IN VITRO PROPAGATION OF TURMERIC (*Curcuma longa* L.)

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IN VITRO PROPAGATION OF TURMERIC (*Curcuma longa* L.)

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CERTIFICATE

*This is to certify that thesis entitled, "**IN VITRO PROPAGATION OF TURMERIC (Curcuma longa)**" submitted to the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in BIOTECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **SAMIUL HOSAIN ANIK** Registration No. **12-05087** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation, has duly been acknowledged.

Dhaka, Bangladesh

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Dedicated To

My Beloved Parents

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SAU, Dhaka **The Author**

IN VITRO PROPAGATION OF TURMERIC (*Curcuma longa* L.)

ABSTRACT

The present study was undertaken in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, during the period from February 2018 to June 2018 to evaluate the effect of different plant growth regulators (BA, IBA) on *in vitro* plantlet regeneration of Turmeric. The healthy, disease free sprouts of turmeric were used as explants which were inoculated in MS media supplemented with different conc. of Benzyl adenine (BA) (1.0, 2.0, 3.0 and 4.0 mg/L) and IBA (1.0, 2.0, 3.0 and 4.0 mg/L) either alone or in combination for shoot and root regeneration. The experiments were arranged in Randomized Completely Block Design (RCBD) with three replications. The maximum shoot induction was obtained with 2.0 mg/L BA. The highest shoot length (5.93 cm) was noticed in BA 2.0 mg/L + IBA 3.0 mg/L and minimum shoot length (1.4 cm) with control MS dose was observed. The highest percentage (81.00%) of shoot induction was recorded with BA 2.0 mg/L in minimum (14.0 days). Where, IBA 3.0 mg/L had produced the highest percentage of root induction (82.00%) required minimum (20 days). The highest length of shoot (3.4 cm, 5 cm and 5.9 cm) at 21, 28 and 42 DAI respectively was found in BA 2.0 mg/L + IBA 3.0 mg/L. The treatment BA 2.0 mg/L + IBA 1.0 mg/L gave the highest number of root (6.0, 8.6, 12.67) at 21, 28 and 42 DAI respectively and the highest length of root (2.7 cm, 4.8 cm and 5.4 cm) at 21, 28 and 42 DAI, respectively was found in BA 2.0 mg/L + IBA 1.0 mg/L. Regenerated plantlets showed, 100% survival during in growth chamber conditions and 86.66% in shade house stage of hardening and 83% in open atmosphere at direct sunlight. Therefore, an efficient protocol has been developed for *in vitro* propagation of Turmeric which has great commercial value for year round production in Bangladesh.

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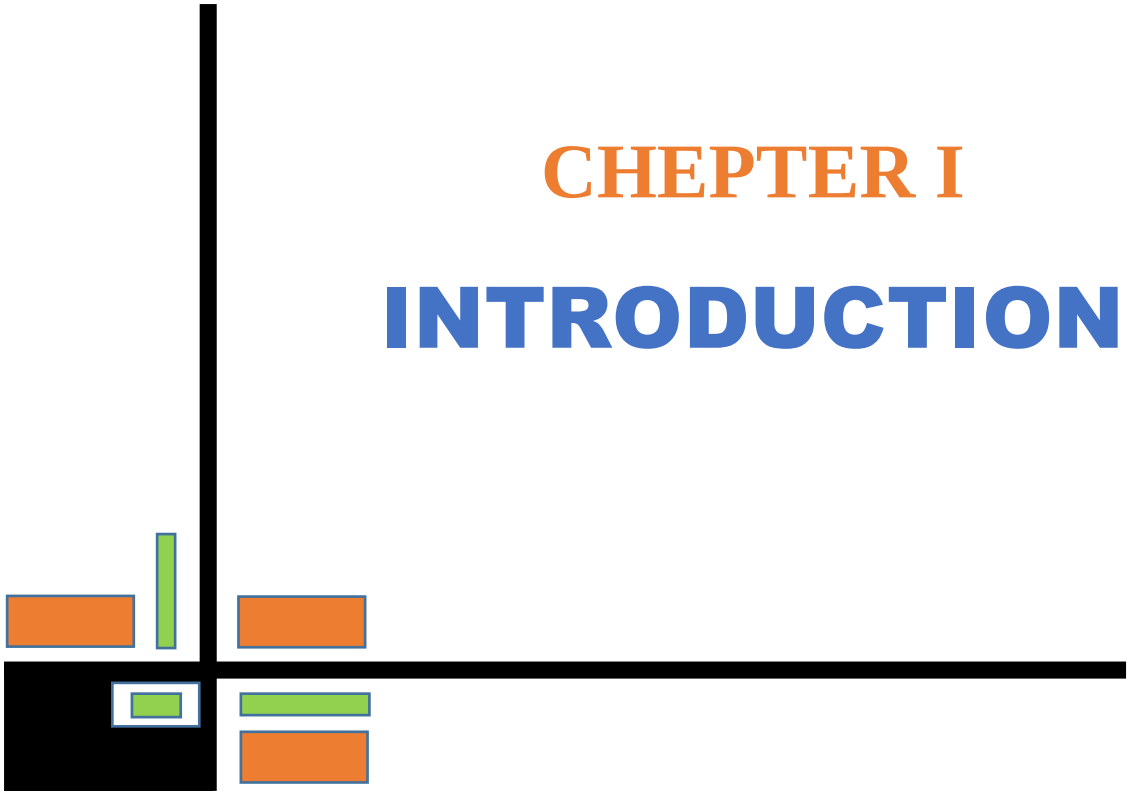
LIST OF ABBREVIATED TERMS

Abbreviation		Full Word
Agril.	:	Agricultural
Biol.	:	Biological
BAP	:	6- Benzyl Amino Purine
BA	:	Benzyl adenine
BARI	:	Bangladesh Agricultural Research Institute
Cm	:	Centimeter
CRD	:	Completely Randomize Design
cv.	:	Cultivar
Conc.	:	Concentration
2, 4-D	:	2,4- Dichlorophenoxy acetic acid
WAI	:	Weeks After Induction
Dw	:	Distilled water
DMRT	:	Duncan's Multiple Range Test
et. al.	:	And others
FAO	:	Food and Agriculture Organization
g/L	:	Gram per litre
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
NAA	:	α-Napthalene acetic acid
Int.	:	International
J.	:	Journal
Kin	:	Kinetin
Mol.	:	Moleculer
mg/L	:	Milligram per litre
Mm	:	Micromole
MS	:	Murashige and Skoog
PGRs	:	Plant Growth Regulators
Res.	:	Research
Sci.	:	Science
CV	:	Co-efficient of Variation
^{o}C	:	Degree Celsius
etc.	:	Etcetera

CHEPTER I

INTRODUCTION



CHAPTER I
INTRODUCTION

Turmeric (*Curcuma longa*) belonging to the Zingiberaceae family is one of the most important rhizomatous herbaceous perennial plant. It is referred as "Queen of Spices" and also known as "Golden spice of life" (BBS, 2013). Because of its beautiful yellow-golden color, it is also called "Indian saffron". Turmeric is an important monocotyledon plant mainly valued all over the world as a spice, coloring agent and also for its medicinal properties (Roses, 1999; Nasirujjaman, 2005). In Bangladesh and other developing countries, it is mainly utilized as spice and culinary additive in curries and other local dishes. It is also used as Ayurvedic medicine where it is called "*haridra*". Turmeric have been identified worldwide as many as 133 species. The word "Turmeric" comes from Latin word "*terra merita*" meaning meritorious earth. The name of the genus, *curcuma*, is derived from the Arabic name "Kurkum".

Turmeric probably originates from Indian sub-continent and South-East Asia. The use of turmeric dates back nearly 4000 years to the Vedic culture in India, where it was used as a culinary spice and had some religious significance. Now-a-days, it is widely cultivated in the tropics and goes by different names in different cultures and countries. In Bangla, it is called "*Holud*" (The Yellow color) and in Sanskrit, it is called "*Haridara*" ("The Yellow One"), "*Gauri*" ("The one whose face is light and shining"), "*Kanchani*" ("Golden Goddess"), and "*Aushadi*" ("Herb").

Turmeric is a warm season crop. It grows well in humid climatic conditions and tropical and sub-tropical regions. Turmeric can be grown at an altitude of 1500 meters (mean sea level), at a temperature range of 20-30^{0}C with a rainfall of 1500-2250 mm per annum. It can be grown well under both irrigated and rainfed conditions. Turmeric crop can be grown on wide variety of soils. However, it thrives best in a well-drained sandy or clay loam rich in humus content but it does not stand under water logging condition (Purseglove, 1976). The ideal soil pH range is 4.5 to 7.5 for this crop. It is exclusively vegetative propagated

crop using rhizomes and the cultivated turmeric is not known to set seeds (Ravindram, 2005).

Turmeric contain more than 100 components. The major component of the rhizome is a volatile oil which contain turmerone, and there are most significant coloring agents called curcuminoids in turmeric. The coloring agent curcuminoids consist of curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants (Ruby, 1995; Selvam, 1995). It contents about 69.43% carbohydrate, 6.30% protein, 5.10% volatile oil and 3.50% mineral and other important element in dry turmeric. Volatile oils include d-α-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes (Ohshiro, Kuroyanag, and Keno, 1990). The secondary metabolites includes bisabolane-type sesquiterpenes like germacrone; termerone; ar-(+)-, α-, and β-termerones; β-bisabolene; α-curcumene; zingiberene; β-sesquiphellanderene; bisacurone; curcumenone; dehydrocurdione; procurcumadiol; bis-acumol; curcumenol; isoprocurcumenol; epiprocurcumenol; procurcumenol; zedoaronediol; and curlone, many of which are specific for a species. Turmerone, arturmerone, and zingiberene are responsible for aroma of turmeric. The rhizomes are also contains four new polysaccharides-ukonans along with stigmasterole, β-sitosterole, cholesterol, and 2-hydroxymethyl anthraquinone (Kapoor, 1990; Kirtikar and Basu, 1993). Nutritionist analyses that 100 g of turmeric contains 390 kcal, 10 g total fat, 3 g saturated fat, 0 mg cholesterol, 0.2 g calcium, 0.26 g phosphorous, 10 mg sodium, 2500 mg potassium, 47.5 mg iron, 0.9 mg thiamine, 0.19 mg riboflavin, 4.8 mg niacin, 50 mg ascorbic acid, 69.9 g total carbohydrates, 21 g dietary fiber, 3 g sugars, and 8 g protein (Balakrishnan, 2007). Turmeric is also rich of the ω-3 fatty acid and α-linolenic acid 2.5% (Goud, Polasa and Krishnaswamy, 1993).

Turmeric is highly valued from the thousands of years for its flavor and aroma. Due to its pungent, spicy aroma and color, turmeric is used in the manufacture of a number of food products like canned beverages, baked products, dairy products, ice cream, yogurt, yellow cakes, orange juice, biscuits, popcorn color, cereals, sauces, gelatin, curry powders, certain curried meat and table sauces. In Bangladesh and Indian sub-continent countries, it is

mainly utilized as spice and culinary additive in curries and other local dishes as a curry powder. It is also used in veterinary medicine and preparing Ayurvedic, Unani, Homeopathic and Allopathic medicine. Due to diversified use of turmeric, it has also great demand in the world market.

Turmeric is highly valuable plant having underground rhizome for its medicinal properties. Turmeric is used as herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments (Dixit, Jain and Joshi, 1988). It helps to improve the complexion and useful in diseases of blood, scabies, leucoderma, inflammations (Arora, 1971; Satoskar, 1986), ozoena, bad taste in the mouth, biliousness, dyspepsia (Thamlikitkul, 1989), ulcer (Vandau, 1998; Kositchaiwat, 1993), elephantiasis, snake-bite, boils and sprains (Kiritikar & Basu, 1996). It is also cured for digestive disorders; to reduce flatus, jaundice, menstrual difficulties, and colic; for abdominal pain and distension (Bundy, 2004).

Turmeric is considered as essential spice in Bangladesh and many of the countries consider curry to be incomplete without it. It has diversified uses. The people of Bangladesh usually use turmeric in all curry preparation like meat, fish, vegetables, pulses etc. for its typical color and flavor. Haldi ceremony (called *Gaye holud* in Bengal) (literally "yellow on the body") is a ceremony observed during wedding celebrations in many parts of the country. Besides, it is also used as dye in textile industries.

Turmeric is grown all over the districts in Bangladesh. But the main growing districts are Khagrachhari, Rangamati, Tangail, Rajbari, Jashore, Jhenaidah, Mymensing, Natore, Pabna, Rajshahi, Nilphamari and Panchagarh. The annual production of turmeric in Bangladesh is 143542 tonne where the area of cultivation is 62746 acres (BBS, 2016-17). It take about 9% of total spices production (BBS annual estimate, 2011-12). Dry turmeric and turmeric powder is exported every year from Bangladesh to abroad. The annual export of turmeric in Bangladesh is 86297 kg in year 2016-17 and earned 30256 taka foreign currency (BBS, 2016-17). Although we are the 4th largest producer of turmeric in the world

but per acre yield and quality is pathetically low. Its commercial cultivation is limited by low rate of rhizome multiplication. In a growing season (8-10 months), only 10-15 lateral buds can be produced. Vegetative propagation of turmeric has the high risk of spreading systemic infections. It is reported that a three-fold increase in the production of rhizomes could be possible by the effective control of the diseases (Balachandran, 1990).

Turmeric is constrained severely by several factors. (1) Turmeric normally propagates by rhizome with a low proliferation rate, and the reproducing part (rhizome) is also the economically used part of the Turmeric plant, which restricts the availability of Turmeric seeds needed for cultivation, (2) preservation of rhizome seeds is a hard job. It requires much attention, time and space, (3) these rhizomes are prone to damages due to different factors such as adverse environment, insect and pathogen attack etc. The high susceptibility of this crop to soft rot (caused by *Pythium myriotylum*, and *P. gramnicolum*) and bacterial wilt (*Pseudomonas solanacearum*) is a major constraint in the production of turmeric, (4) multiplication rate of rhizome is slow and the conventional method of propagation is insufficient to meet the demand of planting material and eventually affect the final cost of rhizomes, (5) limited availability of high yielding genotypes, expensive field maintenance of planting material in turmeric production may despairing the commercial growers (Khader, 1994; Nayak & Naik, 2006).

In vitro approaches for the conservation and the use of plant germplasm can offer some distinct advantage over alternative strategies. Some of these are as follows: (1) It helps to eliminate plant disease from virus, bacterial and fungal effect, (2) clonal material can be produced where this is useful for the maintenance of elite genotypes, (3) rapid multiplication may be possible any time and any season where stocks are required using micro propagation procedures, (4) species that require long germination time, have low levels of seed production or seeds do not readily germinate, rapid propagation is possible, (5) large number of clones from a single seeds or explants, and (6) it shortens time and distribution across the border may be safer, in terms of germplasm health status using *in vitro* cultures. Some more general positive advantages of *in vitro* techniques include the

fact that storage space requirements are vastly reduced compared with field storage. Tissue cultures are not subject to environmental disturbances such as temperature fluctuation, cyclones, insect, pests, and pathogen (Rands, 2010; Nandagopal, 2011).

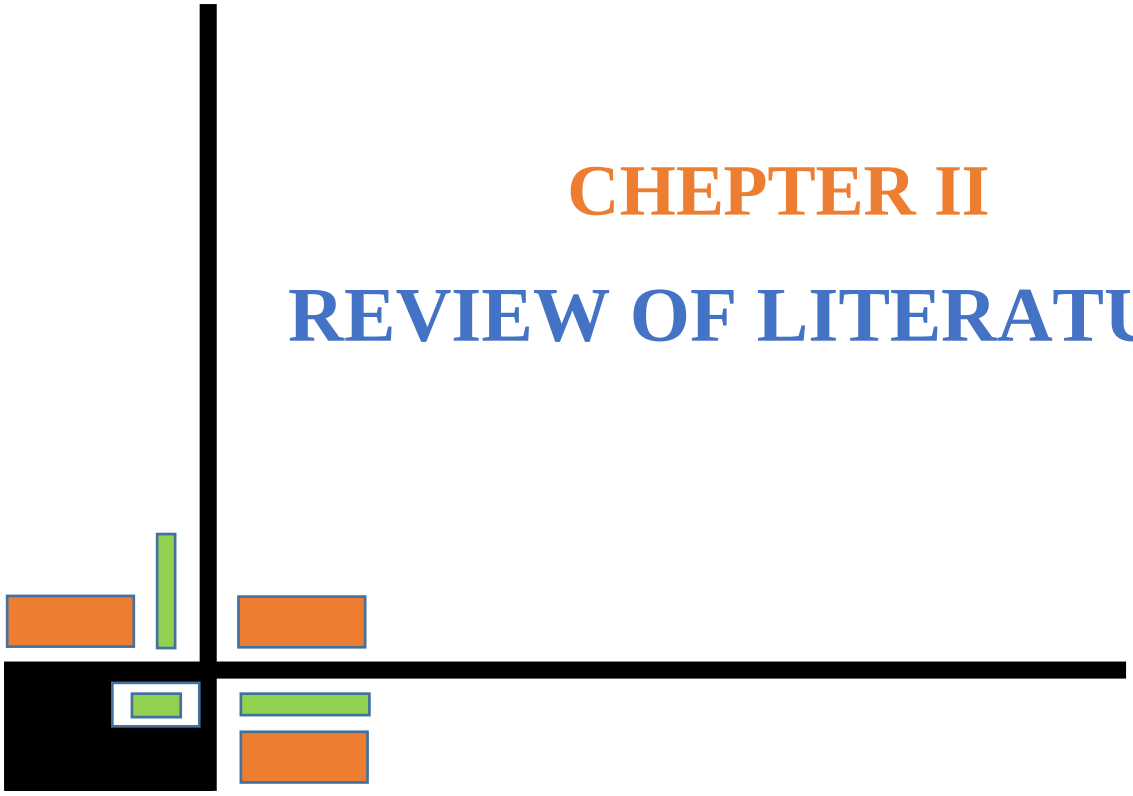
In vitro propagation may help overcome these problems to a great extent. It represents an alternative for massive clonal propagation of rhizome producing species. These techniques are adopted at commercial level in order to fulfill supply gap of huge demand. It increases multiplication rates and also generates material free from pathogens. So due to high popularity and demand for turmeric in Bangladesh it has become one of the first commercial targets for micro propagation and thus tissue culture can be utilized for its large scale production. Therefore, in the present study, an attempt has been made to develop a simple, efficient *in vitro* propagation protocol for clonal propagation of the most popular cultivar of turmeric plants.

Objectives:

i. Establishment of *in vitro* regeneration protocol of Turmeric
ii. Assessment of hormonal effect for *in vitro* response of Turmeric
iii. Identification of best hormonal concentration for *in vitro* regeneration of Turmeric
iv. To study the rapid regeneration of Turmeric within a short period of time.

CHEPTER II

REVIEW OF LITERATURE



CHAPTER II
REVIEW OF LITERATURE

The present investigation involved *in vitro* propagation of Turmeric (*Curcuma longa* L.). It is an important horticultural spice crop valued for its dry rhizomes which impart color, flavor to food and also important for its medicinal properties. Bangladesh is the 4th largest producer of turmeric in the world. But unfortunately, very limited significant work has been done on its value. However, some related works already performed by different institutes home and abroad have been reviewed and some of the most relevant literatures are cited below.

2.1 Concept of plant tissue culture

Plant tissue culture is the growth of plant tissues or cells artificially in the laboratory separate from the plant. It is the backbone of plant biotechnology which is comprised of micropropagation, induction of somaclones, somatic hybridization, cryopreservation and regeneration of transgenic plants. Plant tissue culture was originated from the cell theory that was formulated by Schwann in 1839. Gottlieb Haberlandt (1902), an Austrian botanist, is recognized as father of plant tissue culture. On the other hand this technique was first introduced in Bangladesh with jute in late 1970s in the department of botany at Dhaka University and with potato at Tuber Crops Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI) since 1983.

Morel (1960) was the first to show the potential of clonal propagation in culture by describing *in vitro* multiplication of Cymbidium orchids. Several protocorms formed on each explant, and when divided and transferred, additional protocorms formed. The rate of multiplication was such that several million plants could be produced from one shoot tip in a single year. As a result, many of the economically important orchids are now usually propagated *in vitro*, although techniques have not been worked out for all orchid types. However, credit for the development of the micro-propagation technology in general goes without a doubt to Murashige, who showed that many plants, in addition to orchid, could

be propagated *in vitro*. He 14 along with his colleague was also responsible for devising a suitable medium for tissue culture which is widely used (Murashige and Skoog, 1962).

2.2 *In vitro* Propagation of Turmeric

Micropropagation has been shown to be useful especially in horticultural crops. The first report of micropropagation of turmeric came from National chemical laboratory, Pune using vegetative buds. Nadagouda (1978) reported successful rapid clonal multiplication when buds were cultured on MS medium supplemented with coconut milk and BAP. Successful micropropagation was also achieved by Shetty (1982) who observed both direct organogenesis and callus production when rhizome buds were cultured.

Keshavachandran and Khader (1989) studied the response of Co-1 and BSR-1 cultivars of turmeric for *in vitro* clonal propagation. Buds of these cultivars were cultured on MS medium supplemented with 1 mg Kinetin /L, 1 mg BAP/L and 40 g sucrose/L with an average number of shoots of 2.11 in BSR-1 and 2.5 in Co-1. After 5 weeks, rooted plantlets were transferred to pots and two weeks later plants were well established. A multiplication ratio of 1:2240 was obtained in a year through micropropagation in turmeric (Vidya *et al.*, 1989). They reported profuse rooting on minimal medium. Survival of plantlets was 77%.

Balachandran (1990) carried out *in vitro* clonal multiplication of *Curcuma domestica, C. aeruginosa, C. caesia* and ginger by inoculating excised rhizome buds. For shoot multiplication, 3.0 mg/L of BAP was found to be optimum for all the species. Rhizome buds of both genera produced shoots and roots simultaneously and within four weeks, complete plantlets were formed. They observed that the field established plants were morphologically uniform.

Zuraida (2013) showed that the growth medium supplemented with 5 mg/L BAP and 2 mg/L & 2,4-D promoted callus induction after 70 days of culture. Sub-culturing on the same medium enhanced the production of friable callus. Culture media containing higher concentrations of agar promoted the development of green somatic embryos from the

callus. Respond of somatic embryogenesis was most successful with MS medium in 6.0 g/L agar supplemented with 5 mg/L BAP and 0.2 mg/L & 2,4-D whereby the callus developed into green somatic embryos with an efficiency of 53%. This culture medium also produced the largest number plantlets.

Chirangini and Sharma (2005) discovered that microrhizome were observed at the base of all *in vitro* derived shoots cultured on the media supplemented with a range of sucrose concentration (3-9%) within 8 weeks of incubation. The best response in terms of number (up to 6 micro rhizomes per culture tube) were obtained in the media supplemented with 7% sucrose and highest average fresh weight of 0.81 g with 3-5 buds was observed in MS media supplemented with 5% sucrose .

Rout *et al.* (2001) declared that rhizome formation was initiated on MS media containing 4.44-8.88 μM BA, 2.85-8.57 μM IAA and 3% sucrose within 8 weeks of culture. The high percentage of rhizome formation was noted on MS medium containing 4.44 μM BA, 5.71 μM IAA and 3% sucrose. The medium having sucrose in combination with maltose or fructose caused a decline in rhizome formation as compared to sucrose alone. The results also show that the rhizome frequency was maximum in the medium containing 6-8% sucrose whereas medium supplemented with 1-2% sucrose did not produce rhizomes.

2.3. Sterilization of explant

Sterilization is an important step in the preparation of explants to avoid bacterial and fungal contamination during tissue culture techniques. Therefore optimization of the conditions to avoid this contamination is a prerequisite for in vitro culturing. Different chemicals are used for surface sterilization process e.g. Mercuric Chloride, Sodium hypochlorite and Calcium hypochlorite etc. Moutia and Dookun (1999) used 2.7% solution of sodium hypochlorite and 0.1% mercuric chloride for elimination of bacterial contamination in sugarcane crop and recorded 46.8% and 56.3% clean cultures respectively. The surface sterilization process with antibiotic proved very useful and greatly reduced the bacterial contamination.

Several surface sterilants were used during the micropropagation process with varying duration of treatments. Surface sterilants like calcium hypochlorite, alcohol, mercuric chloride and sodium hypochlorite were used either alone or in combination used chlorine alone to treat the explants. A successful tissue culture protocol starts with effective explants sterilization. Contamination with microorganisms is considered to be the single most important reason for losses during *in vitro* culture of plants. Such microorganisms include viruses, bacteria, yeast, fungi, etc. These microbes compete adversely with plant tissue cultures for nutrients. The presence of these microbes usually result in increased culture mortality but can also result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting.

Gomathy (2014) reported that the explants of turmeric were primarily rinsed in tap water for 20 minutes followed gently rinsed with 70% ethanol for 60 seconds and with 5% NaOCl solution for 10 minutes. After each step of sterilization, the explants were washed with sterile double distilled water for three times. Further sterilization procedures were carried out in laminar air flow chamber by using 0.1% $HgCl_2$ for 5 minutes. The explants were then rinsed five times with sterile distilled water. Finally, in the laminar air flow chamber, the explants were cut into small pieces.

Both NaOCl and $HgCl_2$ are oxidizing agents and damage the microorganism by oxidizing the enzymes. The ineffectiveness of NaOCl may be due to the reason that it is a mild sterilizing agent (Sirivastava et al. 2010). HgCl2 is reported a better sterilizing agent as compared to NaOCl but is more toxic and requires special handling and is difficult to dispose.

Surface sterilization with $HgCl_2$ (0.1%) for 14 minutes to normal sterilization procedure, fungal contamination could be controlled up to 98%. Control of fungal contamination by using $HgCl_2$ has been reported by Rahman *et al*., 2004 in *Curcuma longa*.

2.4. Culture Medium

Success of *in vitro* culture depends largely on the choice of nutrient medium, including its chemical composition and physical form (Murashige, 1962). Several media formulations have been reported for turmeric shoot tip culture but nearly half of them are modified MS media. Other popular media include B5, SH, N6, and Linsmaier and Skoog (LS) media. The culture media vary in both type and concentration of the components, but all have similar basic components of growth regulators, nitrogen, carbohydrates, inorganic macronutrients and micronutrients, vitamins and organic additives.

Generally, the cultures are established on a separate initiation medium, which has a lower concentration of cytokinin than the multiplication medium to which the cultures are subsequently 17 transferred. The composition of initiation, multiplication and rooting media were used. After autoclaving, the culture medium is stored in a clean dust free chamber for 1-2 days before use in order to check for any contamination. Bacterial contamination may be observed, particularly during the rainy season. Use of cefatoxime in the initiation and subsequent subcultures helps to overcome even latent bacterial contaminations.

2.5. Effect of Growth Regulators

In vitro studies carried out at Regional Plant Resource Centre, Bhubaneshwar for PTS 28 and summer cultivar of turmeric indicated that shoot multiplication could be achieved in MS medium supplemented with 4.0 mg/l BAP, 1.0 mg/l IAA and adenine sulfate at 10ppm. Shoots successfully rooted on half strength MS medium supplemented with IAA or IBA at 0.25–0.5 mg/l and 2% sucrose (Rout *et al*., 1995).

At Indian Institute of Spice Research, Calicut, micropropagation was standardized using the bud explants of turmeric on MS medium supplemented with 1 mg/l BAP and 0.5 mg/l NAA. Such explants responded readily to culture conditions producing 8-10 shoots in 40 days (Nirmalbabu, *et al*., 1993 and 1997). They could establish tissue culture plantlets in soil with 80 percent success. However, their opinion was that micropropagated plantlets

take three crop seasons to produce normal seed rhizome and hence the technique cannot be employed for commercial multiplication.

Salvi (2002) developed a protocol for *in vitro* propagation of turmeric cv. Elite using vegetative buds form the rhizome. Multiple shoots were produced on MS solid medium supplemented with BAP and NAA. Liquid medium was more favorable for shoot multiplication. RAPD analysis of regenerated plants using sixteen 10 mer primers did not show any polymorphism.

Amin (2004) showed that the best result towards development of multiple shoot from the cultured explants was obtained when MS medium was supplemented with 2.0 mg/L of BA.

Rahman (2004) obtained optimum development of multiple shoots for culture explants on MS medium with 2.0mg/L BAP. Rooting of shoots was obtained on ½MS medium with 0.1–1.0 mg/L. More than 70 per cent of transplanted plantlets survived in the field.

Dogra (1994) achieved *In vitro* propagation of *Zingiber officinale* using rhizome buds and they also observed that the buds produced multiple shoots when cultured on MS medium with 2.5 mg/L BA and 0.5 mg/L NAA. Mohammed and Quraishi, (1999) used shoot tips (3-5 mm) as explants where maximum number of shoots from single explants was 3.33 when medium contained 3.0 mg/L BAP. Maximum plant height during the same period was on medium containing 2.0 mg/L BA and shoot number was also relatively better.

Ilahi and Jabeen, (1987) showed that stem cuttings from 3 month old plants, young buds, rhizome cuttings with shoot bud primordial and juvenile shoots were cultured on ½ MS medium supplemented with different combinations of growth hormones. Whereas callus could not be induced on the stem explants, callus was induced on the juvenile shoots which on sub culturing to medium with varying concentrations of 2, 4-D and BAP developed bud primordial. Young shoot buds along with a portion of rhizome when inoculated on MS medium containing 2, 4-D and BAP each @ 0.5 mg/l ruptured and some callus was produced. Excised single shoot when transferred to a medium supplemented with 0.1 mg/l

each of 2, 4-D and BAP started rooting within 2-3 days. Young shoots inoculated on MS medium supplemented with 0.5 mg/L both of 2, 4-D and BAP developed greenish white hard callus within 4 weeks. These calli when sub cultured on a media containing 0.1 mg/l of 2, 4-D and 0.5 mg/L BAP produced good callus growth within a week. Shoot buds were observed after 4 weeks which ultimately grew into small plantlets (10-15 plantlets in each culture). Good callus growth was observed within 4 weeks when 2 week old shoot buds were inoculated on a medium supplemented with BAP and 2, 4-D each @ 0.5mg/L.

Pramila *et al*. (2008) showed that MS media supplemented with BAP 4 mg/L + NAA 0.1 mg/L recorded more shoot length and higher number of leaves, indicating its superiority over other treatments in the study of influence of growth regulators on shoot multiplication. Media having various concentrations of BAP and NAA have been evaluated for induction of microrhizome. BAP 2 mg/L + NAA 0.1 per cent mg/L produced highest number of shoots producing microrhizomes whereas early initiation of microrhizomes was observed in MS with BAP 1 mg/L + NAA 0.2 mg/L. BAP 1mg/L alone resulted in highest number of microrhizomes, weight of mirorhizomes and number of nodes per microrhizome.

Hegde (2006) developed *in vitro* studies for clonal propagation of turmeric and indicated that the highest number of multiple shoots in turmeric was obtained in MS medium supplemented with 2.0 mg/L BAP and 0.2 mg/L NAA whereas, better rooting was obtained with 0.5 mg/L IBA.

Nasirujjaman (2004) showed that woody plant medium supplemented with different concentration of BAP or combination of NAA produced varying degree of multiple shoot. A supplementation of 4.0 mg/L BAP+ 1.0 mg/L NAA gave the best result.

Hosoki and Sagawa, (1977) showed about 30% of the explants did not produce shoots, however, an average of 5.7 shoots was produced when scale leaves were removed from these explants 2-3 months after culture. Only a few shoots appeared in 1 ppm BAP+ 1 ppm NAA medium although many roots were formed. Shoots were later transferred individually to 1 ppm BAP medium and shoots elongated and roots also formed within 2 months. Some

plantlets produced additional adventitious shoots. Numerous plantlets with roots were produced by repeated sub culture of individual plantlets on 1 ppm BAP medium.

Chan and Thong (2004) observed that medium used for *in vitro* multiplication of rhizomatous plant, MS medium with 2.0 mg/L BAP and 2.0 mg/L IBA was also suitable for *in vitro* propagation of other Zingiberaceae species.

Prathanturaung (2005) studied the effect of thidiazuron on multiplication of turmeric buds. MS liquid medium supplemented with 7264 μM thidiazuron (TDZ) prior to culture was used. Regeneration rate was up to 11.4+ 1.7 shoots / explant.

Shahinozzaman (2013) showed that the optimum shoot proliferation was obtained from MS medium containing 3.0 μM BA + 0.5 μM NAA in shoots propagation of *Curcuma caesia* Roxb. In this growth regulator combination, maximum 99.97 % explants produced 10.38 shoots with 4.53 cm length after 8 weeks of culture.

Salvi (2002) reported that shoot multiplication rates of 4.2, 3.5 and 6.6 shoots/buds following the culture of shoot-tip explants for 8 weeks in liquid medium supplemented with 1μM NAA and BA, Kin, or 2iP (10 μM each), respectively.

Panda (2007) developed *in vitro* propagation protocol for *Curcuma longa* and gave 7.6 shoots by using medium with 3.0 mg/L BAP. While Prathanturarug (2003) obtained 18.22 shoots/explants after 12 weeks by using 18.17 μM TDZ. Rahman (2004) reported best shoot proliferation from rhizome bud explants in MS + 2.0 mg/L BAP and gave 6.2 average lengths of shoots per culture.

Ali (2004) could get multiple shoots on MS medium supplemented with only BAP and 0.25 mg/L kinetin. Rooting was achieved on MS medium with 1.0 mg/L NAA. All plants were morphologically uniform.

Shahinozzaman (2013) developed *in vitro* propagation of *Curcuma caesia* and gave that spontaneous rooting was observed after 4 weeks of cultivation with all treatments using half strength MS medium containing IBA and NAA at different concentrations, high frequency of rooting (89.76 %) was obtained in 3.0 μM IBA (Indole-3-butyric acid) containing medium. The plantlets, thus developed, were hardened and successfully established in natural soil.

Rahman (2004) showed that rooting experiments with half strength of MS medium and various concentrations of NAA, IBA and IAA revealed that 0.1-1.0 mg/L of any auxins was found to be effective for rooting. But root forming performance of IBA was proved to be the best among the three auxins tested.

Anchalee *et al.* (2012) obtained that explants which were cultured on MS medium supplemented with 1 mg/L NAA and 2 or 3 mg/L BA gave the highest average number of new shoots (2.4, 2.6 shoots, respectively) and number of leaves (5.4 leaves), optimum number of roots (2.6 roots per shoot), and plant height (4.5 cm). MS medium supplemented with only 2 mg/L BA produced the highest average number of shoot (2.6 shoots) and 5.4 leaves per shoot. Explants were trimmed in longitudinal sections (LS) to 2 and 3 sections. At 2 sections gave the highest number of new shoots (4.3 shoots per section). Explants which were cultured on MS medium with 60 gm/L gave the highest average shoots and leaves per bunch, and longest and biggest size of root.

2.6. Hardening

Shagufta (2009) showed that the frequency of shoot induction was 70, 60 and 75 in Faisalabad, Kasur and Bannun varieties respectively. The number of shoots per explant increased with increased BAP concentration while shoot length decreased. These regenerated shoots were further multiplied by sub culturing on fresh medium after 30 days. The regenerated plants of all varieties were transferred to different mixtures of compost for acclimatization. Best hardening response was obtained in Sand + Soil + Peat (1:1:1) after three week of transplantation in glass house. These hardened plants were subsequently shifted into field.

Mohammed (2011) showed augmentation of MS-medium with 4.5 mg/L BAP developed the highest number of shoots and leaves (8.0 and 15.50 respectively). Shoot lets were highly rooted on half strength of B5 medium supplemented with 1.0 mg/L NAA. The maximum percentage of acclimatization, hardening and rhizomes production of *in vitro* derived plants in greenhouse was 80–100%.

Gomathy (2014) reported that Healthy plantlets with 4 to 5 cm long roots were individually removed from the culture tubes. After washing their roots carefully with tap water, plantlets were transplanted into 10 cm diameter plastic pots containing a mixture of sand, soil and vermicompost (1:1:1) and they were placed in the greenhouse for hardening. The plants were watered with half-strength MS salts solution every week and covered with a polythene bag for 2 weeks. Afterwards, the hardened plants were gradually transferred to 20 cm pots containing pure garden soil and kept in the field for developing into mature plants.

Zuraida (2014) showed that rooted explants of turmeric with shoots about 4-5 cm in length were removed from the culture bottles and the roots were washed under running tap water to remove the agar. The plantlets were individually transplanted in soil contained in polybags and kept under controlled conditions in a net house with 75% shading. To maintain humidity, the plants were watered periodically twice a day. The survival rate of the plantlets was recorded after 6 weeks.

CHEPTER III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

3.1 Time and location of the experiment:

The present research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of February 2018 to June 2018.

3.2 Experimental materials:

3.2.1 Source of materials

The planting materials of turmeric (*Curcuma longa*) were collected from Khararchar village, Dhamrai, Dhaka-1822.

3.2.2 Plant material

The healthy, disease free sprout of turmeric of 1-2 cm length were used as explants for the study of *in vitro* propagation of Turmeric (Plate 1)

Plate 1: Sprout of Turmeric used as explant

3.2.3 Instruments

Metal instruments *viz*., forceps, scalpels, needles, spatulas and aluminum foils were sterilized in an autoclave at a temperature of 121^0C for 20 minutes at 1.06 kg/cm^2 (15 PSI) pressure.

3.2.4 Glassware

The Borosil glassware was used for all the experiments. Oven dried (250^0C) Erlenmeyer flasks, culture bottles, flat bottom flasks, pipettes, Petri dishes, beaker and measuring cylinders (25 ml, 50 ml, 100 ml, 500 ml and 1000 ml) were used for media preparation**.** The glassware's were first rinsed with the liquid detergent (Trix) and washed thoroughly with tap water, until the detergent was removed completely. Finally they were rinsed with distilled water and sterilized in oven at 160-180^0C for 3-4 hours.

3.2.5 Culture medium

The degree of success in tissue culture is mainly related to the choice of nutritional components and growth regulators. Presence of plant growth regulators plays a significant role in a successful regeneration of any plant species. Media for tissue culture should contain all major and minor elements, vitamins and growth regulators which are essential for normal plant growth. Explants were inoculated onto media composed of basal MS (Murashige and Skoog, 1962) medium supplemented with the plant growth regulators. Composition of MS media have been shown in appendix I. Hormones were added separately to different media according to the requirements. To do so, stock solutions of hormones were prepared ahead of media preparation and stored at 4^0C temperature.

3.2.6 Plant growth regulators (PGRs)

PGRs or hormones were added separately in different media according to the requirements. Following stock solutions of hormones were prepared ahead of media preparation and stored at 4^0C temperatures.

1. BA (1.0, 2.0, 3.0 and 4.0 mg/L) for shoot induction.
2. BA (1.0, 2.0, 3.0 and 4.0 mg/L) combined with IBA (1.0, 2.0, 3.0 and 4.0 mg/L) for shoot and root formation respectively.
3. IBA (1.0, 2.0, 3.0 and 4.0 mg/L) for root formation.

3.3 Preparation of the stock solution of hormones

In order to prepare hormonal supplements, they were dissolved in proper solvent as shown against each of them below. Generally, cytokinins were dissolved in few drops of basic solutions (1N NaOH) and auxins were dissolved in few drops of basic solutions (1N NaOH) or 70% ethyl alcohol.

Hormone (solute)	Solvents used
BA	1N NaoH
IBA	70% ethyl alcohol

In present experiment, the stock solution of hormones was prepared by following procedure. 100 mg of solid hormone was placed in a small beaker and then dissolved in 10 ml of 70% ethyl alcohol or 1 (N) NaOH solvent. Finally the volume was made up to 100 ml by the addition of sterile distilled water using a measuring cylinder. The prepared hormone solution was then labeled and stored at 4 ± 1^0C for use up to two month. (Growth regulators were purchased from Sigma, USA.)

3.4 Treatments

Four sub experiments were conducted to assess the effect of different concentrations of BA and IBA on shoot proliferation and subsequent rooting of the regenerated.

Sub-experiment 1.

Effect of BA on *in vitro* shoot induction potentiality in Turmeric

Four levels of BA (1.0, 2.0, 3.0 and 4.0 mg/L) and control (0.0 mg/L) were used. The experiments were arranged in Randomized Completely Block Design (RCBD) with three replications.

Sub-experiment 2.

Combined effect of BA and IBA on *in vitro* shoot and root induction potentiality in Turmeric

Treatments:

In this sub-experiment, Four levels of IBA (1.0, 2.0, 3.0 and 4.0 mg/L) were practiced with each level of BA (1.0, 2.0, 3.0 and 4.0 mg/L) Total 16 combinations of BA and IBA were examined in this experiment and control treatment also practiced. The combinations were as follows:

T1 = BA 1.0 mg/L + 1.0mg/L IBA

T2 = BA 1.0 mg/L + 2.0 mg/L IBA

T3 = BA 1.0 mg/L + 3.0 mg/L IBA

T4 = BA 1.0 mg/L + 4.0 mg/L IBA

T5 = BA 2.0 mg/L + 1.0 mg/L IBA

T6 = BA 2.0 mg/L + 2.0 mg/L IBA

T7 = BA 2.0 mg/L + 3.0 mg/L IBA

T8 = BA 2.0 mg/L + 4.0 mg/L IBA

T9 = BA 3.0 mg/L + 1.0 mg/L IBA

T10 = BA 3.0 mg/L + 2.0 mg/LIBA

T11 = BA 3.0 mg/L + 3.0 mg/L IBA

T12 = BA 3.0 mg/L + 4.0 mg/L IBA

T13 = BA 4.0 mg/L + 1.0 mg/L IBA

T14 = BA 4.0 mg/L + 2.0 mg/L IBA

T15 = BA 4.0 mg/L + 3.0 mg/L IBA

T16 = BA 4.0 mg/L + 4.0 mg/L IBA

The experiments were arranged in Randomized Completely Block Design (RCBD) with three replications. Each of replications consisted of five culture vials.

Sub-experiment 3.

Effect of IBA on root induction potentiality of in vitro regeneration in Turmeric

Four levels of IBA (1.0, 2.0, 3.0 and 4.0 mg/L) and control (0.0 mg/L) were used. The experiments also practiced as sub-experiment 1.

Sub-experiment 4.

Acclimatization and establishment of plantlets on soil

Tissue culture derived plantlets were acclimatized in shade house and natural condition to find out the survival percentage.

3.5 Preparation of culture media from ready made MS powder

To prepare 1000 ml of culture media the following steps were followed:

I. 700 ml of sterile distilled water was poured into 1000 ml beaker.

II. 5 gm of MS media (readymade) and 30 gm of sucrose was added and gently stirred to dissolve these ingredients completely with the help of a hot plate magnetic stirrer.

III. Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well.

IV. The volume was made up to 1000 ml with addition of sterile distilled water.

V. The pH was adjusted at 5.8.

VI. 8 gm agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar.

VII. Required volume of hot medium was dispensed into culture vessels. After dispensing and proper cooling of the medium, the culture vessels were plugged with cork and marked with different codes with the help of a glass marker to indicate specific hormonal combinations.

3.6 Steam heat sterilization of media (Autoclaving)

For sterilization, the culture medium was poured in 200 ml culture bottles and then autoclaving was done at a temperature of 121^0C for 30 minutes at 1.06 kg/cm^2 (15 PSI)

pressure. After autoclaving, the media were stored in at 25±2 °C for several hours to make it ready for inoculation with explants.

3.7 Preparation of explants

The healthy diseases free sprouts were washed thoroughly under running tap water and then with sterilized distilled water for several times. Subsequently the explants were transferred to laminar airflow cabinet and kept in a 250 ml sterilized beaker. The beaker with explants was constantly shaken during sterilization. They were treated with 70% ethanol for 1-2 minute and rinsed with autoclave distilled water for 3-4 times. After treating with 70% ethanol, the explants were immersed in 0.1% $HgCl_2$ within a beaker and added 3-4 drops of Tween-20 for about 7 minutes with constant shaking in clockwise and anticlockwise direction. Then explants were washed 3-4 times with autoclaved distilled water to make the material free from chemical and ready for inoculation in culture media.

3.8 Inoculation of culture

The sterilized explants were inoculated carefully following proper sterilization process within laminar airflow cabinet. Prior to use, the surface of the laminar flow bench was swabbed down with 70 % ethyl alcohol and the interior sprayed with the same alcohol. All glassware, instruments and media were steam-sterilized in an autoclave. During the course of the work, instruments in use were placed in a beaker containing 70 % ethanol and were flamed repeatedly using a spirit burner. The worker's hands and forearms were washed thoroughly with soap and water and repeatedly sprayed with 70% alcohol during the period of work. The mouth of all culture vial was flamed before and after positioning of the explants on the medium.

For inoculation, explants were transferred to large sterile glass Petridis or glass plate with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer cover was removed with sterile scalpel blade to make suitable size. After cutting explants into suitable size (0.5-1.0 cm), explants are transferred to culture

bottles containing MS medium with plant growth regulator. After vertically inoculating the explants in culture bottle, the mouth of bottle is quickly flamed and capped tightly. After proper labeling, mentioning media code, date of inoculation etc. the bottles was transferred to growth room.

Plate 2. Explant in the culture vial

3.9 Incubation

The bottles were kept to the culture racks and allowed to grow in controlled environment. The cultures were maintained at 21±1°C with light intensity varied from 3000–5000 Lux (23 W white bulbs). White fluorescent lamps were used for growth of the culture. The photoperiod was generally 14 hours light and 10 hours dark having 70% relative humidity (RH).

3.10 Shoot proliferation

The explants were cultured on MS nutrient medium supplemented with different concentration of BA alone or in combination of IBA. After successful shoot proliferation, subculture was done with newly form shoots. Shoots ware excised in aseptic condition with help of sterile scalpel blade and sterile forceps and transferred to new MS media which was supplemented with same concentration of growth hormones in order to increase budding frequency. The observations on development pattern of shoots were made throughout the entire culture period. Data recording was started after 2rd week from inoculation.

3.11 Rooting of multiple shoots

Newly formed shoots with adequate length were excised individually from the culture vial and transferred to rooting media. The growth regulators IBA was used in different concentration (1.0, 2.0, 3.0 and 4.0 mg/L) along with MS media. The observations on development pattern of roots were made throughout the entire culture period. Data were recorded from 2rd week of inoculation.

3.12 Acclimatization

Acclimatization or hardening is a process by which *in vitro* propagated plants are made to adapt to an *in vivo* environment.After 42-49 days of culture on rooting media, the plantlets were taken out from culture vial with the help of forceps with utmost care to prevent any damage to newly formed roots and dipped in gentle warm water to remove any traces of solidified agar media for acclimatization. Plastic pots (6×6 cm) were kept ready filled with garden soil and compost in the proportion of 1:1 respectively. Immediately after removing solidified agar media from newly formed roots, the plantlets were then transplanted in to the pots with special care. After planting, the plantlets were thoroughly watered and were kept at 21±1°C with light intensity varied from 3000 –5000 lux. The photoperiod was generally 14 hours light and 10 hours dark and 70% RH for 7 days with consecutive irrigation. Then the plants were shifted to shade house with less humidity and indirect sunlight. The top of the pots were covered with transparent plastic sheet and grew at room

temperature and 70% RH for 14 days with periodic irrigation (2days intervals).After 3 weeks, the plants were transferred to the soil following depoting and poting into different pot having bigger pot size. The plants were watered periodically and upper layer of the soil mulched occasionally whenever necessary.

3.13 Data recording:

The observations on development pattern of shoots and roots were made throughout the entire culture period. Five replicates each of them containing 4 bottles (single shoot per culture bottle) were used per treatment. Data were recorded after 2 weeks of culture. Starting from day of inoculation on culture media in case of shoot proliferation. In event of root formation, it was done every week starting from third week to six week of culture. The following observations were recorded in cases of shoot and root formation under *in vitro* condition.

1. Days for shoot induction
2. No. of shoots per explant
3. Length of shoot (cm)
4. Length of longest shoot
5. No. of leaf per explant
6. Days to root induction
7. No. of roots per explant
8. Length of root (cm)
9. Length of the longest root (cm)
10. Percent of explants showing shoot induction
11. Percent of explants showing root induction.

3.13.1 Calculation of days to shoots and roots induction

Days to shoots and roots induction were calculated by counting the days from explants inoculation to the first induction of shoots/roots.

3.13.2 Calculation of percentage of shoot induction

Days to shoot and root induction were calculated by counting the days from explants inoculation to the first induction of shoot/root.

$$\text{Percentage of shoot induction} = \frac{\text{Number of explant induced shoots}}{\text{Number of explants inoculated}} \times 100$$

3.13.3 Days to shoot induction

Days to shoot induction were calculated by counting the days from explants inoculation to the first induction of shoots.

3.13.4 Number of shoot per explant

Number of shoot per explant was calculated by using the following formula,

$$\text{Number of shoot per explant} = \frac{\text{Number of shoots per explant}}{\text{Number of observation}} \times 100$$

3.13.5 Number of leaf

Numbers of leaves produced on the plantlet were counted and the mean was calculated.

3.13.6 Percent of explants showing root induction

The number of roots were produced per explant were recorded and the percentage of root regeneration was calculated as

$$\text{Percentage of root induction} = \frac{\text{Number of shoot induced root}}{\text{Number of shoot incubated}} \times 100$$

Number of days required for initiation of root from the day of inoculation was recorded.

3.13.7 Number of roots/explant

Average number of roots/plantlet was calculated as the following formula -

Where,

X = Mean no. of roots/plantlet

$\sum$ = Summation

x_i = No. of roots in i th observation

n = No. of observation

3.13.8 Length of roots (cm)

Root length was determined in centimeter (cm) from the base to tip of the roots. Average length of the root was calculated by the following formula-Where,

X = Mean no. of roots/plantlet

$\sum$ = Summation

x_i = length of roots in i th observation

n = No. of observation

3.13.9 Percentage of established plantlets

The percentages of established plantlets were calculated based on the number of plantlets placed in the plastic pots and the number of plants finally survived. The percentages of established plantlet were calculated by using the following formula:

$$\text{Percentage of established plantlets} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

$$\text{Percent (\%) of shoot induction} = \frac{\text{Number of explants induced shoot}}{\text{Number of explants incubated}} \times 100$$

The percentage of root induction was calculated as:

$$\text{Percent (\%) of root induction} = \frac{\text{Number of shoot induced root}}{\text{Number of shoot incubated}} \times 100$$

3.13.10 Calculation of number of shoot and root per explant

Number of shoot and root per explants was calculated by using the following formula

$$\text{Number of shoot per explant} = \frac{\text{Number of shoot / root per explant}}{\text{Number of observation}} \times 100$$

3.13.11 Calculation of shoot and root length (cm):

Shoot and root length were measured in centimeter (cm) from the base to the top of the explants by a measuring scale. Then the mean was calculated.

3.13.12 Calculation of survival rate of plantlets:

The survival rate of established plants was calculated based on the number of plantlets placed in the pot and the number of plants finally established or survived by the following equation-

$$\text{Number of established plantlet} = \frac{\text{Survival rate (\%) of plantlet}}{\text{Total number of plantlets}} \times 100$$

3.14 Statistical analysis

The experiment was one factorial set up in a completely randomized design (CRD) with four replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level using STATISTIX 10 program.

CHEPTER IV

RESULTS AND DISCUSSION

CHAPTER IV
RESULTS AND DISCUSSION

Different investigations were made on this experiment was conducted under laboratory condition to evaluate the effect of different plant growth regulators on multiple shoot and root induction in Turmeric. Manipulating the relative ratio of different growth regulators has been successfully used in the current investigation. BA and IBA alone were used for direct multiple shoot proliferation. The results obtained from the experiment are described and discussed here and the analyses of variance (ANOVA) are presented in Appendix II-XII. Presentation of results has been made in three phases.

4.1 Sub-experiment 1: Effect of BA on shoot induction potentiality in Turmeric

The result of the effect of different concentration of BA has been presented under following headings with Figure (1-5).

4.1.1 Days to shoot initiation

Significant variations were observed among different concentrations of BA on days to shoot induction. The maximum days (32.00 days) to shoot induction were recorded in control treatment followed by 1 mg/L of BA (22.00 days). On the other hand, minimum (14 days) was required in 2.0 mg/L BA followed by 3 mg/l of BA (14 days) and 4 mg/L of BA (15 days) (Figure 1)

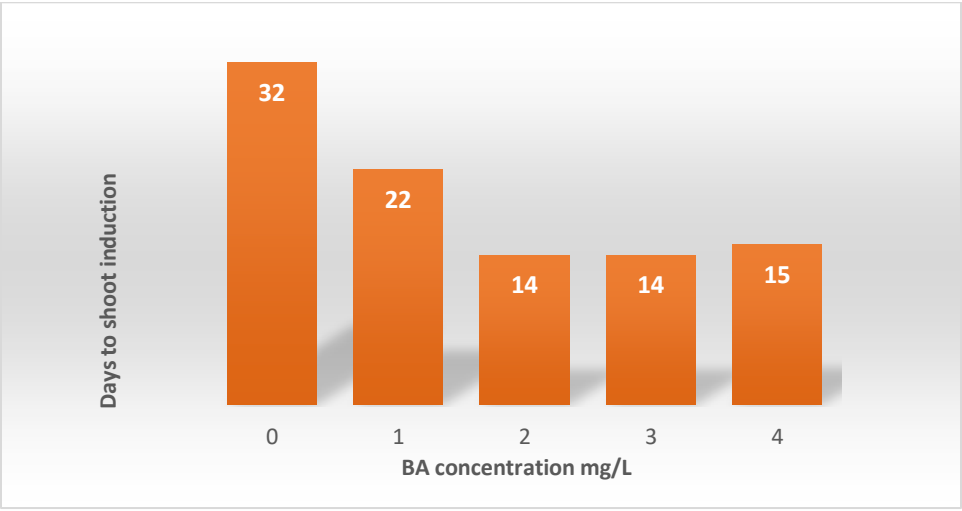


Figure 1 Effect of BA on days to shoot induction in Turmeric

4.1.2 Percentage of shoot initiation

There were significant variations on percent of explants showing shoot initiation at different concentrations of BA. Maximum percentage (81%) of shoot induction was observed in the treatment 2.00 mg/L BA and minimum percentage (31%) was induced in control treatment (Figure 2). Amin (2004) showed that the best result towards development of multiple shoot from the cultured explants of turmeric was obtained when MS medium was supplemented with 2.0 mg/L of BA.

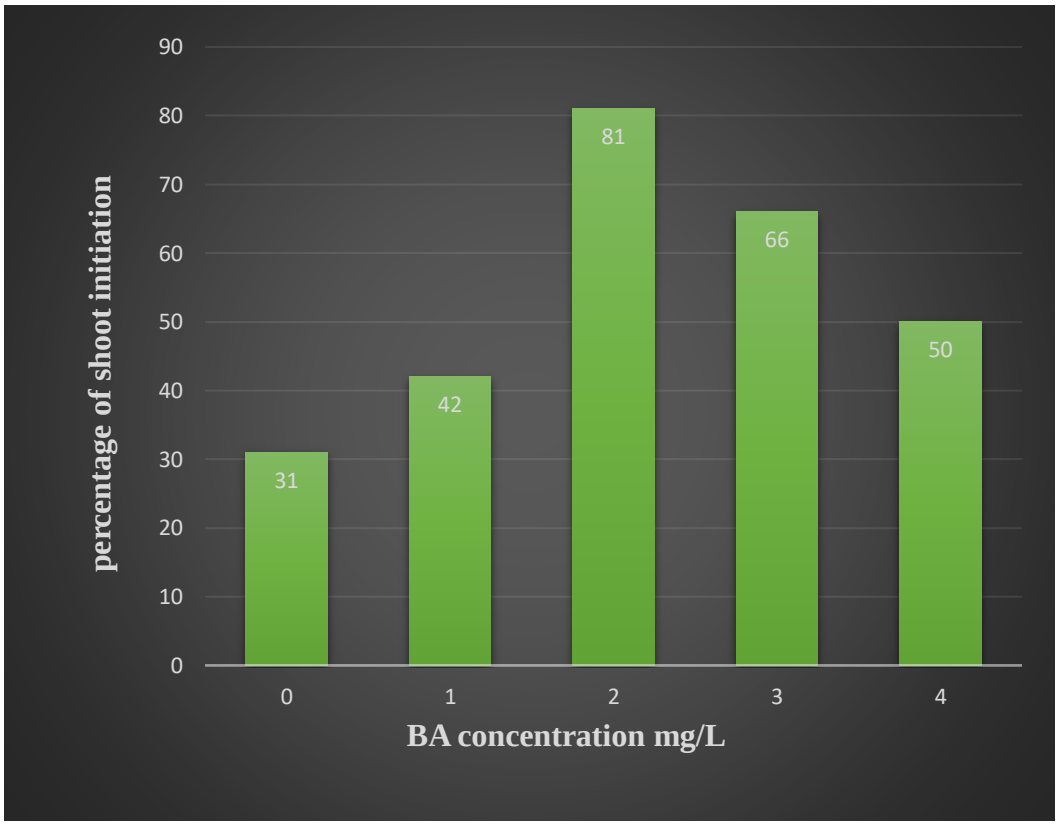


Figure 2: Effect of BA on percentage of shoot induction in Turmeric

4.1.3 Number of shoots per explant

The influence of different concentration of BA on the number of shoots per explant were recorded after 3 and 6 weeks of culture on MS media. The results have been presented in Figure 3 and Table 2. There was no significant variation at 3 weeks after inoculation (WAI) among different concentration of BA 2.0 mg/L BA gave the highest number of shoots (5.75) whereas the lowest number of shoots (1.2) was found at 6 WAI with hormone free media (Figure 3). Anchalee (2012) obtained that explants which were cultured on MS medium supplemented with 2.0 or 3.0 mg/L BA gave the highest average number of new shoots.

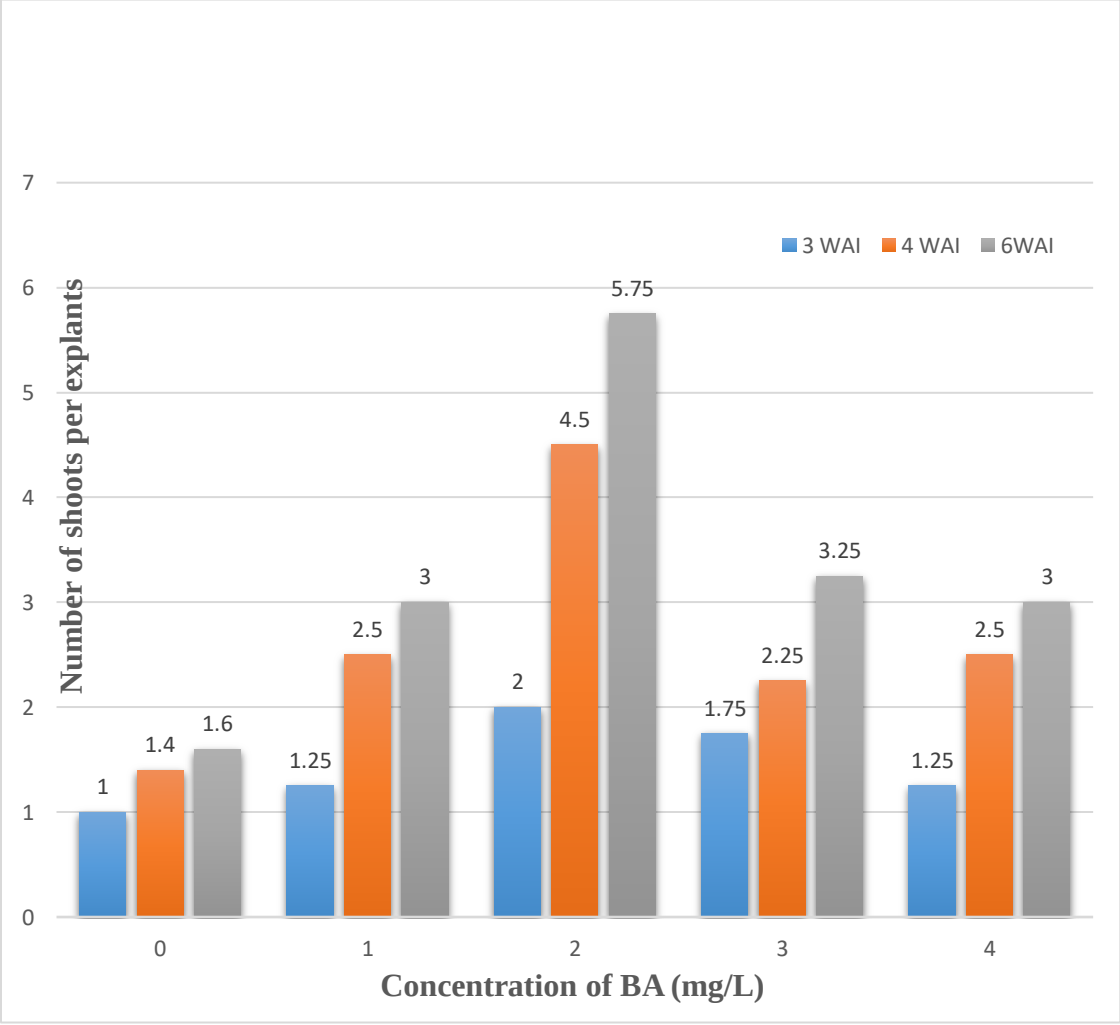


Figure 3: Effect of BA on the number of shoots per explant in Turmeric

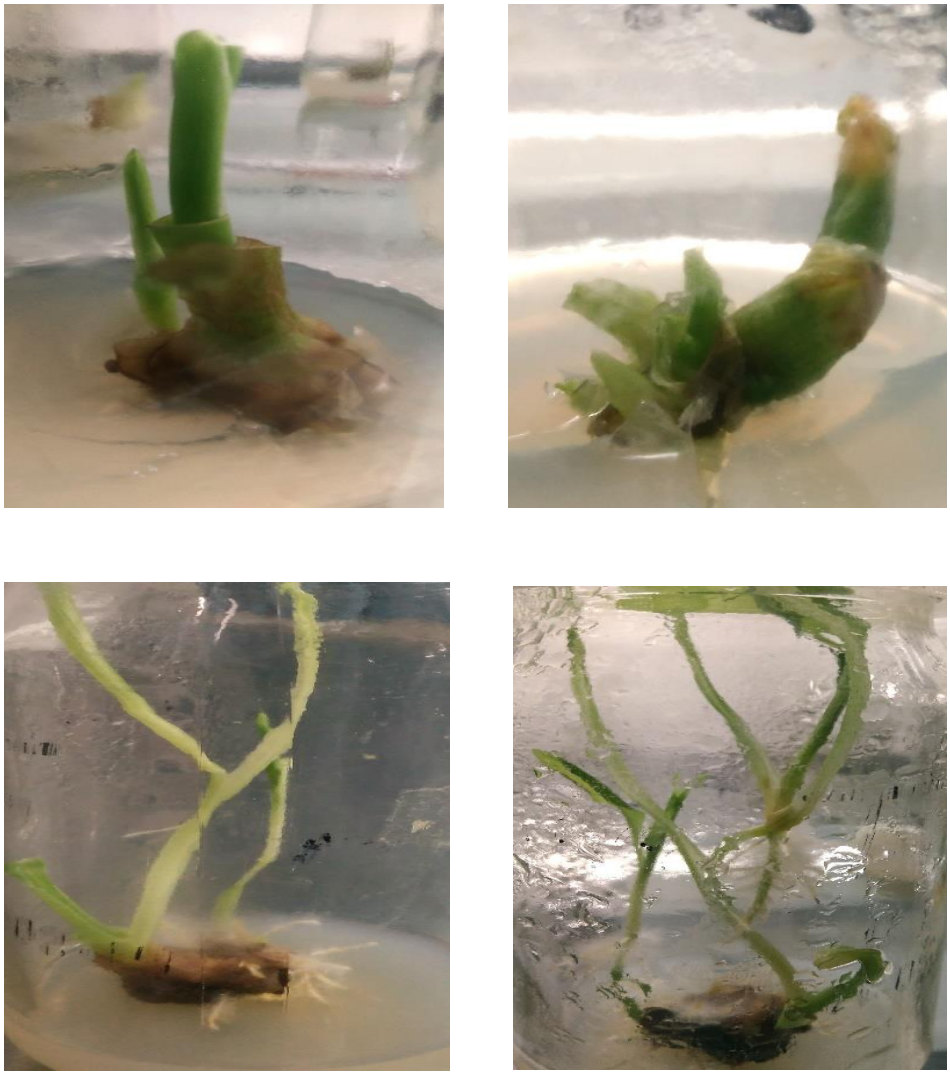


Plate 3: Multiple shoot initiation and development at 42 DAI in the treatment

Panda (2007) developed *in vitro* propagation protocol for *Curcuma longa* which gave 7.6 shoots by using medium with 3.0 mg/L BA. Rahman (2004) reported best shoot proliferation from rhizome bud explants in MS + 2.0 mg/L BA that 6.2 average lengths of shoots per culture. Mohammed and Quraishi, (1999) used shoot tips (3-5 mm) as explants where maximum number of shoots from single explants was 3.33 when medium contained 3.0 mg/L BAP. Maximum plant height during the same period was on medium containing 2.0 mg/L BA and shoot number was also relatively better.

4.1.4 Length of shoot (cm)

The results of length of longest shoots have been presented in Figure 4. The maximum length of longest shoot 5 cm was noticed from the 2.0 mg/L BA in 6 WAI whereas, the minimum length of shortest shoot was 2.2 cm in control treatment.

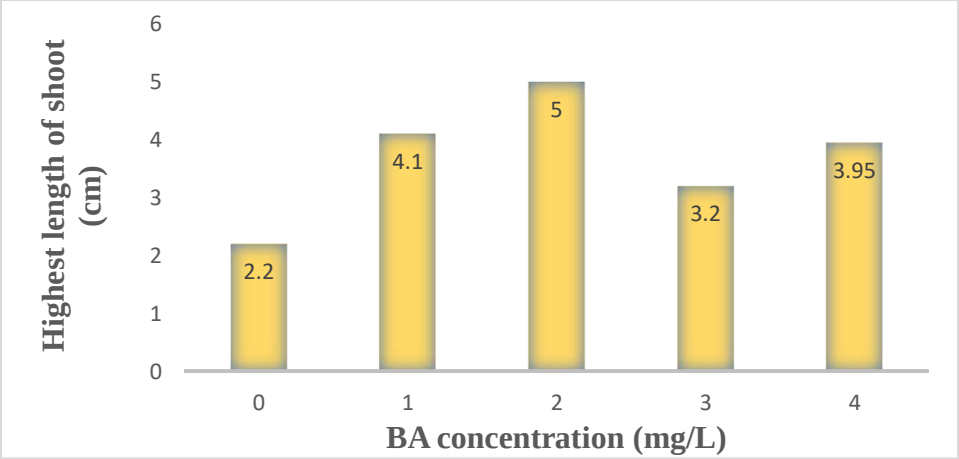


Figure 4: Effect of BA alone on length of shoot in Turmeric

4.1.5 Number of leaf per explants

There was a significant influence of different concentrations of BA on the number of leaves per shoot. BA 2.0 mg/L gave the maximum number of leaves (7.5) and the second highest leaves number (4.5) was found in BA 3.0 mg/L at 42 DAI whereas, the control treatment showed the lowest number of leaves (1.2) at 42 DAI (Figure 4). Anchalee (2012) obtained that explants which were cultured on MS medium supplemented with 2 or 3 mg/L BA gave the highest number of leaf per explant.

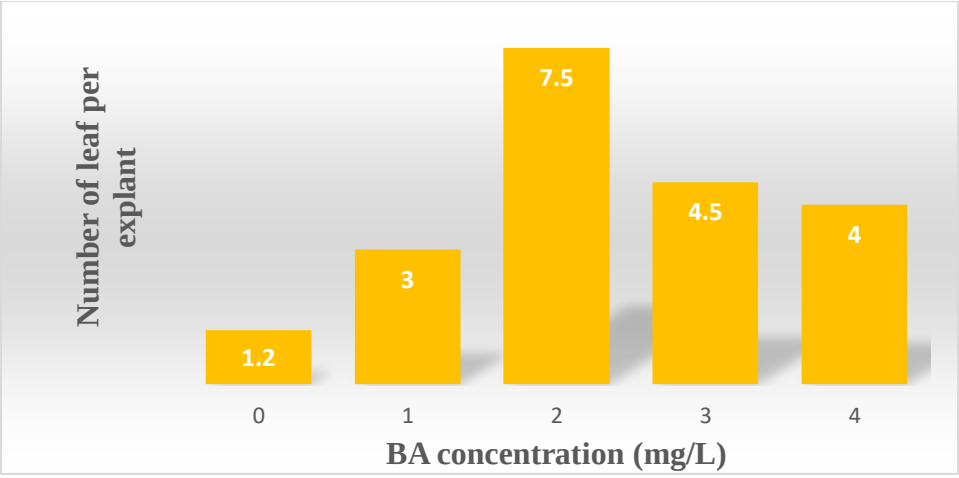


Figure 5: Effect of BA on number of leaf per explant in Turmeric

4.2 Sub-experiment 2: Effect of IBA on root induction potentiality in Turmeric

4.2.1 Days to root initiation

Significant variations were observed among different concentrations of IBA on days to root induction. The maximum days (28 days) to root induction were recorded in control followed by 2.0 mg/L (26.0 days) and 4.0 mg/L (23.75 days). On the other hand, minimum (20.25 days) was required in 3.0 mg/L IBA followed by 1 mg/L (22.5 days) (Figure 6). Pandya. (2014) described that *in vitro* regenerated shoots were shifted to MS medium augmented with 1.0 mg/l indole butaric acid (IBA) for rooting after 4 weeks of sub-culturing. This result may be varied due to the differences of genotype and culture environment (Bolouk *et al.*, 2013).

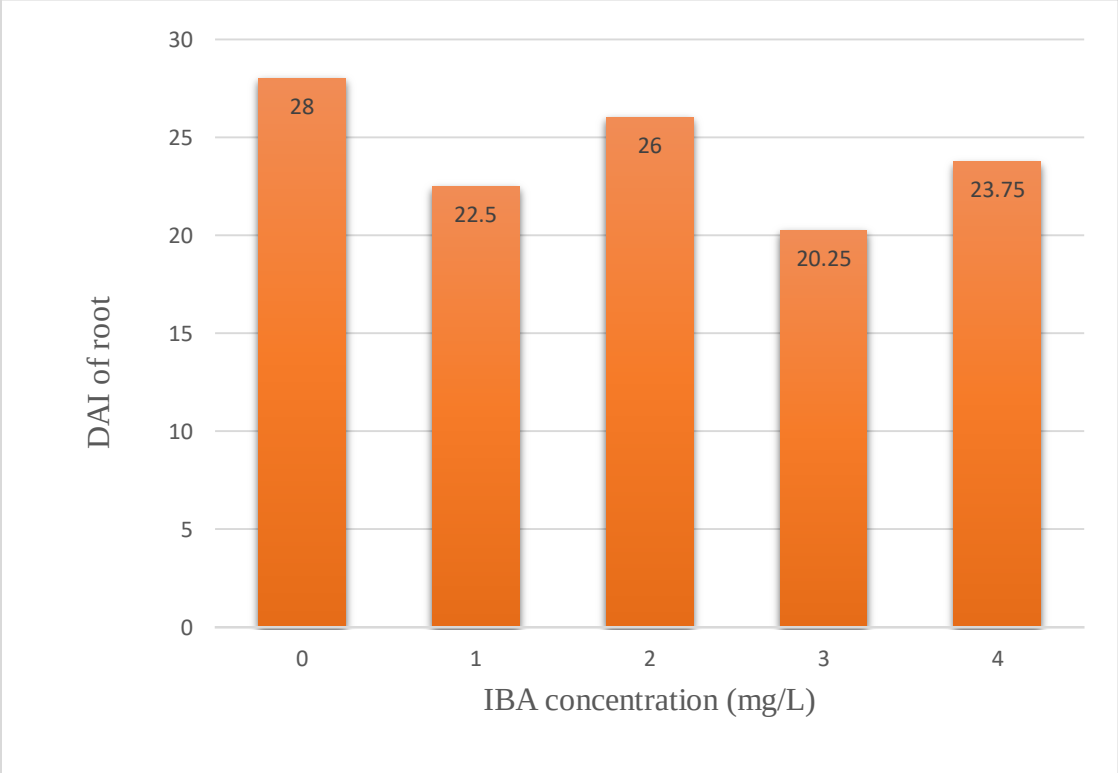


Figure 6: Effect of IBA on days to root initiation

4.2.2 Percentage of root initiation

IBA 3.0 mg/L had produced the highest percentage of root induction (83.00%), while the lowest percentage (35.00%) of root induction was produced in control treatment (Figure 7). Shahinozzaman (2013) developed *in vitro* propagation of *Curcuma caesia* where spontaneous rooting was observed after 4 weeks of cultivation with all treatments using half strength MS medium containing IBA at different concentrations, High frequency of rooting (89.76 %) was obtained in 3.0 mg/L IBA (Indole-3-butyric acid) containing medium.

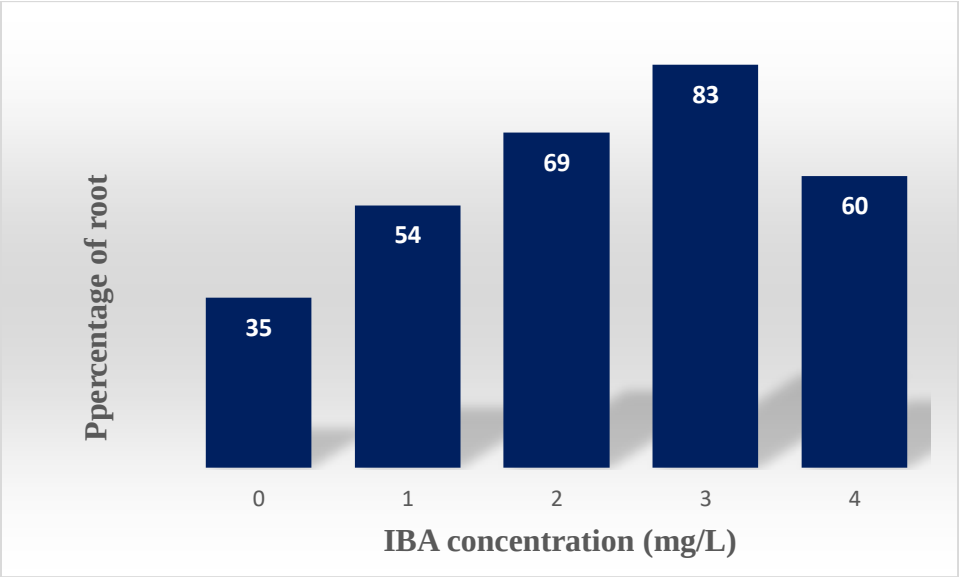


Figure 7: Effect of IBA on percentage of shoot induction in Turmeric

4.2.3 Number of root per plantlet

There was significant influence of different concentrations of IBA on the number of roots per shoot. IBA 3.0 mg/L gave the highest number of root (6.00, 7.50 and 11.5) at 21, 28 and 42 DAI (Plate 4) and the control treatment was found with the lowest number of root (1.0, 2.0, 3 and 4.0 mg/L) at 21, 28 and 42 DAI (Table 1).

Plate 4: Developed roots in the treatment of 3.00 mg/L IBA

Table 1: Effect of different concentration of IBA on number of root at different DAI

IBA (mg/L)	Number of root per plantlet		
	21 DAI	28 DAI	42 DAI
Control (0)	1.0c	2d	2.5e
1.0	1.25c	3c	3.75d
2.0	2.75b	5.5b	7b
3.0	6a	7.5a	11.5a
4.0	3.25b	5b	6c
CV (%)	10.37	7.78	5.9
LSD (0.05)	0.7590	0.9018	0.9204

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD(0.05)= Least significant difference.

4.2.4 Length of root

There was a significant influence of different concentrations of IBA on the length of root at 5% level of significance in laboratory condition. The highest length of root (5.175 cm) at 42 DAI (Plate 3) was noticed from the 3.0 mg/L IBA followed by 2.0 mg/L (4.325 cm) and 4.0 mg/L (3.375 cm). On the other hand, the lowest length of root (1.40 cm) at 42 DAI was noticed in control without hormone followed by 1.0 mg/L (3.2 cm) (Table 3).

Table 02: Effect of different concentrations of IBA on length of root at different days after induction

IBA (mg/L)	Length of root		
	21 DAI	28 DAI	42 DAI
0	1.0c	1.20d	1.40d
1.0	1.525c	2.35c	3.20c
2.0	2.30b	3.375b	4.325b
3.0	2.60a	4.25a	5.175a
4.0	1.65c	2.25c	3.375c
CV (%)	6.41	4.16	3.43
LSD (0.05)	0.2858	0.281	0.3042

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3 Sub-experiment 3: Combined effect of BA and IBA on shoot and root induction potentiality in Turmeric

The results of the combined effect of different concentrations of BA + IBA have been presented under following headings with Table (3-9) and Plate (5-9).

4.3.1 Days to shoot initiation

Significant variations were observed for the different concentrations of BA and IBA on days to shoot induction. The minimum duration (13 days) was obtained in BA 2.0 mg/L + IBA 3.0 mg/L than rest of the treatments. On the other hand, maximum days (32 days) to shoot induction was recorded in control (Table 4).

4.3.2 Percentage of shoot initiation

There was a significant influence of different concentrations of BA and IBA on the percentage of shoot induction per explant. The optimum percentage (80.66%) of shoot induction was noticed in treatment BA 2.0 mg/L + IBA 3.0 mg/L which was best than others and minimum percentage (31.00%) was noticed in hormone free media (Table 3).

Table 03: Combined effect of different concentration of BA and IAA on days to shoot initiation and percent of shoot initiation

Treatment	Days to shoot initiation	Percent of shoot initiation (%)
Control	32.00	31.00
BA 1.0 mg/L + IBA 1.0 mg/L	20.00	49.66
BA 1.0 mg/L + IBA 2.0 mg/L	17.00	56.66
BA 1.0 mg/L + IBA 3.0 mg/L	14.33	52.66
BA 1.0 mg/L + IBA 4.0 mg/L	19.66	46.00
BA 2.0 mg/L + IBA 1.0 mg/L	15.00	52.00
BA 2.0 mg/L + IBA 2.0 mg/L	13.33	69.00
BA 2.0 mg/L + IBA 3.0 mg/L	13.00	80.66
BA 2.0 mg/L + IBA 4.0 mg/L	17.66	61.00
BA 3.0 mg/L + IBA 1.0 mg/L	19.33	41.66
BA 3.0 mg/L + IBA 2.0 mg/L	16.00	55.33
BA 3.0 mg/L + IBA 3.0 mg/L	14.66	70.33
BA 3.0 mg/L + IBA 4.0 mg/L	17.33	50.33
BA 4.0 mg/L + IBA 1.0 mg/L	21.33	43.66
BA 4.0 mg/L + IBA 2.0 mg/L	17.33	49.33
BA 4.0 mg/L + IBA 3.0 mg/L	15.00	64.33
BA 4.0 mg/L + IBA 4.0 mg/L	21.00	43.00
CV (%)	12.97	6.68
LSD(0.05)	6.63	11.22

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.3 Number of shoot per explant

Different concentrations of BA and IBA showed significant variations on the number of shoot. The highest number of shoot (3.0, 6.0 and 7.66) was noticed from the BA 2.0 mg/L + IBA 3.0 mg/L (Plate 5) and second highest number (2.0, 3.66 and 5.66) at 21 ,28 and 42 DAI respectively, were observed in 2.0 mg/L BA + 4.0 mg/L IBA. Whereas the lowest average number of shoot (1.00, 1.5 and 1.8) at 21, 28 and 42 DAI respectively were noticed in control treatment (Table 4).

Plate 5: Multiple shoots in the treatment of 2.00 mg/L BA + 3.00 mg/L IBA after 42 DAI

Table 4: Combined effect of different concentration BA and IBA on number of shoot

Treatments	Number of shoot (cm)		
	21DAI	28DAI	42DAI
Control	1.0c	1.5c	1.8e
BA 1.0 mg/L + IBA 1.0 mg/L	1.0c	2.0d	3.33d
BA 1.0 mg/L + IBA 2.0 mg/L	1.33bc	2.33cd	3.66cd
BA 1.0 mg/L + IBA 3.0 mg/L	2.0b	3.33cd	4.66bcd
BA 1.0 mg/L + IBA 4.0 mg/L	1.0c	2.0d	4.0bcd
BA 2.0 mg/L + IBA 1.0 mg/L	3.0a	5.66ab	6.333ab
BA 2.0 mg/L + IBA 2.0 mg/L	2.0b	4.0bc	6.0abc
BA 2.0 mg/L + IBA 3.0 mg/L	3.0a	6.0a	7.66a
BA 2.0 mg/L + IBA 4.0 mg/L	2.0b	3.33cd	5.66abcd
BA 3.0 mg/L + IBA 1.0 mg/L	1.33bc	2.66cd	4.33bcd
BA 3.0 mg/L + IBA 2.0 mg/L	1.33bc	3.0cd	4.66bcd
BA 3.0 mg/L + IBA 3.0 mg/L	2.0b	3.66cd	5.0bcd
BA 3.0 mg/L + IBA 4.0 mg/L	1.0c	2.33cd	4.33bcd
BA 4.0 mg/L + IBA 1.0 mg/L	1.0c	2.0d	4.0bcd
BA 4.0 mg/L + IBA 2.0 mg/L	1.0c	2.0d	3.66cd
BA 4.0 mg/L + IBA 3.0 mg/L	1.33bc	3.0cd	4.66bcd
BA 4.0 mg/L + IBA 4.0 mg/L	1.0c	2.33cd	3.66cd
CV (%)	17.77	17.93	16.97
LSD(0.05)	0.855	1.692	2.441

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.4 Length of shoot (cm)

There was a significant influence of different concentrations of BA with IBA on the length of shoot at 5% level of significance in laboratory condition. The highest length of shoot (3.4 cm, 5.03 cm and 5.93 cm) at 21 28 and 42 DAI, respectively was noticed from the BA 2.0 mg/L + IBA 3.0 mg/L (Plate 6) followed by BA 2.0 mg/L + IBA 1.0 mg/L (2.86 cm, 4.66 cm and 8.66 cm) at 21, 28 and 42 DAI. On the minimum length of leaves (1.4 cm, 1.8 cm and 2.2 cm) at 21, 28 and 42 DAI, respectively was noticed in control treatment (Table 5).

Plate 6: Length of shoot in the treatment of 2.0 mg/L BA + 3.00 mg/L IBA

Table 5: Combined effect of different concentration of BA and IBA on length of shoot (cm)

Treatments	Length of shoot (cm)		
	21DAI	28DAI	42DAI
Control	1.4h	1.8c	2.2d
BA 1.0 mg/L + IBA 1.0 mg/L	1.43h	2.76c	3.36d
BA 1.0 mg/L + IBA 2.0 mg/L	1.5gh	2.56c	3.53d
BA 1.0 mg/L + IBA 3.0 mg/L	1.6fgh	2.6c	3.53d
BA 1.0 mg/L + IBA 4.0 mg/L	1.66fgh	2.76c	3.73d
BA 2.0 mg/L + IBA 1.0 mg/L	2.86ab	4.6ab	5.6ab
BA 2.0 mg/L + IBA 2.0 mg/L	2.73b	4.3ab	5.23bc
BA 2.0 mg/L + IBA 3.0 mg/L	3.4a	5.03a	5.93a
BA 2.0 mg/L + IBA 4.0 mg/L	2.63bc	4.26b	5.23bc
BA 3.0 mg/L + IBA 1.0 mg/L	2.43bcde	4.23b	5.13bc
BA 3.0 mg/L + IBA 2.0 mg/L	2.4bcde	4.00b	4.96c
BA 3.0 mg/L + IBA 3.0 mg/L	2.6bcd	3.93b	4.83c
BA 3.0 mg/L + IBA 4.0 mg/L	2.06cdefg	3.9b	4.76c
BA 4.0 mg/L + IBA 1.0 mg/L	1.86efgh	2.93c	3.76d
BA 4.0 mg/L + IBA 2.0 mg/L	2.03defg	2.9c	3.76d
BA 4.0 mg/L + IBA 3.0 mg/L	2.1cdef	2.86c	3.83d
BA 4.0 mg/L + IBA 4.0 mg/L	2.0efgh	2.7c	3.63d
CV (%)	17.77	17.93	16.97
LSD(0.05)	0.59	0.76	0.61

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.5 Number of leaves

The number of leaves per shoot showed significant difference with combined concentrations of BA and IBA. The treatment BA 2.0 mg/L+ IBA 3.0 mg/L gave the highest number of leaves (4.0 , 6.0 and 8.0) at 21, 28 and 42 DAI, respectively (Plate 7) whereas the lowest number of leaves (1.0, 2.0 and 3.0) at 21, 28 and 42 DAI respectively was found with hormone free media (Table 6).

Plate 7: Number of leaf in the treatment of 2.0 mg/L BA+ 3.0 mg/L IBA

Table 6: Combined effect of different concentration BA and IBA on number of leaves

Treatments	Leaf number		
	21DAI	28DAI	42DAI
Control	1.0f	2.0e	3.0f
BA 1.0 mg/L + IBA 1.0 mg/L	1.0f	2.33de	4.33de
BA 1.0 mg/L + IBA 2.0 mg/L	1.66def	3.33bcde	5.66abcde
BA 1.0 mg/L + IBA 3.0 mg/L	2.66bcd	4.33abcd	7.0abc
BA 1.0 mg/L + IBA 4.0 mg/L	1.0f	2.0e	4.0e
BA 2.0 mg/L + IBA 1.0 mg/L	2.0cdef	3.33bcde	5.33bcde
BA 2.0 mg/L + IBA 2.0 mg/L	3.33ab	5.33ab	7.66ab
BA 2.0 mg/L + IBA 3.0 mg/L	4.0a	6.0a	8.0a
BA 2.0 mg/L + IBA 4.0 mg/L	2.33bcde	2.66cde	4.66cde
BA 3.0 mg/L + IBA 1.0 mg/L	1.33ef	2.66cde	4.33de
BA 3.0 mg/L + IBA 2.0 mg/L	2.66bcd	3.66bcde	5.33bcde
BA 3.0 mg/L + IBA 3.0 mg/L	3.0abc	4.66abc	6.33abcde
BA 3.0 mg/L + IBA 4.0 mg/L	2.0cdef	3.33bcde	5.66abcde
BA 4.0 mg/L + IBA 1.0 mg/L	1.0f	2.0e	4.0e
BA 4.0 mg/L + IBA 2.0 mg/L	1.33ef	2.66cde	4.66cde
BA 4.0 mg/L + IBA 3.0 mg/L	3.0abc	5.0ab	6.66abcd
BA 4.0 mg/L + IBA 4.0 mg/L	1.0f	2.0e	4.0e
CV (%)	18.33	20.81	15.15
LSD(0.05)	1.66	2.18	2.52

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.6 Days to root induction

Significant variation was observed among different concentrations of BA and IBA on days to root induction. The maximum (28.00 days) to root induction was recorded in control treatment and minimum (17.66days) was required in BA 2.0 mg/L + IBA 1.0 mg/L concentration (Table 7).

4.3.7 Percentage of root induction

Different concentrations of BA and IBA showed the significant variations on percent of explants showing root induction. The highest percentage (82.667%) of root induction was recorded with BA 2.0 mg/L + IBA 1.0 mg/L, whereas the lowest percentage (40.00%) of root induction was recorded in control condition (Table 7). Rahman (2004) showed that rooting experiments with half strength of MS medium and various concentrations of BA and IBA for root forming performance of BA and IBA combination was proved to be the best for percentage of root induction.

Table 7: Combined effect of different concentration BA and IBA on Days to root induction and Percentage of root initiation

Treatment	Days to root induction	Percent of root initiation (%)
Control	28.00	40.00
BA 1.0 mg/L + IBA 1.0 mg/L	25.66	53.33
BA 1.0 mg/L + IBA 2.0 mg/L	24.33	59.66
BA 1.0 mg/L + IBA 3.0 mg/L	23.33	57.66
BA 1.0 mg/L + IBA 4.0 mg/L	24.66	51.66
BA 2.0 mg/L + IBA 1.0 mg/L	17.66	82.66
BA 2.0 mg/L + IBA 2.0 mg/L	18.66	74.33
BA 2.0 mg/L + IBA 3.0 mg/L	19.66	70.33
BA 2.0 mg/L + IBA 4.0 mg/L	20.66	67.33
BA 3.0 mg/L + IBA 1.0 mg/L	21.33	44.00
BA 3.0 mg/L + IBA 2.0 mg/L	22.00	59.00
BA 3.0 mg/L + IBA 3.0 mg/L	23.00	75.00
BA 3.0 mg/L + IBA 4.0 mg/L	20.66	54.66
BA 4.0 mg/L + IBA 1.0 mg/L	22.00	47.00
BA 4.0 mg/L + IBA 2.0 mg/L	24.33	55.00
BA 4.0 mg/L + IBA 3.0 mg/L	24.33	70.33
BA 4.0 mg/L + IBA 4.0 mg/L	25.00	51.66
CV (%)	4.21	6.00
LSD(0.05)	2.85	2.99

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.8 Number of root per plant

There was a significant influence of different concentrations of BA and IBA on the number of root per shoot. The treatment BA 2.0 mg/L+ IBA 1.0 mg/L gave the highest number of root (6.00, 8.66 and 12.66) (Plate 8) and second best result (5.667, 7.66 and 10.33) was noticed from BA 3.0 mg/L + IBA 3.0 mg/L at 21, 28 and 42 DAI whereas the lowest number of root (2.20, 3.50 and 4.5) at 21, 28 and 42 DAI was found with hormone free media (Table 8).

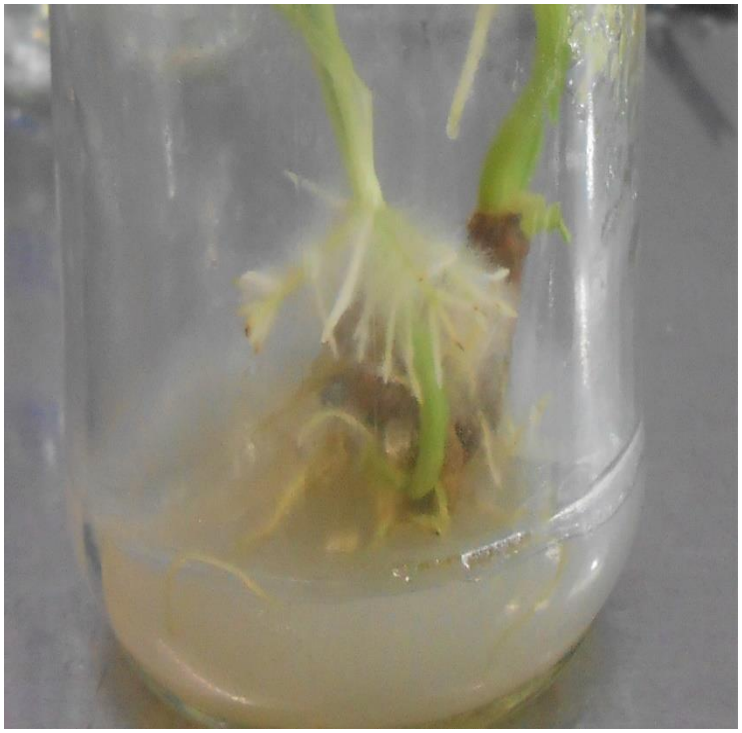


Plate 8: Number of root in the treatment of 2.00 mg/L BA+ 1.00 mg/L IBA

Table 8: Combined effect of different concentration BA and IBA on number of roots at different DAI

Treatments	Number of root (cm)		
	21DAI	28DAI	42DAI
Control	2.2d	3.5e	4.5e
BA 1.0 mg/L + IBA 1.0 mg/L	2.33d	3.66e	4.66e
BA 1.0 mg/L + IBA 2.0 mg/L	2.33d	5.0cde	6.0de
BA 1.0 mg/L + IBA 3.0 mg/L	4.0abcd	6.66abcd	9.33bc
BA 1.0 mg/L + IBA 4.0 mg/L	3.0cd	5.33bcde	6.66cde
BA 2.0 mg/L + IBA 1.0 mg/L	6.0a	8.66a	12.66a
BA 2.0 mg/L + IBA 2.0 mg/L	3.66abcd	5.66bcde	8.33bcd
BA 2.0 mg/L + IBA 3.0 mg/L	4.0abcd	6.66abcd	9.33bc
BA 2.0 mg/L + IBA 4.0 mg/L	3.33abcd	5.0cde	6.66cde
BA 3.0 mg/L + IBA 1.0 mg/L	3.0cd	5.0cde	7.66bcd
BA 3.0 mg/L + IBA 2.0 mg/L	2.33d	5.33bcde	7.33cde
BA 3.0 mg/L + IBA 3.0 mg/L	5.66ab	7.66ab	10.33ab
BA 3.0 mg/L + IBA 4.0 mg/L	2.66cd	5.33bcde	7.33cde
BA 4.0 mg/L + IBA 1.0 mg/L	2.33d	4.33de	5.66de
BA 4.0 mg/L + IBA 2.0 mg/L	2.0d	4.66cde	6.66cde
BA 4.0 mg/L + IBA 3.0 mg/L	5.0abc	7.0abc	10.33ab
BA 4.0 mg/L + IBA 4.0 mg/L	2.0d	5.0cde	6.33de
CV (%)	23.12	13.51	12.49
LSD(0.05)	2.35	2.33	2.79

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05)= Least significant difference

4.3.9 Length of root (cm)

There was significant influence of different combined concentrations of BA and IBA on the length of root. There was a significant variation at 21, 28 and 42 DAI among different concentration of BA and IBA. The highest length of root (2.70, 4.83 and 5.466) at 21, 28 and 42 DAI (plate-9), respectively was found in BA 2.0 mg/L + IBA 1.0 mg/L. The control treatment was found with the lowest number of root (1.2 cm, 2.1 cm and 2.5 cm) at 21, 28 and 42 DAI (Table 9).

Plate 9: Figure showing in the treatment of 2.00 mg/L BA+ 1.00 mg/L IBA

Table 9: Combined effect of BA and IBA on length of root at different DAI

Treatments	Length of root (cm)		
	21DAI	28DAI	42DAI
Control	1.2f	2.1g	2.5g
BA 1.0 mg/L + IBA 1.0 mg/L	1.43f	2.26g	2.7g
BA 1.0 mg/L + IBA 2.0 mg/L	2.0cde	2.83cdef	3.2fg
BA 1.0 mg/L + IBA 3.0 mg/L	2.23bc	3.43bcdef	4.03def
BA 1.0 mg/L + IBA 4.0 mg/L	1.56f	2.33g	3.5efg
BA 2.0 mg/L + IBA 1.0 mg/L	2.7a	4.83a	5.46a
BA 2.0 mg/L + IBA 2.0 mg/L	2.3bc	3.53bcde	4.53bcd
BA 2.0 mg/L + IBA 3.0 mg/L	2.4ab	4.2ab	5.03abc
BA 2.0 mg/L + IBA 4.0 mg/L	1.8def	2.66defg	3.96def
BA 3.0 mg/L + IBA 1.0 mg/L	1.73ef	2.36g	3.46efg
BA 3.0 mg/L + IBA 2.0 mg/L	2.3abc	3.56bcd	4.5bcd
BA 3.0 mg/L + IBA 3.0 mg/L	2.53ab	4.26ab	5.26ab
BA 3.0 mg/L + IBA 4.0 mg/L	1.6f	2.53fg	3.5efg
BA 4.0 mg/L + IBA 1.0 mg/L	1.53f	2.6efg	3.23fg
BA 4.0 mg/L + IBA 2.0 mg/L	2.16bcd	3.06cdefg	4.3cde
BA 4.0 mg/L + IBA 3.0 mg/L	2.36abc	3.76bc	5.0abc
BA 4.0 mg/L + IBA 4.0 mg/L	1.53f	2.63defg	3.36fg
CV (%)	6.27	9.69	7.44
LSD(0.05)	0.38	0.93	0.92

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.4 Experiment 3. Acclimatization of plantlets

The results of acclimatization or hardening have been presented in Table 10 and Plate 10. After 42 days of culture on rooting media, the plantlets were taken for acclimatization.

Table 10. Survival rate of *in vitro* regenerated plantlets of Turmeric

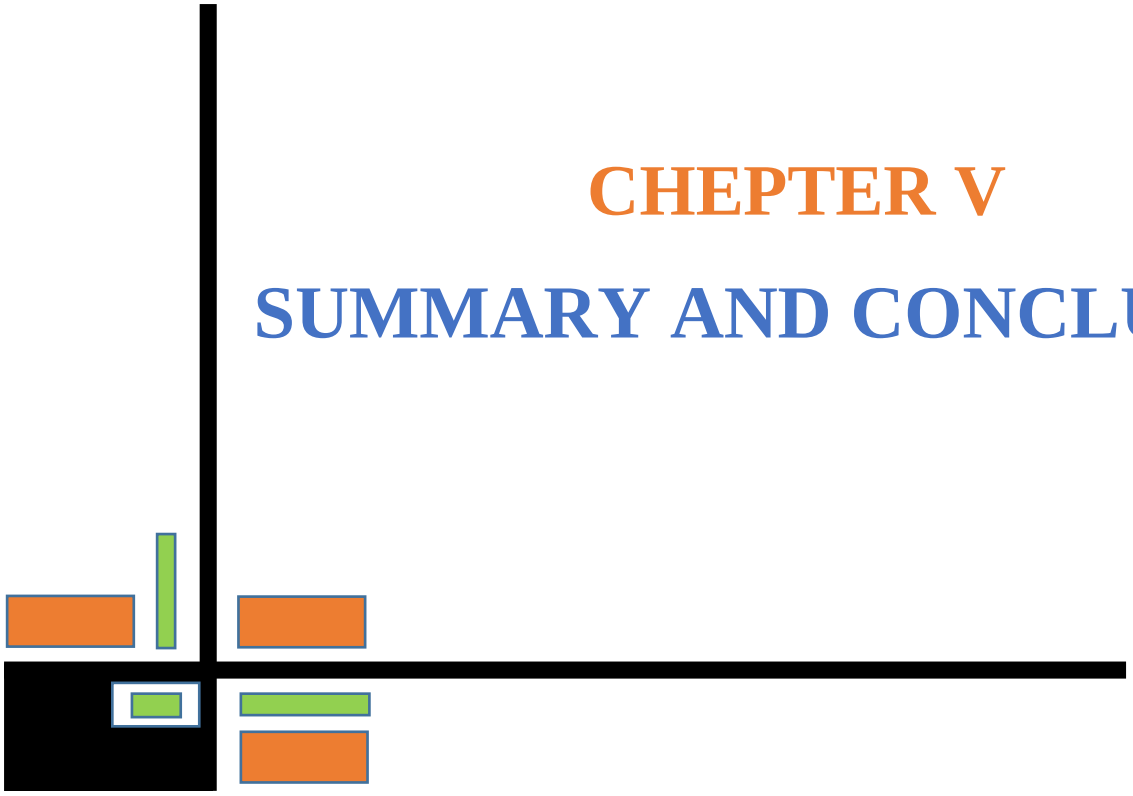
Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate (%)
In shade area with controlled atmosphere	15	13	86.66
In natural condition	15	12	80

The results of acclimatization showed that the 100% of plantlets survived to culture room (Table 10). Then the plantlets were shifted to shade house with less humidity (70% RH) and indirect sunlight (Plate 10). In the shade house, the top of the pots were covered with transparent plastic sheet and grew at room temperature for 14 days with periodic irrigation (2 days interval). In these conditions, 86.66% of the plantlets showed survival (Table 10). After 3 weeks, the plantlets were transferred to the soil following depoting and poting into different pots of bigger size. The plants were watered periodically and upper layer of the soil mulched occasionally whenever necessary. In open atmosphere, survival rate was 80% (Table 10). It was also revealed that regenerated plants were morphologically similar to the mother plant. Dwivedi (2014) found that the plantlets were regenerated in green house, *in-vitro* produced plants were successfully established in soil, with almost 90% survival.

Plate 11: Acclimatization and establishment of turmeric plantlets on soil

CHEPTER V

SUMMARY AND CONCLUSION



CHAPTER V
SUMMARY

The present research was carried out in Biotechnology Lab. of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka -1207 from the period of February 2018 to June 2018 to evaluate the effect of different plant growth regulator on multi shoot proliferation and root formation along with acclimatization for *in vivo* survival.

The healthy, disease free shoot tips of 1-2 cm length were used as explants for the study for *in vitro* regeneration. The major findings have been presented below.

Maximum shoot induction (81%) was found at 2.0 mg/L BA and minimum (31%) in control treatment. Root induction was maximum (83%) at 3.0 mg/L IBA.

Significant variation was observed among different concentration of BA on days to shoot induction. The maximum days to shoot induction were recorded in control (32 days). With different concentration of BA, significant influence was found on the number of leaf. The maximum 8 leaves were recorded with BA 2.0 mg/L+ IBA 3.0 mg/L.

Significant variation was observed in combination of two hormones on days to shoot induction. The maximum days to shoot induction were recorded in control (32.0 days) and BA 2.0 mg/L+ IBA 3.0 mg/L required minimum 13.0 days. The highest number of shoot (7.66) were recorded in BA 2.0 mg/L+ IBA 3.0 mg/L and the lowest number of shoot (1.0 and 1.8 at 3WAI and 6WAI) respectively was found with hormone free media.

The number of leaf per explants was significantly different according to the various concentrations of BA + IBA supplemented. The maximum number of leaf per explants (8.0) was noticed from BA 2.0 mg/L+ IBA 3.0 mg/L and the lowest number of leaf (1.0 and 3.0 at 3WAI and 6WAI) respectively was found with hormone free media.

The maximum length of shoot (5.933 cm) was noticed from the BA 2.0 mg/L + IBA 3.0 mg/L whereas, the minimum 1.4 cm in control treatment.

The maximum 28.0 days to root induction was required in media devoid of growth regulator. At least 17.66 days was required in case of BA 2.0 mg/L + IBA 1.0 mg/L. The highest number of roots (12.66) per explants was recorded in BA 2.0 mg/L+ IBA 1.0 mg/L at 6 WAI.

Regenerated plantlets showed 100% survival during in culture room conditions and 86.66% in shade house stage of hardening and 80% in open atmosphere.

CONCLUSION

Following conclusions can be made from the present study:

i. A regeneration protocol has been developed in Turmeric.
ii. Overall moderate higher dose (BA 2.0 mg/L + IBA 3.0 mg/L) showed better response *in vitro* regeneration in Turmeric.
iii. Combined effect of BA and IBA doses seems to be better than individual effect of BA and IBA for shoot formation and root formation respectively in Turmeric.

CHAPTER VI
RECOMMENDATIONS

Following recommendations could be addressed based on the present experiment:

a) Different combinations of BAP, IAA, NAA and kinetin need to be verified for regeneration of Turmeric.
b) Rather than sprouts, meristem as an explants and callus culture could be practiced.
c) To uncover the influence of genotype if any, research should be carried on with different types of genotype of Turmeric.
d) Precise and detailed investigation on influence of other factors such as different elicitors, antioxidants on *in vitro* regeneration should be considered.

CHEPTER VII

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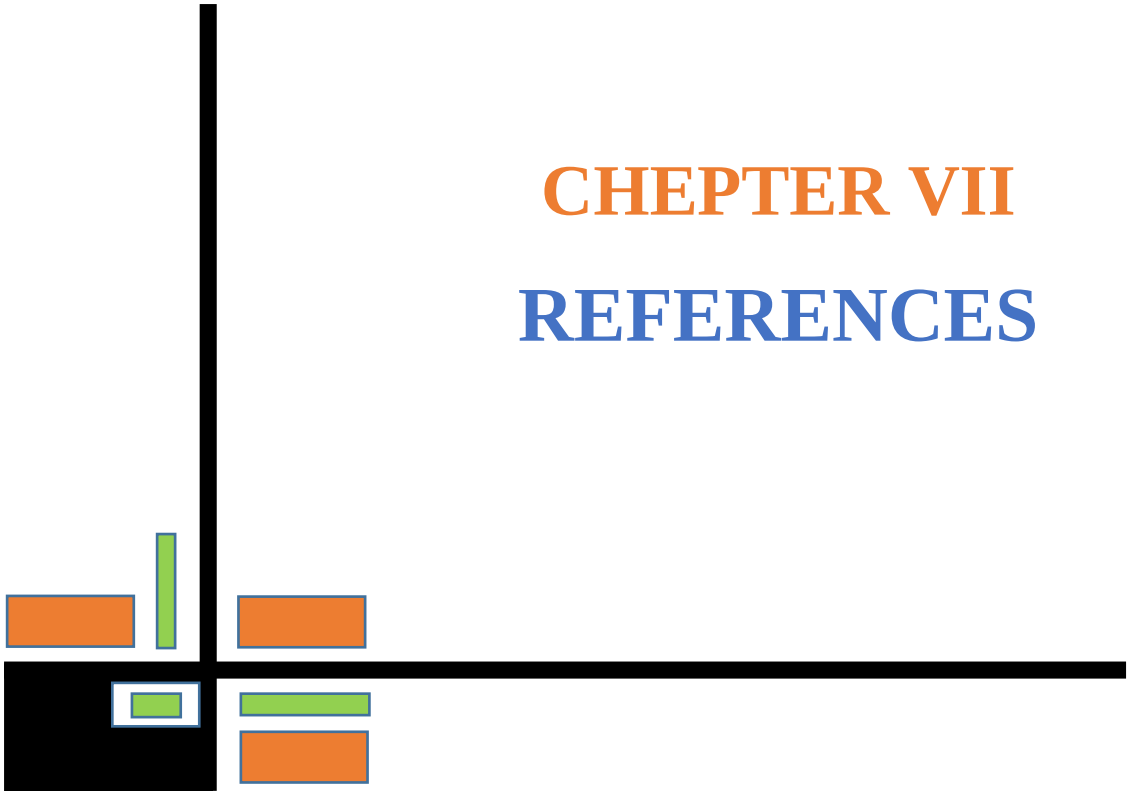

CHAPTER VII

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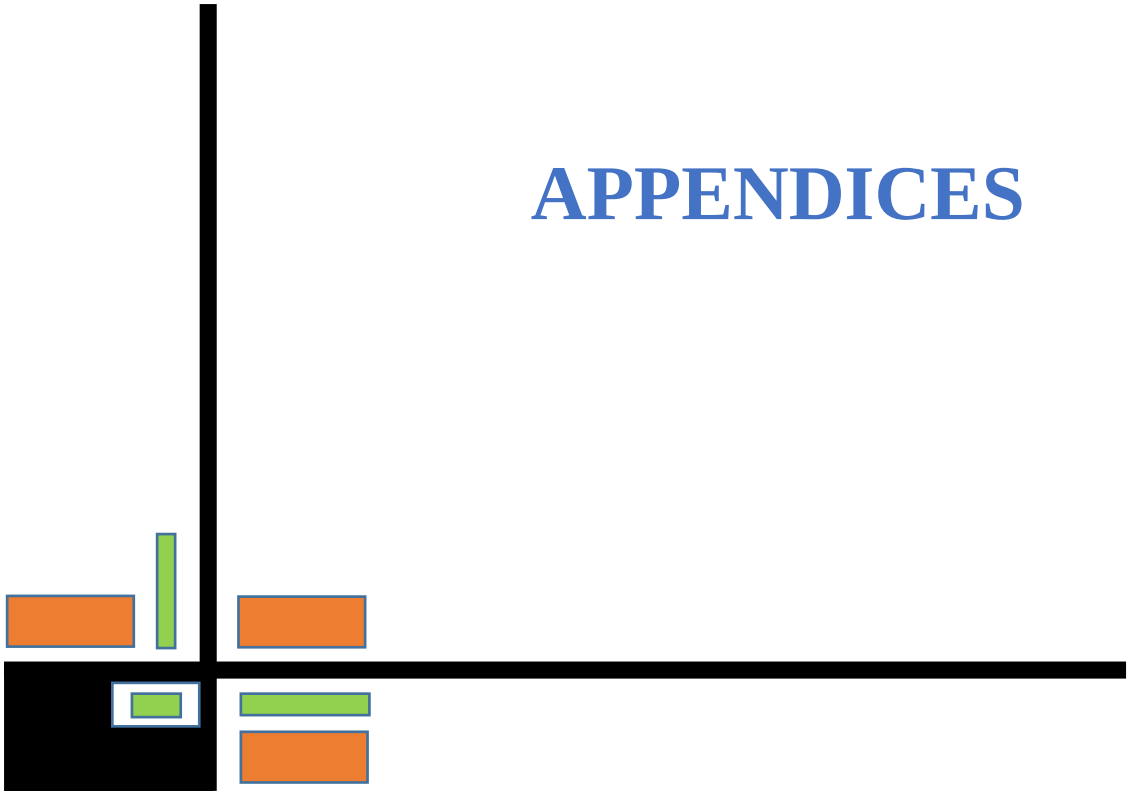
APPENDICES



APPENDICES

Appendix I. Composition of MS (Murashige and Skoog, 1962) medium including vitamins

Components	**Concentrations (mg/L)**	**Concentrations**
Micro Elements	**mg/L**	**µM**
$CoCl_2.6H_2O$	0.025	0.11
$CuSO_4.5H_2O$	0.025	0.10
Fe Na EDTA	36.70	100.00
H_3BO_3	6.20	100.27
KI	0.83	5.00
$MnSO_4.H_2O$	16.90	100.00
$Na_2MoO_4.2H_2O$	0.25	1.03
$ZnSO_4.7H_2O$	8.60	29.91
Macro Elements	**mg/L**	**mM**
$CaCl_2$	332.02	2.99
KH_2PO_4	170.00	1.25
KNO_3	1900.00	18.79
$MgSO_4$	180.54	1.50
NH_4NO_3	1650.00	20.61
Vitamins	**mg/l**	**µM**
Glycine	2.00	26.64
Myo-Inositol	100.00	554.94
Nicotinic acid	0.50	4.06
Pyridoxine HCl	0.50	2.43
Thiamine HCl	0.10	0.30

Total concentration of Micro and Macro elements including vitamins: 4405.19 mg/L

Appendix II. Analysis of variance (ANOVA) of effect of different concentration of BA on days to shoot initiation, percent of shoot and number of leaves at different DAI

Source of variance	d.f	Days to shoot initiation	Percent (%) Shoot Initiation	Number of leaves		
				21 DAI	28 DAI	42 DAI
Treatment	3	74.56	1195.83	3.833**	4.1667**	15.0**
Error	9	1.28	11.11	0.444	0.222	0.5
Total	15					

**=Significant at 1% level of Probability

Appendix III. Analysis of variance (ANOVA) of effect of different concentration of BA on number of shoot at different DAI

Source of variance	d.f	Number of shoot		
		21 DAI	28 DAI	42 DAI
Treatment	3	0.562**	4.395**	6.729**
Error	9	0.229	0.229	0.34
Total	15			

**=Significant at 1% level of Probability

Appendix IV. Analysis of variance (ANOVA) of effect of different concentration of BA on length of shoot at different DAI

Source of variance	d.f	length of shoot		
		21 DAI	28 DAI	42 DAI
Treatment	3	1.154**	29.307**	24.974**
Error	9	0.036	.231	0.263
Total	15			

**=Significant at 1% level of Probability

Appendix V. Analysis of variance (ANOVA) of effect of different concentration of IBA on days to root initiation, percent of root and number of roots at different DAI

Source of variance	d.f	Days to root initiation	Percent (%) root Initiation	Number of root		
				21 DAI	28 DAI	42 DAI
Treatment	3	15.416**	622.563**	15.729**	13.667**	42.3958**
Error	9	0.5883	5.896	0.118	0.166	0.173
Total	15					

**=Significant at 1% level of Probability

Appendix VI. Analysis of variance (ANOVA) of effect of different concentration of IBA on length of root at different DAI

Source of variance	d.f	length of root		
		21 DAI	28 DAI	42 DAI
Treatment	3	1.062**	3.567**	3.353**
Error	9	0.016	0.016	0.018
Total	15			

**=Significant at 1% level of Probability

Appendix VII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to shoot initiation, percent of shoot initiation and number of shoot at different DAI

Source of variance	d.f	Days to shoot initiation	Percent (%) Shoot Initiation	Number of Shoot		
				21 DAI	28 DAI	42 DAI
Treatment	9	2.657**	87. 009**	0. 094**	0.446**	0.444**
Error	30	4.8875	13.622	0. 079	0.309	0.573
Total	47					

**=Significant at 1% level of Probability

Appendix VIII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on number of leaves at different DAI

Source of variance	d.f	Number of leaves		
		21 DAI	28 DAI	42 DAI
Treatment	9	0.24**	0.834**	1.335**
ErrorI	30	0.145	0.518	0.688
Total	47			

**=Significant at 1% level of Probability

Appendix IX. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on length of shoot at different DAI

Source of variance	d.f	Length of shoot		
		21 DAI	28 DAI	42 DAI
Treatment	9	0. 0247**	0.15**	0.468**
Error	30	0.049	0.57	0.718
Total	47			

**=Significant at 1% level of Probability

Appendix X. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to root initiation, percent of root and number of roots at different DAI

Source of variance	d.f	Days to shoot initiation	Percent (%) root Initiation	Number of root		
				21 DAI	28 DAI	42 DAI
Treatment	9	2.657**	94. 04**	0.206**	0.213**	0.324**
Error	30	4.887	12.65	0.234	0.198	0.217
Total	47					

**=Significant at 1% level of Probability

Appendix XI. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on length of root at different DAI

Source of variance	d.f	length of root		
		21 DAI	28 DAI	42 DAI
Treatment	9	0.012**	0.007**	0. 03**
Error	30	0.007	0.003	0.013
Total	47			

**=Significant at 1% level of Probability

Appendix XII. Analysis of variance (ANOVA) of effect of BA and IBA on Days to root and number of shoot at different DAI

Source of variance	d.f	Days to rhoot initiation	Percent (%) Shoot Initiation	Number of Shoot		
				21 DAI	28 DAI	42 DAI
Treatment	9	0. 094**	87. 009**	0.094**	0.446**	0.444**
Error	30	0.333	13.662	0.079	0.309	0.573
Total	47					