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Research Title:

In Vitro Rooting and Acclimatization of *Sorghum bicolor* L.Cultivar wad Ahmed

التجذير داخل الأنابيب وأقلمة نبات الذرة الرفيعة ((الصنف ود أحمد))

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DEDICATION

Dedicatedto my

father and my mother who gave me life.
and my brother(Mohammed and abuzeed).
andsisters(Rayan and Fiyha).

and all friends,

with my love…….

Fatima

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Praise to Allah, the Almighty, who gave me health, ability, and patience to fulfill this work.

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Patience. Support and valuable comment and encouragement during the work special thanks for my faculty Sudan University of science and technology and department of tissue culture commotion of biotechnology and genetic engineering. National center for research.

Also due all members of tissue culture department at the end extend our great thanks to some one dear to us for his help throughout the research period and to our parents for their prayers during this study.

English Abstract.

The research was conducted in the Laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research, to evaluate *in vitro* rooting and acclimatization of sorghum bicolar plantlets cultivar wad Ahmed. Plantlets of *in vitro* maintained stock plantlets come from callus were used as explant material in all experiments. plantlets obtained from *in vitro* callus cultured on hormone free-medium supplemented with two levels ofBAP (0.5 and 1.0) mg/l in combinations with NAA to evaluated multiple shoot formation. The best result for multiple shoots formation (12.8 shoots/explant) was obtained when the plantlets explants were cultured on MS medium supplemented with BAP at 1.0 mg/l combinations with NAA at 1.0 mg/l. *In vitro* regenerated shoots were rooted on MS medium without or with two levels (1.0 and 2.0 mg/l) of NAA and two levels of sugar (20 and 30) g/l. The best results for rooting percentage (90 %) and number of roots (3.5) were obtained when regenerated shoots were cultured on MS basal medium supplemented with NAA at 2.0 mg/l and sugar 30 g/l. Rooted plants were hardened and 70% of them survived under greenhouse conditions.

Arabic Abstract

أجريتالدراسةفيمختبر زراعة الانسجة والخلايا النباتية , هئية التقانة الحيوية والهندسة الوراثية , المركز القومي للبحوث لتقيم التجزير خارج الجسم الحي واقلمة النبيبتات الزرة صنف ود أحمد, أستخدمت النبيبتات المنتجة داخل الانابيب من الكالس كجزء منفصل في جميع التجارب, النبيبتات الناتجة من الكالس تم زراعتها في وسط ّ غذ ائي النبيبتات MS مضافا اليه تركيزين (0.5 and 1.0) mg/l من BAP مخطلط معه NAA لتقييم تكوين الافرع , تم التوصل إليأن BAP بتزكيز 1.0 mg/l مخطلط معه NAA يتركيز /1.0 mg l هو الأكثر فعاليه في تحفيز تضاعف الأفرع الخضرية, حيث تم الحصول علي (12.8فرع عرضي/جذعه) . تم تجذير الأفرع الخضرية داخل الأنابيب علي الوسط الغذائي MS الخالي من منظمات النمو أو مضافا اليه تركيزين (1.0 و2.0)ملجرام من NAA و تركيزينمن السكر(20 و 30) جرام. كون 90% من الأفرع الخضرية جذورا عند زراعتها علي الوسط الغذائي MSالمضاف اليه2.0 ملجرام/ لتر NAA و 30جرام لكل لتر مع اكبر عدد من الجذور(3.5) . تمت اقلمة النبيتات المجذرة و ظلت 70 % منها حية تحت ظروف البيت الزجاجي.

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Abbreviations

MS	**Murashige and Skoog medium**
NAA	**Naphthalene Acetic Acid**
BAP	**Brazil amino purine**
w/v	**Weigh/ Volume**
Cº	**Degree Celsius**
G	**Gram**
%	**Hundred Per Cent**
SE	**Standard error**
mg/l	**Milligram per liter**
g/l	**Gram per liter**

Chapter one

Introduction

Dura sorghum bicolor(L.) Family (*gramineae,Poaceae*) is one of five top cereals in the world, along with wheat (*TritiumaestivuL.)* oats (*Avonsativa L.)* and burly (*Humdrumvulgar L.)* House, (1985) it is usually grown under hot and dry conditions the crop is also one of the oldest cultivated renter of origin is thought tope the African continent. the crop is primarily growth in tropical and subtropical regions of the world with minimal rainfall (300-400 mm).it is particularly important in hot and dry tropical regions where maize, wheat and other crops are hot adapted (fAo,2001). More the 35% of sorghum is grown directly for human consumption the rest is used primarily for animal feed alcohol production and industrial products (Badi,*et al.*,1990). total world annual sorghum production is about to million tan from a cultivated of 46 million ha. the most important produces are the united states, Nigeria, Sudan, Mexico, China, India, Ethiopia, Argentina, (fAo,1985). Burkina Faso is the world Ledger in sorghum production and consumption (fAo,2005).

Historians believe that sorghum originated in Africa more precis Ely in Ethiopia between 5000and7000 years ago (Doggett, 1988) from there it was distrusted along the trade and shipping routes around the Africa continent and through the middle east India at least 3000 years ago it then journeyed along the silk route into china. Sorghum was first taken to north American in the (700-1800`s) through the slave tradefrom west re-introduced in to Africa in the late 19” century for commercial cultivation the crop further respreads to south America and Australia.

In most of the west eastern African countries sorghum alone accounts for abut50% of the total area under cereals there for true food security will be hard to achi vein in those countries without a significant improvement of

the production use and marketing of this major staple cereal. The yield is 105-126Kg\ha (fAo, 1985) the low production in Africa is essentially due to biotic factors in sect's fungal diseases weed act.

Millions of people through not the semi a red regions of sup shoran depend on sorghum as staple crop in many household's sorghum is the primary source of energy, protein, vitamins and minerals. As the fifth most abundant crop worldwide. sorghum plays ahuge role in the world market as means of livelihood for millions of subsidence formers and as on important part of food security. furthermore, sorghum is used as the us. World word 31million tons or48% of sorghum produced are used for lives tock feed (Doggett,1988).

Despite its extensive use sorghum is lowing protein digest ability when cooked with 81% in wheat 73%in mazed66% in rice (Maclean*et al.*,1981) sorghum alone is not consider as bread making cereal because of the lack of gluten,bread.(Anglani,1994).Amon"

g interesting features of sorghum utilization is biscuits and other cooked products in the USA and japan. Sorghum utilization as human food is increasing as it is in as shacks and cookies. Sorghum has been intentionally introduced in China for food needs and it is becoming one of the most important crops. the future promise of sorghum in the developed world is for wheat substation for people allergic to gluten in addition pasta products such as spaghetti and macaroni, made from semolina wheat could be made with mix bures of compositeflour consisting of 30-50% sorghumwheat bran cooked sorghum flours mixed with vitamins and exogenous sources of protein (peanuts or soya bans) are Commercial available in many Africa countries for preparation of instant so it porridge for infants. Sorghum can be buffed popped shredded and flaked to produce ready –to-eat breakfast cereals.

Sorghum starch is successfully applied for the production bio-ethanol (Suresh,1998) In Nigeria and south Africa. Sorghum is in duster ally used for the production of lager beer tailor and sorghum also is reported to be on abortive epilepsy flux and stomachache

(Duke and lydon,1989) the root is used for malaria in southern Rhodesia. The seeds have been used for breast biases and diarrhea the stem for tuber collar swelling in India the plant is considered on the (mythicand insecticidal).

In Sudan sorghum is one of the most important crops it is cultivated on area of over 6.3milion ha

(Babiker,2007) the crop cons the main staple food for 80% of the populace as animal feed and stocks as building materials.

Methods of growing plant tissue have been used recently in propagation economical plants by placing plant darts in different concentration in of hormones and follow their growth.

Objectives of the study

1. Determine the best concentrations of BAP combinations with NAA suitable for regenerated shoot formation.
2. Determine auxin NAA and optimum concentrations suitable for *in vitro* rooting of the regenerated shoots.
3. Acclimatization of *in vitro* regenerated plants and transfers to grow under field conditions.

Chapter tow

Literature review

2.1. Origin and distribution

Sorghum is the 5th most important grain crop after wheat, maize, rice and barley. It is indigenous to Africa. Globally, it produces approximately 70 million tons of grain from about 50 million ha of land. It is the dietary staple of more than 500 million people in more than 30 countries. “For all that, however, sorghum now receives merely a fraction of attention of what it could. Not only is it inadequately supported for the world’s fifth major grain crop, it is under supported considering its vast untapped potential” National Research (Council,1996). Sorghum could contribute more to food supplies than at present, especially to those regions and peoples in greatest need.

Sorghum is a tropical grass grown primarily in semi-arid parts of the world. In Africa, a major growing area runs across West Africa south of the Sahara, through Sudan, Ethiopia and Somalia. It is grown in upper Egypt and Uganda, Kenya, Tanzania, Burundi, and Zambia. It is important crop in India, Pakistan, Thailand in central and northern China, Australia, in the drier areas of Argentina and Brazil, Venezuela, USA, France and Italy. The crop has spread over the drier areas of the world; it does better when it is dry and cool, whereas pearl millet is better adapted to dry hot conditions. Sorghum is a staple food for about 300millions people worldwide.

2.2. Cultivars

Cultivar planning aims to reduce risks by avoiding drought periods during the most critical growing stages of the plant growth, such as flowering and seed set. Cultivars differ in their reaction to the environment and the climate, which can be used in planning the seed package. The yield

potential of the farm or field should be known as well as the long-term rainfall pattern to be able to make the best cultivar choice. The long-term rainfall data will be a guide for the choice of the correct growing season length of the cultivars suitable for that area. Isolated or small areas of sorghum are prone to bird damage. When selecting bird resistant cultivars for such areas, contracts should be negotiated prior to planting, as this grain is not accepted easily by industry (Hussien,2006). Cultivars with a wide adaptability would be a good first choice when starting with sorghum production. Mutesseasonal results can be used to select specific cultivars, which can be incorporated into the cultivar package after proper testing onsite.

2.3. Taxonomy

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae(Grass)

Genus : Sorghum

Pliny (ca. 60 to 70 A. D.) was the first to give a written description of sorghum and after that there was hardly a mention of it until the sixteenth centuryestablished the genus Sorghum and brought the sorghums under the name S. bicolor. (IT IS, 2006).

developed a simplified classification that is in common use. There are a total of 15 races The basic races are bicolor, guinea, caudated, kefir, durra, and there are ten hybrid races under S. bicolor subsp. bicolor. Sorghum is a

cereal of remarkable genetic variability—more than 30 000 selections are present in the world and it is very difficult to classify them. Sorghum belongs to the order of Pales and to the family of *Gramineaece. The* species Sorghum bicolor covers a wide range of varieties, from white and yellow.

2.4. Botany

Sorghum is classified under the family of Phocaea, tribe *Andropogonea*, subtribe Sorghum, genus Sorghum. All cultivated sorghum belongs to Sorghum bicolor subsp. bicolor. The morphological characteristics of sorghum differ, based on the variety and environment in which it is grown. Sorghum is a perennial by nature and, hence, a very suitable multi-cut forage crop, but where the end product is grain it is grown as an annual rain fed crop.

Sorghum is an erect plant with a solid stem, which can grow from 0.8 m to 5 m high depending mainly on its photoperiod sensitivity. The sorghum leaf has a prominent midrib; typical leaf blades are on average 8-12 cm wide and 50-90 cm long. Leaf sheath and stem are often covered with a waxy bloom. The plant can tiller depending upon the variety (or hybrid), temperature conditions and nitrogen supply. Sorghum has an extensive fibrous root system which can grow as deep as 3m (Patil*et al*,.1998)

2.5. Insect Pests

Pests and Potential Problems of bacterial, fungal, and viral diseases of sorghum (Kucharek,1992). Common fungal diseases include anthracnose, leaf blight, sorghum downy mildew, zonate leaf spot, rough spot, sorghum rust, charcoal rot, and stalk rot/grain mold. Grain can also be affected by fungal smut. Most viral diseases of sorghum are mosaics with the most important being maize dwarf mosaic (Taylor *et al.,* 2001). One of the most common bacterial diseases of sorghum is bacterial leaf stripe (Kucharek,1992).

2.6. Seeds and Plant Production

When planting for seed production, a firm weed free seedbed is needed. Seeds can be planted up to 2 inches deep depending on soil textures. The seeding rate may vary widely depending on the variety being planted and row spacing. A general recommendation is to calculate seeding rates based on desired plant populations per acre rather than pounds of seed per acre due to the large variances in the seed sizes of sorghum varieties (Kansas State University, 1998). Sorghums cross pollinate, requiring
seed production fields to be isolated by approximately 3,000 ft. from other sorghum crops (FAO, 2012).

Fertilizer applications should be based upon soil tests. The nutrient requirements of sorghum seed production are similar to that of corn. Sorghum seed is sensitive to fertilizer burn. Fertilizer should be incorporated into the soil prior to planting or otherwise applied to avoid seedling damage. Fertilizer should be applied so that nitrogen is available during the vigorous growth stages. By the boot stage of sorghums, 65–70% of the total nitrogen has been taken into the plant.

2.7. Uses

Sorghum is an important crop for food and fodder in the semi-arid tropics of the world. Sorghum is a staple food in African and Asian subcontinents. Most of the grain produced in these countries is utilized for human consumption. Though sorghum is known for its nutritional quality, the consumption of this cereal is decreasing due to easy availability of rice and wheat through public distribution system and easy methods of processing and cooking of fine cereals (such as rice). The various foods that are made in different parts of the world especially in Indian and African sub-continent are described in this review. The objective of this review is to explore the global utilization of sorghum as a food. The requirement of special skill in preparing sorghum rotes and non-availability of ready-made sorghum flour

and soju in the market are deterrents for wider use of sorghum as food. The grain sorghum is utilized in preparation of many traditional foods and in bakery preparations like bread, cakes and biscuits. Dough prepared with cold water has poor adhesiveness and is difficult to roll thin. Higher water uptake, low gelatinization temperature, high peak paste viscosity and high setback are the starch properties that have been shown to be associated with good quality of roti, the unleavened bread that is the most common form in which sorghum consumed on the Indian subcontinent. Technologies for production of shelf-stable refined flour, grits and semolina from sorghum and millet have been developed and laboratory studies have demonstrated their successful utilization and incorporation into various traditional foods (idli, dosa, chakli, papad, etc.) and newer convenience health products (vermicelli, noodles, plain and ready-to-eat flakes, extruded products, weaning and supplementary foods, and bakery products).Efforts are being made for popularization and wider adoption of the successful technologies to promote sorghum for diversification of their utilization among the non-traditional urban population. Sorghum plays an important role in crop rotation systems. Sorghum is a C4 species with high photosynthesis efficiency. It can achieve higher yields with a lower input of resources in compared to other crops. Most grain sorghum in China is used as food to make various breads, cakes, dumplings and noodles. But sorghum is commonly called "coarse food "because of its amino acids imbalance and high tannin content. (Hulse*et al.*,1980). (Subramanian and Jambunathan,1984).

2.8. Tissue culture of sorghum:

Sorghum tissue culture has been challenged by threeprédominant obstacles for decades, namelytoxic pigments (phenolics), lowregenerationfrequencies and short duration of callusregenerability. Here, we report a robust tissue culture system for sorghum, which has minimizedthese major impediments. To optimize media, different concentrations of various plant growthregulators, such as 2,4-dichlorophenoxyacetic acid (2,4-D), N6-benzyladenine (BA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and α-naphthaleneaceticacid (NAA) wereevaluated. Additionalingredients, including KH2PO4, CuSO4·5H2O, L-asparagine, L-proline and polyvinylpyrrolidone (PVP) werealsoassessed. Resultsshowedthatcallusagehad a conspicuouseffect on itsgrowth and regenerability, withcallusweeklygrowth ratio and regenerabilitypeakedattwoweeksafter induction. Acallus induction rate up to 100% wasachieved in inbred line Tx430, whereasregeneration rates up to 100% wereobtainedfrom SA281 and 91419R. This highly efficient system has been utilized for sorghum transformation for severalyears and has been proven to bereliable and reproducible.

Although sorghum tissue culture has been studied formany decades, a highly efficient and comprehensive system has remained elusive till date. Poor long-term callus regenerability is considered as one of the major obstacles in sorghum tissue culture (Raghuwanshi and Birch,2010). Sorghum inbred line SA281, which regenerated embryogenic callus well but produced a lot of phenolics in previous experiments, has

been utilized to study sorghum tissue culture in our laboratory for many years. So we started with SA281 to optimize medium and subsequently used another two lines (Tx430 and 91419R) in our tissue culture system. Hence, the aim was to define a robust tissue culture system for sorghum by

optimizing media and investigating the effect of callus age on regenerability.

2.8.1. Multiple shootformation:

Twenty-four diverse genotypes of sorghum were evaluated for response to callus induction and plant regeneration with two media viz., MS and NBKNB using shoot tips as the start material to identify a model genotype Altpeter and Varshney, (2001). None of the genotypes tested showed promising results. Therefore, alternative methods of in vitro pathways using shoot meristem isolated from shoot tips were explored (Harshavardha*et al*2002). Shoot apical meristems were isolated and were induced to multiple shoots or multiple shoot bud's pathway by manipulation of thidiazuron (TDZ), 6-benzyl adenine (BAP) and 2, 4-dichlorophenoxy acetic acid (2, 4-D). Choice of the pathway whether large-scale multiplication of shoots or production of target tissues for transformation can be exercised based on the needs and applications (Murty*et al.*,1990). A simple procedure, for large scale handling of shoot tips is described in detail. Electron microscopic studies revealed that meristems isolated from 7-day-old seedlings are superior because of possessing greater number of transformation competent cells.

2.8.2. Regeneration of sorghum

A system for rapid plant regeneration through somatic embryogenesis from shoot tip explants of sorghum [*Sorghum bicolor* (L.) Moench] is described Elhag and Butler, (1992). Somatic embryogenesis was observed after incubation of explants in dark for 6–7 weeks through a friable embryogenic callus phase. Linsmaier and Skoog medium supplemented with 2,4-dichlorophenoxyacetic acid (2 mg l^{-1}) and kinetin (0.1 mg l^{-1}) was used for induction of friable embryogeniccalli and somatic embryos (Nahdi and

de Wet,1995). Germination of somatic embryos was achieved about 5 weeks after transfer onto Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (2 mg l^{-1}) and indole-3-acetic acid (0.5 mg l^{-1}) under light. Seeds from *in vitro*-regenerated plants produced a normal crop in a field trial, and were comparable to the crop grown with the seeds of the mother plant used to initiate tissue culture (Sairam*et al.*,1999). The simplicity of the protocol and possible advantages of the system for transformation over other protocols using different explants are discussed.

2.8.3. Callus

Callus induction is the first step of *in vitro* regeneration, and the main influence factors of callus formation are genotype, basic medium and hormone (Elkonin*et al.,* 2000), as well as the type of explant used Carvalhoet al, (2011). Most researchers have focused on tissue culture using immature embryos, for example, from Tx430. However, little work has been conducted using mature embryos as explants (Wei et al,2004). An appropriate variety is the main obstacle in sorghum tissue culture and genetic transformation using mature embryos as explants. In the present research, 120 sorghum varieties were screened to select an appropriate variety for tissue culture. Our results showed that 79.17% of the varieties could not be induced to form calluses. Only one variety was found to be a good material for tissue culture. The results also revealed why there are fewer successful studies using mature embryos as explants in sorghum tissue culture.

2.8.4. Rooting

Elongated, welldevelopedindividualregenerated shoots afterattaining 3-6 cm length and eachwithtwo to four leaveswereseparatedfrom the medium and used for root induction. The media used for root induction washalfstrength MS media supplementedwith (0.1 mg 0.2mg) -1 NAA. Culturing for rootingwasalsocarried out in glass vesselsunder the same conditions as for shoot elongation. Wellrooted shoots (ten plants for eachgenotype) weretransferredintosoil and kept in the greenhouse to check for normal development.**(**Sairam*et al.*,1999).

Chapter three

MATRIALS AND METHODS

This study was carried out at the Laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan in (2018).

3.1. Plant material

The plant material was an *in vitro* stock of sorghum cultivar Wad Ahmed. Explants of 1.5-2.0 cm were obtained from *in vitro* established plantlets from callus.

3.2. Tissue culture techniques

3.2.1. Media compositions and Preparation

Murashigeand Skoog, (1962) (MS) medium was used in this study. It consists of five inorganic macro elements prepared separately in stock solutions (1000) ml and micro elements concentrated 100 times and prepared in stock solutions (100 ml). The stock was added to the medium at the rate of 1.0 ml/l for the normal concentration (1X), and NaFe EDTA mineral salts concentrated 100X in stock solution (1000 ml), and supplemented with 30g/l sucrose, MS vitamins which includes thiamine, nicotinic acid, pyridoxine and glycine concentrated 100 times and prepared in stock solution (100 ml) the vitamins were added to the medium at the rate of 1.0 ml/l for the normal concentration(1X), and myo-inositol was added at 100 mg/l. The pH was adjusted to 5.8 using HCL or NaOH before adding 6g/l agar dissolved by heating in a microwave oven. The medium was then dispensed in glass bottles in measured amount of 25ml/bottle, and autoclaved at 121°C and 15 psi for 15 minutes and stored to cool in the incubation room.

3.2.2. Sterilization

3.2.2.1. Sterilization of equipments and glassware

All operations for *in vitro* culture were carried out inside a laminar air flow cabinet (Chemiphar Industries Indian Limited) with HEPA filters and using sterilized plant materials, equipment's, glass ware materials and chemicals. The hood surface was wiped clean with cotton soaked in 70 % ethanol and sterilized by germicidal ultraviolet light for at least 15 min prior to use. All surgical instruments, glassware and other accessories were sterilized in an autoclave at 121 ºC at 15 psi for 15 min and then dried in an oven. Surgical instruments like scalpel, forceps, and scissors were again sterilized by dipping in 100 % ethanol and flaming prior to use.

3.3. Effect of BAP in combinations withNAA on multiple shoot formation

Plants excised from *in vitro* established plantlets from callus were cultured in culture bottles containing MS basal media supplemented with BAP at a concentration 0.5 and 1.0 mg/l in combination with NAA at concentration 0.5 and 1.0 mg/l, to determine the best concentration required to induce the highest multiple shoot number per explants and the highest shoot lengh. All media were supplemented with 3 % sucrose and 0.6 % agar and pH was adjusted to 5.8 before autoclaving. Cultures were incubated for four weeks at 25°C±2 under cool white fluorescent light with a 16 h photoperiod.

3.4. *In vitro* rooting of regenerated shoots

Shoots derived from shoots regenerated from callus were excised and cultured on full - strength MS media supplemented with three concentrations (0.0, 1.0, and 2.0 mg/l) of NAA. Media were supplemented with suger at two concentrations 20 and 30g/l, to evaluate the effects of NAA on *in vitro* rooting formation and root lengh.

3.5.Acclimatization of plantlets

In vitro rooted plantlets were carefully taken out of the culture bottles and washed gently under running tap water to remove the agar and the remains of medium sticking to the roots. Plants were transferred to plastic pots containing a mixture of autoclaved soil and sand at the rate of 1:1. The pots were covered with glass bottles to prevent rapid loss of water and were kept under room conditions. The plants were watered three times a week. After 3-4 weeks, the glass bottles were removed and the plants were transferred to the plastic house and placed under shade.

3.6. Culture conditions

All media used in this study were supplemented with 30g/l sucrose, solidified with 6% (w/v) agar and the pH was adjusted to 5.8 before addition of the agar and autoclaved at 121C and 15 ib psi for 15 min. The cultures were incubated at a temperature of 25±2 º C under 16 h daily illuminations with white fluorescent light (1000 lux).

Chapter four

RESULTS AND DISCUSSION

4.1. Effect of BAP in combinations with NAA on multiple shoot formation

Table 4.1. Showed that the shoot obtained from callus of sorghum were cultured on MS medium supplemented with 3% sucrose and (0.5 and 1.0) mg/l BAP and combination of NAA (0.5 and 1.0) mg/l. multiple shoot formation and shoot length were observed under 16 h photoperiod after 4 weeks of culture (Table 4. 1). The highest regeneration percentage (100%), the regeneration dependent (Baskaran and Jayablan,2005) number of shoot/explants (12.8±02.3) and shoot length were obtained with BAP at 1.0 mg/l when combined with 1.0 mg/l IBA as shown in Table 4.3. Regeneration percentage, shoot number and shoot length increased with increase of NAA concentration. Them, 2003 reported that callus growth on explants usually interfere with the propagation process. The inhibitory effect of addition of auxins on multiple shoot induction has been demonstrated in a number of plants including faba bean (Khalafalla and Hattori, 2000).mung bean (Gulati and Jaiwal, 1992). cotton (Abdellatef and Khalafalla, 2007). and sesame (Magda et al., 2008) where the, addition of auxins to medium containing cytokinin did not improve shoot multiplication rate. similar results were recorded while using shoot tipes from *in vitro* germination (Baburaj*et al., 2000).*

Table (4-1-1) Effect of BAP in combination with NAA on multiple shoot formation:

BAP (mg/l)	NAA (mg/l)	Regeneratig (%)	Number of shoot (mean±se)	Shoot length (mean±se)
Control		100	1.5±0.6	1.2±0.6
0.5	0.5	100	10 ± 1.9	4.6±0.4
1.0	1.0	100	12.8±2.4	4.7±0.4

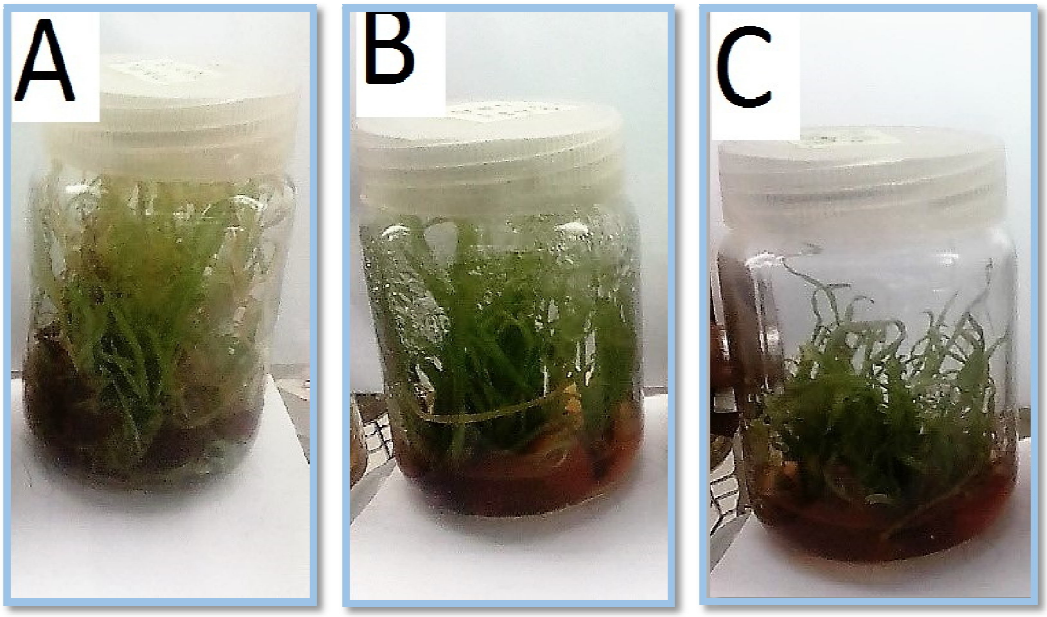

Figure (4:1). **Effect of BAP combinations with NAA on multiple shoot formation**

A: multiple shoot regenerated from shoot on MS medium containing 1.0 mg/l BAP comnation and 1.0mg/l NAA.

B: multiple shoot regenerated from shoot on MS medium containing 0.5mg/l BAP and 0.5 mg/l NAA.

C: multiple shoot regenerated from shoot on MS basal medium.

4.2. *In vitro* rooting of regenerated shoots

Induction of roots on calli-regenerated shootsexcised and transferred to full-strength MS basal medium without or with two levels (1.0 and 2.0mg/l) of NAA and media supplemented with (20 and 30) g/l sugar. Norooting of shoots was obtained on explants cultured on media without auxins, however, the explants became chlorotic and eventually die. MS basal medium containing 2.0 mg/l NAA gave the highest rooting percentage (90 %) similar result was reported by Patil et al. (1998). the highest number of roots per shoot (3.5±0.8) and the highest roots length (1.2±0.3) (Table 4.2), (Fig 4.2). Similar response was observed in *S.bicolor* (Saradamani*et al.*, (2003). Baskaran and Jayabalan(2005).

Table (4.2.1) Effect of NAA on rooting of *in vitro* derived shoots of sorghum after 4 weeks of culture on full-strength MS medium

NAA (mg/l)	Rooting(%)	Number of root (mean ±se)	Root length (mean ±se)
Control	0	0	0
NAA1+ suger20	80	1.8±0.4	1.1 ±6.2
NAA2+ suger20	70	1.7±0.5	1.0±0.3
NAA1+ suger30	40	0.9±0.4	0.8±0.4
NAA2+suger30	90	3.5±0.8	1.2±0.3

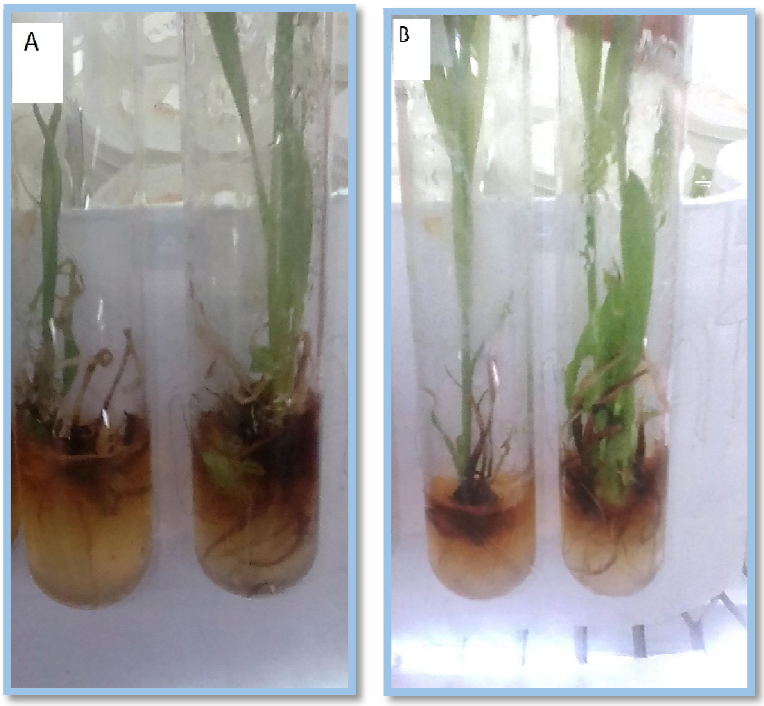

Figure 4.2. *In vitro* rooting of sourghum regenerated shoots.

A: Rooted shoot on MS basal medium supplemented with 1.0 mg NAA

B: Rooted shoot on MS basal medium supplemented with 2.0 mg/l NAA.

4.3. Acclimatization of plantlets

For acclimatization, plantlets were removed from rooting medium after six weeks of incubation and transferred to plastic pots containing autoclaved soil (soil: sand 2:1) and covered with glass bottle to maintain humidity and were kept under culture room conditions for one week Roussos *et al.*, *(*1999). After two weeks, glass bottles were removed and transferred to greenhouse and placed under shade until growth was observed. 70 % of sourghum regenerated plants survived and all were morphologically normal (Fig 4.3). Similar results were reported by Roussos et al., (1999) who recorded high survival rates of transplanted plantlet.

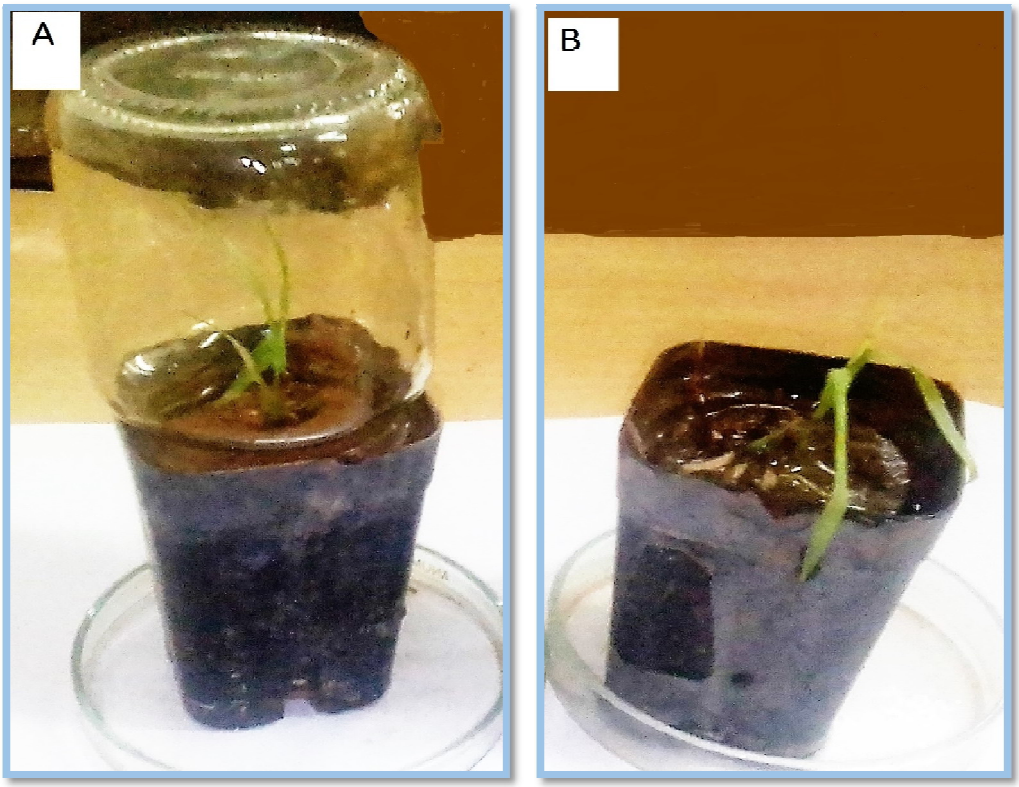

Figure 4.3. Acclimatization of *in vitro* regenerated sorghum plants.

A: Hardening of plantlets under room conditions.

B:sorghum plant established in soil under greenhouse conditions

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