988). In our study, stem explant resulted in callus tissue formation only, no other differentiation was observed. Aasim *et al*., (2010) showed that cotyledon nodal explants regenerate more compared to hypocotyl explants which induced callus only. Jayanand *et al*., (2003) developed an efficient and reproducible protocol for the regeneration of shoots at high frequency using explants derived from the axillary meristems in chickpea plant.

Different tissues when grown on the same media show variability in differentiation. This probably could be due to either endogenous levels of various growth hormones or relative sensitivity to various auxins (Chandra *etal*., 1993). Hence, there is a need to standardize regeneration protocol for each type of explant.

In our study, shoot regeneration was observed when lower levels of TDZ were used, however, no regeneration was observed with Kinetin. Plant growth regulators had effective role on the induction of callus, shootsand the concentration of the growth regulators; play a vital role in regeneration of plants in vitro (Aloni *et al*., 2006).

Our result agreed with the findings of other researchers, who reported that TDZ at lower concentration is better than BA, Kinetin or 2i-P (Saini and Jaiwal, 2002; Keneda *et al*., 1997; Yoshida, 2002; Genga and Allavena, 1991).

In addition,Anwar *et al*., (2010b) indicated that TDZ at 4.0 µM in combination with 2i-P (10.0 µM) and Kinetin (2.0 µM) was found to be optimal for the expansion of merisematic zone followed by shoot induction of chickpea. On the other hand Huda *et al*., (2003) reported the maximum percentage (40.0) of shoots bud formation was obtained on MS medium with 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA. In our study, shoot vitrification was observed with Baladi and EinAl-Bayda landraces with 3.0 and 4.0 µM of TDZ (Fig 4.8, 4.9), similar finding was reported with Aasim *et al*., (2008) and Asaim *et al*.,(2009b)on cowpea plant.

In our study more callus was observed with NAA containing media, this is agreed with the findings of Aasim *et al*., (2009c) who also reported a positive effect of the presence of NAA in the culture medium on callus induction in cowpea. The addition of NAA in the culture medium resulted in decreasing shoot regeneration. This finding agrees with Brar *et al*., (1997) who reported a negative effect of NAA along with BA on shoot regeneration in cowpea.

In Ein Al –Bayda landrace, stunted shoots were observed with TDZ application (Fig 4.4) might be due to inhibitions and less active cell division, which agreed with Chen et al., (1995) who observed stunted shoots on unconditioned explants of bean.

Thidiazuron (TDZ) has been reported as a potent growth regulator for *in vitro* morphogenesis in many plant species including grain legumes (Wang *et al*., 2008).Thidiazuron has been demonstrated as a better

induction factor for organogenesis and somatic embryogenesis in chickpea (Rizvi and singh, 2000). Although the exact mechanism of action of TDZ is not clear, it is believed to be involved in regulating endogenous levels of various growth regulators (Malik and Sexena, 1992). The high activity of a low concentration of TDZ has not been investigated, we assumed that TDZ is persistent in plant tissue and is metabolized in manner similar to that reported with *phaseolus luntus* L. (Mok and Mok, 1985). TDZ when interacted with endogenous hormones, it reprograms the mode of morphogenesis (from organogenesis to somatic embryogenesis) possibly by releasing, synthesizing, protecting or even inhibiting auxins *in situ* in combination with other sub culture metabolic change (Gill and Saxena, 1992).

In this study stem and leaf explants showed no response to shoot regeneration in all five cultivars when Kinetin was used in the media. However, the internode explants was found to be the best for callus induction with different combinations of 2.0 mgl^{-1} kinetin and 2.0 mgl^{-1} IAA (Huda *et al*., 2000).

Organogenic differentiation in cell and tissue culture is due to hormonal manipulation of the culture medium, morphogenesis of shoot *via* organogenic is highly affected by the plant hormones (George *et al*., 2008).

In our experiment, the media supplied with 1.0 and 2.0 µM TDZ, showed variation response among the 5 genotypes studied. The organogenic response varied greatly with the genotypes, the significance of

the genotype in determining cultures response was recognized (Cardinale *et al*., 2007). Our findings agree with (Singh *et al*., 1997) who reported variation in shoot regeneration (48-85%) among 3 chickpea lines (BG-256, C235, PDG).

In another study, 39% shoot regeneration was obtained with the MCC764 chickpea line, no shoot regeneration was observed with other lines (Mirkabad *et al*., 2010). Moreover, Sayem *et al*., (2010) reported different shoot regeneration of chickpea genotypes.

In our study all chickpea genotypes (Baladi landrace, Ein Al-Bayda landrace, HUDAS variety, FLIP05-100c variety, FLIP03-147c variety) showed no response when cultured on MS containing Kin.

In contrast to our study, good shoot health and expanded leaf were observed on MS medium containing 1.0 mgl^{-1} Kin, Binachhola-3 chickpea failed to produce any shoot, other three local verities (Barichhola-4, Binachhola-4, Hyprochhola) showed better response in this medium (Banu *et al.,* 2011).

Chapter Six

Conclusions and Recommendations

6.1 Conclusions

The results of the present investigation demonstrated that the high variability in the growth and regeneration of local chickpea varieties, BA at 2.2 µM was good for shoot multiplication from shoot tip in Baladi and Ein Al-Bayda local landraces, while TDZ at 2.0 µM alone was the best for shoot regeneration from leaf explant in FLIP05-100c variety, leaf explants were better source than stem section for shoot regeneration. The establishment of an *in vitro* multiplication and regeneration protocol for Palestinian chickpea landraces could be effectively used for the improvement of these landraces through biotechnology technique.

6.2 Recommendations

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

References

1. Aasim, M., K.M. Khawar, and S.Özcan. 2008. ***In Vitro* micropropagation from shoot meristems of Turkish Cowpea (*Vigna unguiculata* L.)** cultivar Akkız. Bangladesh J. Bot, 37: 149-154.

2. Aasim, M., K.M. Khawar, and S. Özcan. 2009a. ***In vitro* micropropagation from plumular apices of Turkish cowpea (*Vignaun guiculata* L.)** cultivar Akkiz. Sci. Hort, 122: 468-471.

3. Aasim, M., K.M. Khawar, and S. Özcan. 2009b. ***In vitro* shoot regeneration of Fenugreek (*Trigonella foenum graceum* L.).** Am-Eu J. Sustain. Agric, 3: 135-138.

4. Aasim, M., K.M. Khawar, and S. Özcan. 2009c. **Comparison of shoot regeneration on different concentrations of TDZ from shoot tip explant of cowpea on gelrite and agar containing medium.** Not. Bot. Hort. Agro Bot. Cluj, 37: 89-93.

5. Aasim, M., K.M. Khawar, and S. Özcan. 2010. **Efficient *in vitro* propagation from preconditioned embryonic axes of Turkish cowpea (*Vignaun guiculata* L.)** cultivar akkiz. Arch. Biol. Sci, 62: 1047-1052.

6. Aasim, M., N. Hussain, E.M. Umer, M. Zubair, S.B. Hussain, S. Saeed, T.S. Rafique, and C. Sancak. 2010. ***In vitro* shoot regeneration of fenugreek (*Trigonella foenum-graecum* L.)** using different cytokinins.African J of Biot. Vol. 9(42), pp. 7174-7179.

7. Aasim, M., S. Day, F. Rezaei, M. Hajyzadeh, S, Tahir, and S. Özcan. 2011. ***In vitro* shoot regeneration from preconditioned explants of chickpea (*Cicer arietinum* L.)** cv. Gokce. African J of Biot. Vol. 10 (11), pp. 2020-2023.

8. Aasim, M., S. Day, F. Rezaei, and M. Hajyzadeh. 2013. **Multiple shoot regeneration of plumular apices of chickpea**. Turk J Agric, 37:33-39.

9. Abbo, S., D. Shtienberg, J. Lichetzveig, S.L. Yadun, and A. Gopher. 2003. **The chickpea, summer cropping, and a new model for pulse domestication in the ancient near east.** Q. Rev. Biol, 78, 435e448.

10. Abdelwahd, R., N. Hakam, M. Labhilili, and S.M. Udupa. 2008. **Use of an adsorbent and antioxidants to reduce the effects of leached phenolic*in vitro* plantlet regeneration of faba bean.** African. J. Biot, 7:997-1002.

11. Ahmadi, A., D. Azadfar, and A.J. Mofidabadi. 2010. **Study of inter-generic hybridization possibility between Salix aegyptica and *Populus caspica* to achieve new hybrids**. Int.J Plant Prod, 4(2): 143-147.

12. Ali, S., and A. Bano. 2008. **Leaf and AdoLeScence in chickpea (*Cicer arietinum* L.) and the role of plant growth regulators**. Pak. J. Bot, 40(6): 2481-2492.

13. Aloni, R., E. Aloni, M. Langhans, and C.I. Ullrich. 2006. **Role of cytokinin and auxin in shaping root architecture: regulating vascular**

differentiation, lateral root initiation, root apical dominance and root gravitropism. Annals of Bot, 97: 883-893.

14. Anwar, F., P. Sharmila, and P. Saradhi. 2008. **An ideal protocol for *in vitro* regeneration, efficient rooting and stable transplantation of chickpea.** Physiol. Mol. Biol. Plants, 14: 329-335.

15. Anwar, F., S. Alghamidi, H. Ammar, and K.H.M. Siddique. 2010a. **An efficient *in vitro* regeneration protocol for faba bean (*Vicia faba* L.).** Journal of Medicinal Plants Res. Vol. 5(28), pp. 6460-6467.

16. Anwar, F., P. Sharmila, and P. Saradhi. 2010b. **NO more recalcitrant: Chickpea regeneration and genatic transformation.** African J of Biot. Vol. 9 (6), pp. 782-797.

17. Arditti, J., and R. Ernst. 1993. **Micropropagation of orchids**. John Wiley & Sons, New York.J.Bot of the Linnean Society, 122: 183–241.

18. Arnaldos, T.L., R. Munoz, M.A. Ferrer, and A.A. Calderon. 2001. **Changes in phenol content during strawberry (Fragaria x ananasa, cv. Chandler) callus culture**. Physiol. Plant, 113: 315-322.

19. Bairu, M.W., W.A. Stirk, and J.V. Staden. 2009. **Factors contributing to *in vitro* shoot-tip necrosis and their physiological interactions**. Plant Cell Tissue and Organ Culture, 98:239–248.

20. Banu, T.A., R.H. Saker, and M.L. Hoque. 2011. ***In vitro* regeneration of four local varieties chickpea grown in Bangladish**. Bangladish J. Sci. Ind. Res, 46 (3).379-384.

21. Barik , D.P., S.K.U. Naik, A. Nudgal, and P.K. Chand. 2004. **Rapid plant regeneration through *in vitro* axillary shoots proliferation of butterfly pea (*Clitoria ternatea* L.)**. *In vitro* cell. Dev. Biol. Plant, 43:144-148.

22. Barik, D.P., S.K.U. Naik, M. Ohapatra, and P.K. Chand. 2007. **High-frequency plant regeneration by *in vitro* shoot proliferation in cotyledon node explant of Grasspea (*Lathyrus sativus* L.)** *in vitro* cellular. Dev. Biol plant, 40: 467-470.

23. Barna, K.S., and A.K. Wakhlu. 1993. **Somatic embryogenesis and plant regeneration from callus cultures of chickpea (*Cicer arietinum* L.).** Plant cell reports, 12(9): 521-524.

24. Barna, K.S., and A. K. Wakhlu. 1995**. Modified single node culture method – A new micropropagation method for chickpea**. *In vitro* Cell Developmental Biol Plant, 31: 150.

25. Batra, P., N.R. Yadav, A. Sindhu, R.C. Yadav, and V. K. Chowdhury. 2002. **Efficient protocol for *in vitro* direct plant regeneration in chickpea (*Cicer arietinum* L.)**. Indian J. Exp. Biol, 40 (5):600-602.22.

26. Bhojawani, S.S., and M.K. Razdan. 1996. **Clonal Propagation. In: Bhojwani S.S. and Razdan M.K. (Eds.). Plant Tissue Culture: Theory and Practice,** A Revised Edition. Elsevier, Amsterdam, pp. 483-536.

27. Boef, W.S., T. Berg, and B. Haverkort. 1996. **Crop genetic resources in J. Bunders, B. Haverkort and W. Hiemstra (eds). Biotechnology; building on farmers' know-ledge**. Macmillan, London and Basingstoke, PP. 103-128.

28. Brar, M.S., J.M. Al-Khayri, C.E. Shamblin, R.W. Mcnew, T.E. Morelock, and E.J. Anderson. 1997. ***In vitro* shoot tip multiplication of cowpea (*Vigna unguiculata* L.)**. *In Vitro* Cell Dev. Biol, 33:111-118.

29. Brenes, A., A. Viveros, C. Centeno, I. Arija, and F. Marzo. 2008. **Nutritional value of raw and extruded chickpeas (*Cicer arietinum* L.)** for growing chickeans. Journal of Agric Res, 6(4), 537-545

30. Cardinale, B.J., J.P. Wright, M.W. Cadotte, I.T. Carroll, A. Hector, D.S. Srivastava, M. Loreau, and J.J. Weis. 2007. **Impacts of plant diversity on biomass production increase through time because of species complementarity**. Proc Natl Acad Sci USA, 104:18123–18128.

31. Chakraborti, D., A. Sarkar, andS. Das. 2006**. Efficient and rapid *in vitro* plant regeneration system for Indian cultivars of chickpea (*Cicer arietinum* L.)**. Plant cell tissue organ cult, 86: 117-123.

32. Chandra, R., A. Chatrath, R. Polisetty, and S. Khetarpal. 1993. **Differentiation of *in vitro* grown explants of chickpea (*Cicer arietinum* L.)** Ind. J. Plant Physiol, 36: 121-124.

33. Chatenet, M., C. Delage, M. Ripolles, M. Irey, B.L.E. Lockhart, and P. Rott. 2001. **Detection of sugarcane yellow leaf virus in quarantine and production of virus-free sugarcane by apical culture**. Plant Disease, 85(11):1177-1180.

34. Chaudhry, Z., A. Afroz, and H.Rashid. 2007. **Effectof varietyand plant growthregulatorson callus Proliferationand regeneration responseof three tomato cultivars (***Solanum lycopersicum* **L.)**. Pak. J. Bot, 39(3): 857-869.

35. Chen, J., F.H. Witham, and C.W. Heuser. 1995. **Inhibition of NAA-induced adventitious roots in mung bean cuttings by Kinetin, Zeatin, Ethidium bromide and other DNA intercalators**.World wide web J Biol, 1:1-8.

36. Christensen, B., S. Sriskandarajah, M. Serek, and R. Muller. 2008. ***In vitro* culture of Hibiscus rosasinensis L.: Influence of iron, calcium and BAP on establishment and multiplication**. Plant Cell Tissue and organ Culture, 93(2):151-161.

37. Conceicao, L.F., F. Ferreres, R.M. Tavares, and A.C. Dias. 2006. **Induction of phenoliccompounds in *Hypericum perforatum* L. cells by colletotrichum gloeosporioides elicitation**. Photochem, 67: 149-55.

38. Corby, R. 1981. **Seeds of Leguminosae**. Pages 913-915 in Advances in legumes systemic. Part 2 (Polhill, R.M., and Raven, P.H., eds.). Kew, UK: Royal. Bot Gar dens.

39. Cubero, J.I. 1975. The research on chickpea (*Cicer arietinum* L.) in Spain (1975). **Proceedings of the International Workshop on Graih Legumes, ICRISAT Hyderabad, India**: International Crops Research Institute for the Semi-Arid Tropics. Pages 117-122

40. Cubero, J.I. 1987. **Morphology of chickpea**. Pages 35-66 in the chickpea (Saxena, M.C., and Singh, K.B., eds.). W allingford, Oxon, UK: CAB International.

41. Davey, M.R., V. Kumar, and N. Hammatt. 1994. ***In vitro* culture of legumes. In: Vasil IK, Thrope TA (eds) plant tissue culture**. Kluwer, Dordrecht, pp313-329.

42. Debergh, p. 1994. ***In vitro culture of ornamental. In : Vasil IK, Thrope TA (eds) plant cell and tissue culture***. *Kluwer, Dordercht, pp 561-573.*

43. Donmez, A.A. 2011. ***Cicer* uludereensis Dönmez: a new species of *Cicer* (Chickpea) (Fabaceae) from around the Fertile Crescent, SE Turkey**. Turkish Jof Bot, 35, 71-76.

44. Elke, B., and B.D. Hess. 1994. ***In vitro* regeneration and propagation of chickpea (*Cicer arietinum* L.)** from meristem tips and cotyledonary nodes. *In vitro* cellular Dev. Biol, 30(1):75-80.

45. Fan, L., R. Linker ,S. Gepstein, E. Tanimoto, R. Yamamoto, and P.M. Neumann. 2006. **Progressive inhibition by water deficit of cell wall extensibility and growth along The elongation zone of maize roots is related to increased lignin metabolism and progressive stellar accumulation of wall phenolic**. Plant Physiol, 140: 603-12.

46. FAO. 2008. **Food and Agricultural Organization Statistical Database**. Rome: FA Food andAgric Organization. www.faostat.org .

47. FAO. 2008. **The FAO Statistical Databases and Datasets (FAOSTAT) Production Statistics**. Rome: FAO.

48. Fernandez-Orozco, R., J. Frias, H. Zielinski, M. Munoz,M.K. Piskula, H. Kozlowska, and C. Vidal-Valverde. 2009. **Evaluation of bioprocesses to improve the antioxidant properties of chickpeas**. Food Res. Technol, 42: 885-92.

49. Ford, L.B., and M. Jackson. 1986. **Plant genetic resources: an introduction to their conservation and use**. Edward Arnold, London.

50. Fraguas, C.B., M. Pasqual, L.F. Dutra, and O.J. Cazetta. 2004. **Micropropagation of fig (*Ficus carica* L.) 'Roxo de Valinhos' plants**. *In Vitro* Cellular and Dev Biol-Plant, 40(5):471-474.

51. Fraguas, C.B., C.M.D. Dornelles, and G.P.P. Lima. 2009. ***In vitro* bud induction and multiplication of cv. 'IAC Gomo-de-mel' pineapple fruit with benzyl amino purine and naphthalene acetic.** Ciencia Rural, 39(6):1682-168b.

52. Franklin, G., R. Jeyachandran, G. Melchias, and S. Lgnacimuthu. 1998. **Multiple shoot induction and regeneration of pigeonpea (*Cajnus cajan*L. Millsp)** cv. Vamban 1 form apical and axillary meristem. Curr. Sci,74 : 936-937.

53. Gamborg, O.L., T. Murashige, T.A. Thorpe, and I.K. Vasil. 1976. **Plant tissue culture media.***In Vitro,* 12, 473–478.

54. Gamborg, O.L. 2002. **Plant tissue culture Biotechnol Milestones**. *In Vitro* Cell Dev Biol, Plant, 38: 84–92.

55. Gaspar, T.H., C. Penel, F.J. Castillo, and H. Greppin. 1985. **A two-step control of basic and acid peroxidases and its significance for growth and development**.Physiologia Plantarum, 64:418-423.

56. Gautheret, R. J. 1934. **Culture du tissus cambial. C.R. Hebd**. Seances Acad. Sci, 198, 2195-2196.

57. Genga, A., and A. Allavena. 1991. **Factors affecting morpho- genesis from immature cotyledons of *Phasolus coc- cineus*.** Plant Cell Tissue Organe Culture, 27:189–196.

58. George, E.F., M.A. Hall, and G.J.D. Klerk. 2008. **Plant Propagation by Tissue Culture 3rd Edition,** Vol. 1. Springer, Dordrecht, the Netherlands, 501 p.

59. Gill, R. and P. K. Saxena. 1992. **Direct somatic embryogenesis and regeneration of plant from seedling explant of peanut (*Arachis hypogae*L):** Promotive role of Thidiazuron. Can J Bot, 70: 1186-1192.54.

60. Girga, M., M.K. Kovaj, and E.T. lovie. 1986. **Somatic embryogenesis in (*Vicia Fablel* L)**. Plant Cell Tissue and Organ Culture, 3:319-324.

61. Gonzalez-Olemedo, J. L., Z. Fundora, L.A. Molina, J.A. bdulnour, Y. Desjardins, and M. Escalona. 2005. **New contributions to propagation of pine-apple (*Ananas comosus* L) in temporary immersion bioreactors**. *In vitro* Cellular and Dev Biol plant, 41:87-90.

62. Gopalakrishnan, S., M.H. Beale, J.L. Ward and R.N. Strange. 2005. **Chickpea wilt: identification and toxicity of 8-O-methly-fusarubin from Fusarium acutatum.** Phytochem, Vol.66, pp. 1536-1539, Iss, 0031-9422.

63. Haberlandt, G. 1902. **Kulturversuche mit isolierten pflanzenzellen. Stizungsber Akad .** Wiss. Wien. Math-Naturwiss. KI., Abt. J. 111,69-92.

64. Hassan, S., and I. Khan. 1991. **Improvement in chickpea production through induce mutation.** International chickpea Newsletter, 25:12-13.

65. Hinchee, M.A.W., D.V. C.A. Connor-Ward Newell, R.E. Mcdonnell, S.J. Sato, C.S. Gasser, D.A. Fischhoff, D.B. Re. R.T. Fraley, and R.B. Horsch. 1988. **Production of transgenic soybean plants using *Agrobacterium tumefaciens-mediated* DNA transfer**, Biot, 6 915-922.

66. Hofmann, N., R.L, Nelson, and S.S. Korban. 2004. **Influence of media components and pH on somatic embryo induction in three genotypes of soybean**. Plant Cell Tissue and Organ Culture, 77: 157-163.

67. Hossain, M.D.A. 2009. **Management of chickpea pod borer, *Helicoverpa armigera* (Hubner) through intercropping's and insecticide spraying.** Thiland. J. Agric. Sci, 36 (1): 51-65.

68. Huda, S., R. Islam, and M.A. Bari. 2000. **Shoot regeneration from internode derived callus of chickpea (*Cicer arietinum* L.)**. International Chickpea and Pigeon pea Newsletter, 7:28-29.

69. Huda, S., R. Islam, and M.A. Bari. 2003. **Shoot differentiation from cotyledon derived callus of chickpea (*Cicer arietinum* L.)**. Plant tissue Culture, 13 (1): 53-59.

70. ISLAM, R., S. RIAZUDDIN, and H. FAROOQUI. 1995. **Clonal propagation from seedling nodes and shoot apices of chickpea (*Cicer arietinum L.*)**. Plant Tissue Culture, 5: 53–57.

71. Jayanand B, G. Sudarsanam, and K.K. Sharma. 2003. **An efficient protocol for the regeneration of whole plants of chickpea (*Cicer arietinum* L.)** by using axillary meristem explants derived from *in vitro* germinated seedlings. *In Vitro*: Cell. Dev. Biol. Plant, 39: 171-179.

72. Jayashree, B., K.B. Hutokshi, S. Sanjeev, and H.C. Jonathan. 2005. **A legume genomics resource: The Chickpea Root Expressed Sequence Tag Database**. Elect. J. Biot, 8(2): 128-133.

73. Jiang, B., Y. Yang, Y. Guo, Z. Guo, and Y. Chen. 2005. ***Thidiazuron*-induced *in vitro* shoot organogenesis of the medicinal plant *Arnebia euchroma* (Royle) Johnst**. *In Vitro* Cell. Dev. Biol. Plant, 41: 677–681.

74. Jones, M.G.K. (1994).***In vitro* culture of potato**.In :Vasil IK, Thrope TA (eds)plant cell and tissue culture. Kluwer, Dordercht, pp 363-378

75. Kefeli. V.I., M.V. Kalevitch, and B. Borsari. 2003. **Phenolic cycle in plants and environment**. J. Cell Mol. Biol. 2: 13-18.

76. Keneda, Y., Y. Tabei, S. Nishimura, K. Harada, T. Akihama, and K.Kitamura. 1997**. Combination of Thidiazuron and basal media with low salt concentrations increases the frequency of shoot organogenesis in soybean (Glycine max)**. Plant Cell Rep 17:8–12.

77. Kevers, C.T., R.J. Franck, J.Strasser, andT. Gaspar. 2004. **Hyperhydricity of micropropagated shoots: a typically stress-induced change of physiological state. Plant tissue culture** .And Organ Culture, 77 (2):181-191.

78. Khalisi, A.A.H. 2009. **The Effect of Different levels and kinds of Cytokinins on Buds proliferation of Iraqian Date Palm Cultiver (Barhi)** ***in vitro***. Department of Bio, college of education Ibn Al-Haitham, university of Baghdad. AL- HAITHAM J. for pure and Appl. Sci Vol.22 (2)

79. Khawer, M.K., G. Sancak, S. Uranbey, and S. Özan. 2004. **Effect of Thidiazuron on shoot regeneration from different explants of Lentil (*Lens cutlinaris medik*) *via* organogenesis** .Turk. J. Bot, 2.

80. Kiran, G., C.P.G. Kaviraj, P.B.K. Jogeswar, and S. Kishor. 2005. **Direct and high-frequency somatic embryogenesis and plant regeneration from hypocotyls of chickpea (*Cicer arietinum* L.)** a grain legume. CurrSci, 89: 1012–1018.

81. Kottapalli, P.P., M. Gaur, S.K. Katiyar, J.H. Crouch, H.K.P. Buhariwalla, andK. Gali. 2009. **Mappingand validation of QTLs for resistance to an Indian isolate of ascochyta blight pathogen in chickpea**. Euphytica 165: 79–88.

82. Krikorian, A.D. 1994. ***In vitro culture of root, tuber crops. In: Vasil IK, Thrope TA (eds) plant cell and tissue culture***. *Kluwer, Dordercht, pp 293 -312.*

83. Krishna, K., and S.D. Joshi. 2008**. Rapid multiplication of *rauvolfia serpentine* benth**. Ex. Kurz through tissue culture, scientific world, Vol. 6, No. 6.

84. Kumar, V.D., P.B. Kirti, J.K.S. Sachan, and V.L. Chopra. 1994. **Plant regeneration *via* somatic embryogenesis in chickpea (*Cicerarietinum*L.)**.Plant Cell Rep*,* 13: 468-472.

85. Kumar, S., V. Kapoor, K. Gill, K. Singh, I. Xess, S.N. Das, and S. Dey. 2006. **Characterisation of N-Acetyl D-galactosamine-specific lectin possessing medicinal properties purified from *Cicer arietinum* L.**Department of Biophysics, Biotand Microbiology, All India Institute of Medical Sciences, New Delhi-110029.

86. Laibach, F. 1929. **Ectogenesis in plants.Methods and genetic possibilities of propagating embryos otherwise dying in the seed**. J. Hered. 20, 201–208.

87. Lainé, E., and A. David. 1994. **Regeneration of plants from leaf explants of micropropagated clonal Eucalyptus grandis.** Plant Cell Rep, 13: 473-476.

88. Laukkanen, H., H. Häggman, S. Kontunen-Soppela, and A. Hohtola. 1999. **Tissue browning of *in vitro* cultures of scots pine: Role of peroxidase and polyphenol oxidase. Physiol.** Plant, 106: 337-343.

89. Lopez-Amoros, M. L., T. Hernandez, and I. Estrella. 2006. **Effect of germination on legume phenolic compounds and their antioxidant activity.** J. Food Comp. Anal. 19: 277-83.

90. Malik, K.A., and P.K. Saxena. 1992. **Thidiazuron induces high frequency shoots regeneration in intact seedlings of pea (*Pisumsativum*), Chickpea (*Cicer arietinum* L) and lentil (*Lens culinaris*)**. Chickpea (*Cicer arietinum* L). Plant Physiol; 19:731-740.

91. Mirakabad, Z.H., R.A. Baghrei, and Z.M. Mehrjerdi. 2010. **Efficient protocol for break impasses of regeneration via callus for 20 genotypes of chickpea.International j of plant production**, 4(2).

92. Moghaleb, R.E.A., H. Saneoka, and K. Fujita. 1999. **Plant regeneration from hypocotyls and cotyledon explants of tomato (*Lycopersicone sculentum*)** . Soil Sci. Plant Nutr, 45:639-646.

93. Mohamed, F., P.E. Read, and P.C. Dermot. 1992. **Dark preconditioning, CPPU, and Thidiazuron promote shoot organogenesis on seedling node explants of common and Faba Bean.** J. A Mer. Soc. Hort. Sci, 117(4):668-672. 1.

94. Mohan, J.S. and H. Hoggman. 2007. **Protocols for Micropropagation of Woody Trees and Fruits**, Publisher: Springer-Verlag, the Netherland, pp: 559.

95. Mok, M.C. and D.W.S. Mok. 1985. **The metabolism of [14C]-Thidiazuron in callus tissue of *Phaseoluslunatus*.**Physiol Plant, 65: 427-432.

96. Monnier, M. 1995**. Culture of zygotic embryos, *in vitro* embryogenesis in plants (Thorpe, T. A., ed.),** Kluwer Academic, Dordrecht, The Netherlands, pp 117-153.

97. Murashige, T., and F. Skoog. 1962. **A revised medium for rapid growth tobacco and bioassays with tissues cultures**. Physiologia Plantarum 15:473-497.

98. Murthy, B.N.S., J. Victor, R.P. Singh, R.A. Flectcher, and P.K. Saxena. 1996. ***In vitro* regeneration of chickpea (*Cicer arietinum* L.): stimulation of direct organogenesis and somaticembryogenesis by Thidiazuron**, Plant Growth Reg, 19 (1996)233-240.

99. Murty, C. and J.K. Pittaway, and M.J. Ball. 2010. **Chickpea supplementation in an Australian diet affects food choice, satiety and bowel health,** Appetite, 54, (2) pp. 282-288.

100. NAZ, S., A. ALI, F. SIDDIQUE, and J. IQBAL. 2008. **Somatic embryogenesis from immature cotyledons and leaf calli of chickpea**. Pak. J. Bot., 40 (2): 523-531.

101. Nguyen, T.T., P.W.J. Taylor, R.J. Redden, and R. Ford. 2004. **Genetic diversity estimates in *Cicer* using AFLP analysis**. Plant Breed. 123: 173-179.

102. Noshad, D., S. Miresmaili, A. Riseman, and A. Ekramoddoullah. 2009. ***In vitro* propagation of seven Daphne L. species**. Plant Cell Tissue and Organ Culture 96(2):201-209.

103. Odutayo, O.I., F.B. Akinirinusi, I. Odunbososye, and R.T. Oso. 2005. **Multiple shoot induction from embryo derived callus cultures of cowpea (*Vigna ungimculata* L.)**. Walp. African J. of Biot, 4: 1214-1216.

104. Oudhia, P. 2003. **Traditional and me dicinal knowledge about pudina (Mentha sp. family: Labiatae) in Chhattisgarh**, India. Bot. http:// botanical.com.

105. Polisetty, R., P. Patil, J.J. Deveshwar, S. Khetarpal, K. Suresh, and R. Chandra. 1997**. Multiple shoot induction by benzyl adenine and complete plant regeneration from seed explants of chickpea (*Cicer arietinum* L.).** Plant Cell Rep, 16: 565-571.

106. Polowick, P.L., D.S.Baliski, and J.D. Mahon. 2004. ***Agrobacterium tumefaciens*-mediated transformation of chickpea (*Cicer arietinum* L.),**

gene integration, expression and inheritance. Plant cell Rep, 23 (7): 485-491.

107. Reed, B.M. (1999). **Design a micropropagation system: Workshop presentation from the 1998 SIVB Congr**. *In Vitro* Cell Dev. Biol. Plant., 35: 275-284.

108. Rehman, M.M., M.N. Amin, H.S. Jahan, and R. Ahmed. 2004. ***In vitro* regeneration of plantlets of Curcuma longa Linn**. Valuable spice .Asian J. of plant Sci, 3:306-309.

109. Rekha, K.T. and M. Thiruvengadam. 2009. **Efficient micropropagation of chickpea (*Cicer arietinum L.*)**. Philippine Agric Sci. Graduate Inst. of Biotechnol.

110. Reynolds, J.F. 1994.***In vitro* of culture of vegetable crops. In: Vasil Ik, Thrope TA (eds) plant cell and tissue culture**. Kluwer, Dordrecht, ppt, 331-362.

111. Richa, C., and N.P. Singh. 2002. **Plant regeneration *via* somatic embryogenesis in chickpea (*Cicer arietinum L.*).**The Indian Journal of genetics and plant breeding. Vol :62. Iss : 4

112. Rizvi, S.M.H. and R.P. Singh. 2000. ***In vitro* plant regeneration from immature leaflet derived callus cultures of (*Cicer arietinum* L*).* *via* organogenesis.** Plant Cell Biotechnol. Mol. Biol, 1:109-114.

113. Sagare, A.P. and K.V. Krishnamurthy 1991**. Protoplast regeneration in chickpea (*Cicer arietinum* L.*)*. Indian J. Exp. Biol. 29: 930-932.

114. Sagare, A.P., K.V.Krishnamurthy, andK. Suhasini. 1993. **Plant regeneration *via* Somaticembryogenesis in chickpea (*Cicer arietinum* L.).** Plant Cell Rep, 12: 652-655.

115. Saini, R., andP.K. Jaiwal. 2002. **Age, position in mother seedling, orientation, and polarity of the epicotyl segments of blackgram (*Vignamungo* L. *Hepper*) determines its morphogenic response**. Plant Sci, 163:101–109.

116. Saleem, K., A. Mehboob, G. Mehnaz, F. Mehvish, and A. Zahoor. 2010. ***In vitro* micro-propagation of indigenous chick pea (*Cicer arietinum L.*)** cultivars, African J of Agric Research Vol. 5(21), pp. 2934-2938.

117. Sanyal, L., A.K. Singh, K. Meetu, and D.V. Alma. 2005. **Agrobacterium-mediated transformation of chickpea (*Cicerarietinum L.*) with *Bascillusthuringiensis* Cry1Ac gene for resistance against pod borer insect *Helicoverpa armigera*** . Plant Sci, 168 (4):1135-1146.

118. SAS Institute. 1990. **SAS user guide. Statistics. SAS Institute, Cary, N.C.**

119. Sawardekar, S.V. 2007. **Transformation studies in chickpea (*Cicer arietinum* L.)**. Department of genetic and plant breeding college ofagriculture, Dharwad university of agriculture sci, Dharwad – 580005.

120. Sayem, M.A., S.S. Siddique, and M. Zaman. 2010. ***In vitro* regeneration through anther culture of Brassica SPP**. Bangaladish J of Agri. Research, 35 (2): 331-341.

121. Sengar, R.S., R. Chaudhary, and S.K. Tyagi. 2010. **Present status and scope of floriculture developed through different biological tools.** Research J. of Agri. Sci. 1(4): 306-314.

122. Senthil, G., B. Williamson, R.D. Dinkins, and G. Ramsay. 2004. **An efficient transformation system for chickpea (*Cicer arietinum* L).** Plant Cell Rep., 23(5):297-303.

123. Shagufta, N., A. Fayyaz, S. andI. Javed. 2007. **Multiple shoot formation from different explants of chick pea (*Cicerarietinum* L.)** *pak. j. bot*., 39(6): 2067-2073.

124. Shaheena, P., V. Enkateshwarlu, D. Srinivas, k. Jagan, and M.Ugandhar. 2012. **Direct *in vitro* shoots proliferation of chick pea (*Cicer arietinum* L.) from shoot tip explants induced by Thidiazuron, Bio**. Sci discovery, 3(1):01-05.

125. Shalini, S., P. Batra, A. Sindhu, and V.K. Chowdhuray. 2001. **Multiple shoots inductor and complete plant chickpea (*Cicer arietinum* L.).**Crop Research, 21 (3):308-311.

126. Sharma, K.K. and R. Ortiz. 2000. **Program for the application of genetic transformation for crop improvement in the semi-arid tropics**. *In Vitro* Cell. Dev. Biol. Plant, 36: 83-92.

127. Sheila, V.K., J.P. Moss, C.L.L. Gowda, and H.A.V. Rheenen. 1992. **Plant regeneration in chickpea.** Page 153 in Biot and crop improvement in Asia (Moss. J.P., ed).

128. Singh, A., N.P. Singh. and A.N. Asthana. 1997. **Callus induction and direct regeneration from immature embryo in chickpea. International Chickpea and Pigeon pea Newsletter**, 4: 39-40.

129. Singh, R.B. 2000. **Environmental consequences of agricultural development: a case study from the green revolution state of Haryana, India**. Agricultural Ecosystem and Environment, 82: 97-103.

130. Singh, N. and N. Kataria. 2012. **Role of potassium fertilizer on nitrogen fixation in Chickpea (*Cicer arietinum* L.) under quantified water stress**. J of Agric Technol 2012, Vol. 8(1): 377-392.

131. Smith, V. and J. Jmmerson. 2005. **Chickpeas (Garbanzo beans).** Agric Marketing Policy Center Montana State University, Briefing. No. 55.Taylor.

132. Sounder, R.V., D.H. Telavathi, and B.H.M. Nijalingappa. 1989. **Shoot tip culture in (*Dolichos biflorus* L)**.Cur-rent Sci, 58:1385-1388.

133. Sujatha, G.N., B.D. Jayabalan, and R. Kumari. 2007. **Rapid *in vitro* micropropagation of *Cicer arietinum* L.** Department of plant science, school of life Sciences, Bharathidasan University, India. Hortic. Sci, 34(1): 1-5.

134. Taji, A., P.P. Kumar, and P. Lakshmanan. 2002. ***In Vitro* Plant Breeding, Food Pro ducts Press**, New York, pp 167.

135. Tang, X. M. and L. Chen. 2011. **A review on regeneration in cowpea (*Vigna unguiculate* L. Walp)**. J of Agric Sci and Technology, Vol 5 , No 4. Iss 1939-1250, USA.

136. Thorpe, T. 2007. **History of plant tissue culture**. J. Mol. Microbial Biotechnol, 37: 169-180.

137. Toker, C. 2009. **A note on the evolution of kabuli chickpeas as shown by induced mutations in *Cicer aretinum* L.** Genetic Resources and Crop Evolution 56, 7-12.

138. Toth, S., P. Scott, S. Sorvari, and O. Toldi. 2004. **Effective and reproducible protocols for *in* vitro culturing and plant regeneration of the physiological model plant (*Ramonda myconi* L.)** Rchb. Plant Sci, 166(4):1027-1034.

139. Tukey, H. B. 1934. **Artificial culture methods for isolated embryos of deciduous fruits**. Proc. Am. Soc. Hortic. Sci, 32 313–322.

140. Tyagi, R.K., A. Agrawal, C. Mahalakshmi, Z. Hussain, andH. Tyagi. 2007. **Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers**.*In Vitro* Cell. Dev. Biol. Plant, 43: 51-58.

141. Upadhyaya, H.D., S.L. Dwivedi, M. Baum, and R.K. Varshney. 2008. **Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.)**. Bmc plant. Biol, 8: 106.

142. Upadhyaya, H.D., M. Thudi, N. Dronavalli, N. Gujaria, S. Singh, S.Sharma, and R.K. Varshney. 2011. **Genomic tools and germplasm diversity for chickpea improvement. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)**.Vol 9.

143. Vani, A.K.S. and Y.V.D. Reddy. 1993. **Somaticembryogenesis and plant regeneration in chickpea (*Cicer arietinum* L.).** *In vitro*, 30A (3): 65.

144. Vani, A.K.S.and Y.V.D. Reddy. 1996. **Morphogenesis from callus cultures of chickpea (*Cicer arietinum* L.).** INDIAN j Exp Biol, 34 (1996) 285-287.

145. Vasil, I.K. and V. Vasil. 1994. ***In vitro* culture of cereals and grasses. In: Vasil IK, Thrope TA (eds) Plant cell and tissue culture**. Kluwer, Dordrechet, ppt. 313-329.

146. Veraplakorn V, M.N. Nakorn, L. Kaveeta, S. Suwanwong, and L. Bennett. 2012. **Variation and long term regenerative capacity of two important tropical forage legumes: Cavalcade (*Centrosema pascuorum* cv. Cavalcade) and stylo 184 (*Stylosanthes guianensis* CIAT184) *in vitro*.** African J of Biotechnol. Vol. 11(92), pp. 15843-15851.

147. Wang, H.M., M.H. Liu, M.H. Wang, Y.G. Zu. 2008. **Effects of Thidiazuron, basal medium and light quality on adventitious shoot regeneration from in vitro culture stem of Populus alba x P**. berolinensis. J of Forestry Res, 19(3): 257-259.

148. White, P.R. 1963. **The Cultivation of Animal and Plant Cells, Ronald Press**, New York. 2nd ed.

149. Yadav, S.S., R.J. Redden, W. Chen, and B. Sharma. 2007. **Chickpea breeding and management**.Website: www.cabi.org.

150. Yoshida, T. 2002. **Adventitious shoot formation from hy- pocotyls sections of mature soybean seeds**. Breeding Sci, 52:1–8.

151. Zhu, H., H.K. Choi, D.R. Cook, and R.C. Shoemaker. 2005. **Bridging model and crop legumes through comparative genomics Plant Physiol**. 137: 1189–1196.

152. Zimmerman, R.H., and H.J. Swartz. 1994. **In vitro culture of temperate fruit. In: Vasil IK, Thrope TA (eds) plant cell and tissue culture**. Kluwer, Dordercht, pp 457-474.

153. Ziv, M. (1991). **Vitrification: morphological and physiological disorders of *in vitro* plants. In: Debergh P. C.; Zimmerman, R. H. (eds.).** Micropropagation – technology and application. Dordrecht Kluwer Academic Publishers. p. 45–79.

جامعه النجاح الوطنيه

كلية الدراسات العليا

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ا . حسان ابو قاعود

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

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الملخص

تم في هذه التجارب دراسه أثر مستويات مختلفه على نمو، مضاعفه وتمايز بنات الحمص داخل الأنابيب . أستخدم 3 أصناف (HUDAS, FLIP03-147c and FlIP05-100c) و كذلك اثنان من السلالات المحليه عين البيضاء ، بلدي . أستخدمت تراكيز مختلفه من هرمونات حامض النفثالين (NAA) والبنزل أدنين (BA) و الكاينتين (Kin) و الثيادرززرون (TDZ) في تجارب المضاعفه والتمايز . تم استخدام سويقات منبته داخل الأنابيب من الأصناف المختلفه لدراسه تأثير الهرمونات على مضاعفتها ، تم الحصول على أكبر عدد من السيقان (3) باستخدام وسط موراشيج وسكوج بدون اضافه هرمون وكذلك مع استخدام 2.2 ميكرومولار في سلاله البلدي، قلل استخدام kin مع NAA عدد السيقان النتجه في تجارب التمايز . تم مقارنه استخدام أجزاء النبات أوراق وسيقان) مع تراكيز مختلفه من TDZ وKin و NAA . أظهرت نتائج التمايز اختلاف واضح بين الأصناف وتم الحصول على أعلى نسبه من تمايز الأوراق 2 % عند استخدام 2 ميكرومولار TDZ في صنف FlIP05-100c .