IN VITRO REGENERATION OF DRAGON FRUIT (*Hylocereus undatus*)

NARAYAN CHAKRABORTY

DEPARTMENT OF BIOTECHNOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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BY

NARAYAN CHAKRABORTY

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Approved by:

Fahima Khatun
Assistant Professor
Department of Biotechnology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

Dr. Md. Ekramul Hoque
Professor
Department of Biotechnology
Sher-e-Bangla Agricultural University
Dhaka-1207
Co-supervisor

Dr. Mohammad Nazrul Islam
Chairman
Examination Committee
Department of Biotechnology
Sher-e-Bangla Agricultural University
Dhaka-1207

DEPARTMENT OF BIOTECHNOLOGY
Sher-e-Bangla Agricultural University
Sher-e -Bangla Nagar, Dhaka-1207

CERTIFICATE

*This is to certify that the thesis entitled, "**IN VITRO REGENERATION OF DRAGON FRUIT (Hylocereus undaus)**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN BIOTECHNOLOGY**, embodies the results of a piece of bona fide research work carried out by **NARAYAN CHAKRABORTY,** Registration No. **18-09218** under my supervision and guidance. No part of this 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.

Dated:

Dhaka, Bangladesh

Fahima Khatun
Assistant Professor
Department of Biotechnology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

DEDICATED TO

MY

BELOVED PARENTS

ABBREVIATIONS AND ACRONYMS

Acronym		Full form
Agril.	:	Agriculture
Biol.	:	Biological
Biot.	:	Biotechnology
cm	:	Centimeter
CRD	:	Completely Randomized Design
Conc.	:	Concentration
DAI	:	Days After Inoculation
DMRT	:	Duncan's Multiple Range Test
WAI	:	Week After Inoculation
et al.	:	And others
i.e.	:	id test (That is)
FAO	:	Food and Agricultural Organization
g/L	:	Gram per litre
BAP	:	6- Benzyl Amino Purine
BA	:	Benzyladenine
KIN	:	Kinetin
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
NAA	:	a- Napthalene acetic acid
2, 4-D	:	2,4- Dichlorophenoxy acetic acid
Int.	:	International
J.	:	Journal
Mol.	:	Molecular
mg/L	:	Milligram per litre
µM	:	Micromole
MS	:	Murashige and Skoog
PGRs	:	Plant Growth Regulators
Res.	:	Research
Sci.	:	Science
TDZ	:	Thidiazuron
CV	:	Co-efficient of Variation
^{o}C	:	Degree Celsius
etc.	:	Etcetera
w/v	:	Weight by volume

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IN VITRO REGENERATION OF DRAGON FRUIT (*Hylocereus undatus*)

ABSTRACT

The present experiment was carried out in the Laboratory of Biotechnology, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of February, 2020 to December, 2020 to study the effect of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), NAA (0.25, 0.50, 0.75 and 1.00 mg/l) individually or in combination of both for *in vitro* regeneration of Dragon fruit. The shoot tips of Dragon plants were used as explants which were sterilized with 70% ethanol and 0.1% $HgCl_2$. The highest percentage of shoot induction (76.00%) was observed in MS medium with BA 2.0 mg/l in minimum 12.2 days. The maximum number of shoot (3.20) and the highest shoot length (4.02 cm) were obtained at 42 days with the treatment BA 2.0 mg/l and BA 2.50 mg/l respectively. The treatment NAA 0.50 mg/l produced the highest percentage of root (68.00%) and minimum 12.40 days required to produce root with the treatment NAA 0.25 mg/l. The maximum number of root (5.40) and the highest length of root (5.38 cm) at 42 days were recorded with the treatment NAA 0.50 mg/l and NAA 0.25 mg/l respectively where the control treatment gave the lowest result. In combined effect, BA 2.0 mg/l+ NAA 0.50 mg/l showed the highest percentage of shoot (86.67%) and root (93.33%) in 11.67 days and 12.67 days respectively. The maximum number of shoot (3.67) and the highest shoot length (4.36 cm) were obtained at 42 days in BA 2.0 mg/L+ NAA 0.50 mg/l. The maximum number of root (5.67) and the highest length of root (6.27 cm) at 42 days were recorded with BA 2.0 mg/l+ NAA 0.50 mg/l where the treatment BA 1.0 mg/l + NAA 0.25 mg/l showed the lowest result. Finally, the survival rate of *in vitro* regenerated plantlets was recorded 84% in natural condition. Overall, feasible protocol has been developed for *in vitro* rapid regeneration of Dragon fruit having potential application in large scale multiplication for commercial purpose.

CHAPTER-I

INTRODUCTION

CHAPTER I

INTRODUCTION

Dragon fruit (*Hylocereus undatus*) is one of the tropical fruits belonging to the family *Cactaceae*. It is also known as pitaya or pitahaya or strawberry pear or night blooming cereus. The origin of *Hylocereus* sp. is the tropical and sub-tropical forest regions of Mexico and Central and South America (Mizrahi *et al.,* 1997). The genus *Hylocereus* contains 16 species (Innes and Glass, 1992) and it can be categorized into three different species based on skin and pulp color, i.e., *Hylocereus undatus* (red skin, white pulp), *Hylocereus polyrhizus* (red skin and red pulp), *Hylocereus costaricensis* (red skin and red pulp) and (yellow peel and white pulp) (Nerd *et al.,* 2002). Dragon fruit is now widely cultivated as fruit crops in Southeast Asian countries (Haber *et al.,* 1983; Mizrahi *et al.,* 1997). Among the main countries cultivating dragon fruit are Vietnam, Colombia, Mexico, Costa Rica, and Nicaragua (Vinas *et al.,* 2012). Now a days it is also a popular fruit in Bangladesh.

It is an edible vine cactus species which has received worldwide recognition as an ornamental plant as well as fruit crop. It is perennial and fast growing climber with triangular or rarely four or five ridged stems. The stem is fleshy, vine like with many branched segments. Each segment has three wavy wings and 1-3 spines or sometimes spineless. These spines form aerial roots which adhere, help to climb and keep the plant erect. Its fruit is beautiful with bright red skin studded with green scales and white or red flesh with numerous tiny black seeds. The flower is so beautiful that its nick name is “Novel woman” or “Queen of the night”. *Hylocereus* sp. has fleshy stems and may grow from the ground or climb onto trees using aerial roots.

It is well known for its rich nutrient contents specially vitamin C, phosphorus, calcium as well as its antioxidant characteristics (Morton, 1987). The fresh fruit contains 82.5-83.0% moisture, 0.16-0.23% protein, 0.21-0.61% fat and 0.7-0.9% fiber. Hundred gram fresh fruit pulp contains 6.3-8.8 mg calcium, 30.2-36.1 mg phosphorous, 0.5-0.61 mg iron and 8-9 mg vitamin C (Taiwan Food

Industry Development and Research Authorities, 2005). Dragon fruit contains high amount of vitamin C and water-soluble fiber (Ruzainah *et al.,* 2009).

This cacti species shows high potential as an ornamental and fruit crop and its demand is high in national and international markets. They have the potential to be a profitable new crop for farmers. The market is already established in Asian countries with a much larger demand for it. Plants of the *Hylocereus* begin to produce significant yield two to three years after planting and reach full production after five years (Jacobs, 1999). Therefore, it becomes an important commodity among the local communities and even fetches higher price than 'The King of Fruits'-Durian (Gunasena *et al.,* 2006). The Dragon fruit growing industry has expanded greatly in the last five years.

In Bangladesh, dragon fruits were introduced by some private entrepreneurs from different countries for growing as ornamental plants. Some of the elite farmers in different places of Bangladesh like Ashulia, Dhaka and Halda Valley Tea State, Fatikchari, Chittagong already started its commercial cultivation. Research on it has already been started at Bangladesh Agricultural Research Institute (BARI), Gazipur & the Germplasm Center, Bangladesh Agricultural University (BAU), Mymensingh. Already mass media like different TV channels and newspapers have drawn public attention of Bangladesh regarding the cultivation of this nutritious fruit. However, published information on this crop in Bangladesh is very scanty. Hence, research on this crop should be emphasized. Thus, in near future, it could find its way to international market if the growing areas could be extended for production of quality produces. So, large amount of plant materials are needed for area extension.

Dragon fruit can be propagated by seeds or stem cuttings. Seeds are not true to type and it has low seed viability. But cutting is the most common method because it gives true to type plant and fruiting stage is reached more rapidly than seed propagation (Gunasena *et al.,* 2006). Dragon fruit is propagated by stem cuttings which may be planted directly in the field or in pots (Zee *et al.,*

2004). In many plant species, vegetative propagation is normally practiced using stem cuttings (Abdullah *et al.,* 2005; Henrique *et al.,* 2006). However, these conventional methods of propagation are not efficient for producing a large number of plants within short period. Plant tissue culture became a tool to propagate desire elite planting materials for commercial production. High number of aseptic plantlets could be obtained within short period using plant tissue culture protocols. *In vitro* regeneration is considered as reliable and rapid technique for propagating the plantlets (Thomas and Philip, 2005; Thomas and Jacob, 2004). Instead of conventional propagation, plant tissue culture is an alternative propagation method which plays a very essential role in large scale plantlet production program. *In vitro* culture techniques become vital tools in recent years for rapid mass multiplication and germplasm conservation of rare, endangered, and threatened medicinal plants (Anis and Faisal, 2005; Uppendra *et al.,* 2005). Plant tissue culture also is being increasingly applied for clonal propagation of selected tree species to supplement conventional methods which have their limitations, especially when a large number of genetically uniform propagules are required (Mascarenhas *et al.,* 1981; Mascarenhas and Muralidharan, 1989). *In vitro* regeneration can increase the availability of disease-free plants and reduces culture space requirement thus lowers the cost of production. The development of efficient tissue culture protocol is necessary not only for rapid mass propagation but also for conservation of genetic resources and genetic improvement (Tao *et al.,* 2002). Advanced propagation method through tissue culture can facilitate efficient clonal propagation. The success of *in vitro* regeneration is influenced by genotype, explant types and the composition of culture medium, especially combinations and concentrations of plant growth regulators (PGRs). Use of plant growth regulators (PGRs) in cactus tissue culture is of fundamental importance. Suitable initial explant and correct plant growth regulators are significant features for better shoot formation and callus production in order to develop protocol for indirect organogenesis or somatic embryogenesis for a large scale production of healthy planting materials. There were few reports which

evaluated regeneration capability of explants derived from *in vitro* shoot explants of *Hylocereus undatus* (Chen *et al.,* 2006).

Tissue culture technique has been used in the multiplication of "good clones" of agricultural crops and medicinal plants. The use of *in vitro* culture techniques can ensure speedy multiplication of valuable varieties (Sunandakumari *et al.,* 2004) and the possibility to obtain biological material free from pathogens in large-scale (Kane, 2014). A balance between auxin and cytokinin determines the *in vitro* regeneration of plants grown in artificial medium. Generally, cytokinin helps in shoot proliferation and auxin helps in callus formation and rooting of proliferated shoots. The presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to obtain adventitious shoot formation (Caboni and Tonelli, 2009). However, the requirement of cytokinin and auxin depends on the plant species, genotype, explant types and culture conditions. Requirement of growth regulators depends on type of explants and its physiological condition also. The best media with optimum growth regulator, growth condition and suitable explants are needed to be standardized for large scale production of plants.

Therefore, this study was conducted to examine the effect of different levels of plant growth regulators on *in vitro* regeneration of dragon fruit. Therefore, attempt was made to develop protocols for large-scale *in vitro* propagation of locally available dragon fruit. Considering the above facts, the present investigation has been undertaken to find out the performance of different hormones with the optimum concentration of BA and NAA for *in vitro* plantlets regeneration of dragon fruit. The experiment was conducted to fulfill the following objectives.

Objectives:

i. Assessment of hormonal effect on *in vitro* response of Dragon fruit.

ii. Identification of the best hormonal concentration for *in vitro* regeneration of Dragon fruit.

iii. Establishment of *in vitro* regeneration protocol of Dragon fruit.

iv. To study the rapid regeneration of Dragon fruit within a short period of time.

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The present investigation involved *in vitro* micropropagation of *Hylocereus undatus.* Plant regeneration from culture media of shoot tip explants followed by genetic stability of plantlets seems to be meager. However, information available in these aspects have been reviewed and presented in these sections:

2.1 Description of Dragon Fruit

Dragon fruit (*Hylocereus undatus*) is a perennial climbing cactus with green stems and it grows in tropical and subtropical countries. They are epiphytic or hemi-epiphytic in nature (Barbeau, 1990). The fleshy succulent stems are three sided (occasionally four or five) and lobed along the ridges, which have small swellings equipped with short spines. They are long day plants and flowers only bloom at night. The attractive, large white flower opens rapidly at 7 pm and completely opens by 10 pm. They gained recognition as an ornamental plant and are also well known for their edible fruits (Le Bellec *et al.*, 2006). Most of the varieties of dragon fruits from Asia are predominantly *Hylocereus undatus* that are self-compatible and self-incompatible, while some of these are autogamous and will set fruits without the involvement of a pollen vector. Fruit pulp is an edible part of dragon fruit. It is generally used in fruit salad and is a natural source of antioxidants. The flesh of dragon fruit is mildly sweet, low in calories and contain high amount of vitamin C and antioxidant. It has gained more attention due to its health benefits including prevention of memory losses, prevention of cancer, control of blood glucose level in diabetic patients, prevention of oxidation, aiding in healing of wounds etc. In addition, it has the ability to promote the growth of probiotics in the intestinal tract (Zainoldin and Baba, 2009).

2.2 Propagation of Dragon Fruit

Dragon fruit plants can be propagated through *in vitro* tissue culture, seed propagation, vegetative propagation and grafting. Despite acceptable seed

germination efficiencies of between 71 and 83 % for *Hylocereus undatus* (Elobeidy, 2006), such proliferation is not commercially feasible, because seed-derived plants have a long juvenile phase, delaying fruit production for several years (Le Bellec *et al.,* 2006). Currently, the conventional methods such as seed propagation and vegetative means through stem cuttings and grafting could not meet the current market demand (Gunasena *et al.,* 2006). *In vitro* culture technique is an alternative way of rapid multiplication of disease-free improved planting materials and also it is a faster and efficient process compared to other conventional plant propagation methods.

2.3 *In Vitro* Regeneration

Micropropagation is the process of vegetative growth and multiplication from plant tissues or seeds. It is carried out in aseptic and favourable conditions on growth media, using various plant tissue culture techniques (Zhou and Wu, 2006). Tissue culture is based on concept of totipotency; the ability of plant cells and tissues to develop into a whole new plant (Fowler *et al.,* 1993). It is widely used and carries immense potential in a broad spectrum of plant biotechnology and plant genetics applications. *In vitro* propagation is a promising method of rapidly producing numerous, uniform plants that are free of microbial contamination. The development of an in vitro protocol is advantageous not only for maintainable utilization of a species, but also for germplasm conservation and genetic improvement (Chen *et al.,* 2006). Micropropagation of the cacti facilitates for the production of large numbers of plants from a relatively small stock (Drew and Azimi, 2002). In conventional cultivation, many plants do not germinate, flower and produce seed under certain climatic conditions or have long periods of growth and multiplication. Micropropagation ensures a good regular supply of medicinal plants, using minimum space and time (Prakash and Van Staden, 2007). The advantages of *in vitro* micro propagation are higher rate of multiplication, environment can be controlled or altered to meet specific needs of the plant, plant available all year round (independent of regional or seasonal variation), identification and

production of clones with desired characteristics, production of secondary metabolites, new and improved genetically engineered plant can be produced, conservation of threatened plant species and preservation of genetic material by cryopreservation.

In addition, micropropagation of medicinal plants is a mean of producing disease free superior quality planting material. It is a viable alternative for species where elites have been identified based on their potential for yielding higher amount of active principles; which are difficult to regenerate by conventional methods, and, where conventional methods are inadequate to meet the demand of quality planting material (Chen *et al*., 2006).

2.3.1 Shoot culture

Shoot culture refers to the *in vitro* propagation by repeated enhanced formation of axillary shoots from shoot tips or lateral buds cultured on medium supplemented with growth regulators, usually a cytokinin (George and Debergh, 2008). The term shoot culture is now preferred for cultures started from explants bearing an intact shoot meristem in shoot multiplication by the repeated formation of axillary branches. In this technique, newly formed shoots or shoot bases serve as explants for repeated proliferation; several shoots (or shoot clumps) are finally rooted to form plantlets which can be grown *in vivo*. It is the most widely used method of micropropagation (Gahan and George, 2008). The most efficient procedure, propagation from axillary shoots, has proved to be a reliable method for the micropropagation of a large number of species (Kurtz, l99l and Mauseth, 1977) reported that cytokinin is generally considered to be essential for the development of cactus axillary shoots. The axillary shoots produced are either subdivided into shoot tips and nodal segments that will serve as secondary explants for further proliferation or are treated as microcutting or rooting. Compared to other micropropagation methods, shoot cultures can provide reliable rates and consistency of multiplication following culture stabilization, less susceptible to genetic

variation and may provide for clonal propagation of *Periclinal chimeras* (Michael, 201l).

2.3.2 Subculturing

The rate of *in vitro* shoot multiplication depends greatly on subculturing of proliferating shoot cultures. Apostolo (2005) reported that in prolonged cultures, the concentrations of nutrients in the medium is gradually exhausted and at the same time, the relative humidity of culture vessel decreases leading to drying of developed shoots, as the high humidity in culture vessel helps to promote the rapid shoot growth. Since, it is desired to establish cultures that could be continuously multiplied in order to produce regular output of desired number of shoots for field transfer, a regular subculturing has been recommended in order to maintain rejuvenility of tissues during the period of culture (Debnat, 2004).

2.3.3 *In vitro* rooting

The success of a micropropagation protocol depends strongly on the rooting efficiency of regenerated shoots and their subsequent acclimatization to the field condition (Caboni *et al.,* l997). Some micropropagated shoots easily form root *in vitro.* This can be an advantage or disadvantage. *In vitro* roots are not always functional and they can easily damage during planting out. These roots usually die and new roots have to be formed once the plant is established. In such case, allowing the plants to root outside the glasshouse would be better. However, some plants may need roots to survive the transplantation, although their roots are not fully functional. They usually recover faster than plants that still have to form roots (Sanette, 2003). Auxins played major role in rooting process and their efficiency depends on several factors such as the affinity for auxin receptor protein involved in rooting, the concentration of free auxin that reaches target competent cells, the amount of endogenous auxin and the metabolic stability (De Klerk *et al.,* 1999). Different types of auxin such as Indole 3-butytic acid (IBA), Indole 3-acetic acid (IAA) and Naphthalene acetic

acid (NAA) were added to MS medium to promote adventitious root formation. Dahanayake and Ranawake (2011) observed that root induction of proliferated shoots was obtained on MS medium supplemented with 0.05 µM NAA regardless of the shoot induction medium in dragon fruit crop. Brasil (2005) discussed that reduction of BAP levels during the shoot proliferation phase might have been the trigger for rooting, as observed in other studies. Vinas (2012) reported that spontaneous rooting is especially important to accelerate acclimatization thereby avoiding an extended *in vitro* rooting phase.

2.4 Factors Affecting *In Vitro* Multiplication of Dragon Fruit

2.4.1 Explant materials

Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile condition. Using the appropriate growing conditions with the addition of suitable plant growth regulators (PGRs) for each explant type, plant can be induced to rapidly produce new shoots and new roots (Chen *et al.,* 2006). Selection of explant material is a crucial aspect of micropropagation that requires three important considerations such as genetic and epigenetic characteristics of the source plant, pathogen control and physiological conditions of the plant prior to explant excision in order to optimize its ability to establish in a culture (Hartmann *et al.,* 1990). Explants can influence growth and morphogenetic potential of culture. Mathews (1987) reported that the efficient micropropagation depends not only on the selection of the most suitable explant, but also on the suitable combination of growth regulators, and/or the best nutritional composition of the medium. Choice of suitable explant is important when propagation is to be based on direct or indirect shoot initiation. Although in some species, explants from many organs are capable of producing adventitious shoots, it is usually found that those excised from different organs or from different tissues within an organ, varied strongly in morphogenetic capacity (Gahan and George, 2008).

2.4.2 Plant growth regulators (PGRs)

Hormones also known as plant growth regulators are chemicals used to alter the growth of a plant or plant parts. Growth regulators play a key role for developing a specific mode of growth in the cultured cells or tissues, which may be due to accumulation of specific biochemical contents in them. In tissue culture, they are important media components in determining the development and developmental pathway of the plant cells. They are used in different proportions to break dormancy and enhance shoot formation since it is well demonstrated that the apical dormancy is under control of these growth regulators (Madhulatha *et al.,* 2004). In tissue culture, five major classes of growth regulators are important: auxins, cyokinins, gibberellins, abscisic acid and ethylene (Razdan *et al.,* 2003, Gaba *et al.,* 2005). Among these classes, auxins and cytokinins are the most important for regulating growth and morphogenesis in plant tissue and organ cultures. The cytokinins and auxins are of importance in *in vitro* culture as the later are concerned with root formation, the former is mainly required in the media for shoot formation and growth of buds (North *et al.,* 2012). These growth regulators are required in combination in the media as it is always the manipulation and variation of auxins and cytokinins levels that can successfully change the growth behavior of plant cultures (Dixon and Gonzales, 1994). N-6 benzyl aminopurine (BAP) with the formula $C_{12}H_{11}N_5$ for cytokinins and 1-naphthaleneacetic acid (NAA) with molecular formula $C_{12}H_{10}O_2$ for auxins are the most favored and common hormones employed in various experiments for tissue culture and micropropagation (George *et al.,* 1993). The single or combination of different hormones in the medium causes maintenance of specific and balanced inorganic and organic contents in the growing tissue. This leads the cells or tissues to develop either into shoots or roots or even death (Ikram-ul-Haq *et al.,* 2007). Auxins are generally used to stimulate callus production and cell growth in a culture medium or to initiate shoots, particularly roots, and to induce somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures (George et *al.,* 1993). Auxins and other growth regulators such as

gibberellins play important roles in the growth and differentiation of cultured cells and tissues (Bohidar *et al.,* 2008). Auxins such as 1-Naphtalene acetic acid (NAA) have been reported to promote plant rooting *in vitro* (Hussein *et al.,* 2012). Cykonins can stimulate protein synthesis. The cytokinins are used to stimulate cell division in the culture medium and induce shoot formation or auxillary shoot proliferation (George *et al.,* 1993). Cytokinins such as benzyl aminopurine (BAP) and kinetin are known to reduce the apical meristem dominance and induce both axiliary and adventitious shoot formation from meristematic explants in banana (Jafari *et al.,* 2011). However, the application of higher BAP concentrations inhibits elongation of adventitious meristems and the conversion into complete plants (Buising *et al.,* 1994). The use of cytokinin in plant nutrient media for in vitro culture depends on plant tissue growth stage and expected end product.

Moreover, Kishor and Devir (2009) reported that exogenous application of different cytokinins, such as BAP, BA, Kinetin, TDZ, Zeatin etc. has become obligatory for induction of multiple shoot in many plants. Vinas *et al.* (2012) described that shoots that grew continuously on high concentrations of BAP resulted in apical death. On the other hand, the basal medium containing lower doses of BAP promoted development of normal shoots without symptoms of apical necrosis. Many other studies have reported the use of auxins and cytokinin in tissue culture. Gubbuk and Pekmzci (2004) reported that moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation. Also they reported higher shoot proliferation and elongation with Thidiazuron (TDZ) than with BAP. However, BAP above 20 μM and TDZ over 2 μM decreased shoot elongation. The use of TDZ is known to inhibit shoot elongation. In another study, it was found that TDZ at 0.91 μM induced the largest number of shoots, but at higher concentration of TDZ (9.1 μM), elongation of shoots was inhibited and clumps of small globular buds appeared at the base of shoots (Shirani *et al.,* 2011). The influence of plant growth regulators and their interaction played an important role in shoot proliferation

in many plant species. Cytokinins was required in an optimal quantity for shoot proliferation in many genotypes but inclusion of low concentration of auxins along with optimal concentration of cytokinin triggered the rate of shoot proliferation (Perveen *et al.*, 201l; Jahan *et al.*, 2011).

2.4.3 Acclimatization

Acclimatization is the adaptation of plantlets developed *in vitro* to new external uncontrolled environment during which normal photosynthetic activity and water relations have to be developed (Desjardins, 1995). Micropropagation allows rapid production of high quality, disease free and uniform planting material irrespective of the season and weather. However, plants cultivated *in vitro* are different from field grown plants. High mortality is observed upon transfer of microshoots to *ex vitro* conditions as the cultured plants have nonfunctional stomata, weak root system and poorly developed cuticle (Mathur *et al.*, 2008). Microshoots on being transferred to ex vitro conditions are exposed to abiotic (altered temperature, light intensity and humidity conditions) and biotic stress conditions i.e. soil microflora, so need acclimatization for successful establishment and survival of plantlets (Deb and Imchen, 2010). Plantlets supplied with an excess of phytohormones show abnormalities in morphology and anatomy and are called vitrified plants or hyperhydrich plants (Hronkova *et al.*, 2003). The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field (Hazarika, 2003). In many plant species, the leaves formed *in vitro* are unable to develop further under ex vitro conditions and they are replaced by newly formed leaves (Preece and Sutter, 1991; Diettrich *et al.*, 1992). *In vitro* grown plantlets have large stomata with changed shape and structure. Guard cells have thinner cell walls and contain more starch and chloroplast (Marin *et al.*, 1988). Ticha et al. (1999) found that acclimatization of tobacco plantlets to ex vitro conditions decreased stomatal density and also changed the size and morphology of stomata on both sides of newly formed leaves. During acclimatization to *ex*

vitro conditions, leaf thickness generally increases, leaf mesophyll progresses in differentiation into palisade and spongy parenchyma, stomatal density decreases and stomatal form changes from circular to elliptical one. Development of cuticle, epicuticular waxes, and effective stomatal regulation of transpiration occur leading to stabilization of water potential of field transferred plantlets. (Pospı´sˇilova´ *et al.,* 1999).

CHAPTER-III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Time and location of the experiment

The experiment 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, 2020 to December, 2020.

3.2 Experimental materials

3.2.1 Source of material

The planting materials of Dragon fruit were collected from Agargaon Nursery, Sher-e-Bangla Nagar, Dhaka-1207.

Plate 1: Donor Dragon fruit plant for explants collection

3.2.2 Plant materials

Fresh, healthy and disease free shoot tips of Dragon fruit were harvested in a beaker filled with water. The explants were washed thoroughly with running tap water. Shoot tips were collected from the elite plants showing good biomass yield and shoot were trimmed to size of 2-3 cm for further work.

Plate 2: Shoot tips of Dragon fruit

3.2.3 Instruments

Metal instruments viz., forceps, scalpels, needless, 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 Erlen meyer flasks, culture bottles, flat bottom flasks, pipettes, petridishes, beaker and measuring cylinders (25 ml, 50 ml, 100 ml, 500 ml and 1000 ml) were used for media preparation. The glassware 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 an autoclave at a temperature of 121^0C for 30 minutes at 1.06 kg/cm^2 (15 PSI) pressure

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. Compositions 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 Sterilization of Instruments and Glassware

All glassware and instruments were first rinsed with the liquid detergent (Trix) and washed thoroughly with tap water until the detergent was removed completely. Then the glassware and instruments were sterilized in an autoclave at a temperature of 121^0C for 30 minutes at 1.06 kg/cm^2 (15 PSI) pressure.

3.3 Preparation of the stock solution of hormones

The first step of the preparation of the medium was the preparation of hormone stock solutions. To expedite the preparation of the medium separate stock solutions for growth regulators were prepared and used. Growth regulators and concentrations used in for *in vitro* regeneration of are presented below:

1. BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) alone were used for shoot proliferation.
2. NAA (0.25, 0.5, 0.75 and 1.0 mg/L) were applied for root formation.
3. BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) in combination with NAA (0.25, 0.5, 0.75 and 1.0 mg/L), were used for shoot and root proliferation.

To prepare the above 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).

Hormones (Solute)	Solvents used
BA	1 N NaOH
NAA	96% ethyl alcohol

In present experiment, the stock solution of hormones were prepared by following procedure

3.3.1 Stock solution of BA

A 100 mg of powder hormone was placed in a small beaker and then dissolved in 10 ml of 1 (N) NaOH solvent. Finally the volume was made up to 100 ml by the addition of sterile distilled water using a measuring cylinder.

3.3.2 Stock solution of NAA

A 100 mg of powder hormone was placed in a small beaker and then dissolved in 10 ml of 70% ethyl alcohol 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 $(4\pm1)^0$C for use up to two month.

3.4 Treatments

Three sub experiments were conducted to assess the effect of different concentrations of BA on shoot proliferation and NAA on subsequent rooting of the multiplied shoot and BA with NAA for shoot and root induction.

Sub-experiment 1: Effect of BA on *in vitro* shoot induction potentiality in Dragon fruit

Five levels of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and control (0.0 mg/l) treatments were used. The experiments were arranged in Completely Randomized Block (CRD) with three replications.

Sub-experiment 2: Effect of NAA on root induction potentiality of *in vitro* regeneration in Dragon fruit

Four levels of NAA (0.25, 0.5, 0.75 and 1.0 mg/L) and control (0.0 mg/l) were used.

Sub-experiment 3: Combined effect of BA and NAA on *in vitro* shoot and root induction potentiality in Dragon fruit

In this sub-experiment, four levels of NAA (0.25, 0.5, 0.75 and 1.0mg/l) were practiced with each level of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l). Total 20 combinations of BA and NAA were examined in this experiment and control treatment also practiced. The combine treatments were as follows:

T_1 = BA 1.0 mg/l + 0.25 mg/l NAA

T_2 = BA 1.0 mg/l + 0.5 mg/l NAA

T_3 = BA 1.0 mg/l + 0.75 mg/l NAA

T_4 = BA 1.0 mg/l + 1.0 mg/l I NAA

T_5 = BA 1.5 mg/l + 0.25 mg/l NAA

T_6 = BA 1.5 mg/l + 0.50 mg/l NAA

T_7 = BA 1.5 mg/l + 0.75 mg/l NAA

T_8 = BA 1.5 mg/l + 1.0 mg/l NAA

T_9 = BA 2.0 mg/l + 0.25 mg/l NAA

T_{10} = BA 2.0 mg/l + 0.50 mg/l NAA

T_{11} = BA 2.0 mg/l + 0.75 mg/l NAA

T_{12} = BA 2.0 mg/l + 1.0 mg/l NAA

T_{13} = BA 2.5 mg/l + 0.25 mg/l NAA

T_{14} = BA 2.5 mg/l + 0.50 mg/l NAA

T_{15} = BA 2.5 mg/l + 0.75 mg/l NAA

T_{16} = BA 2.5 mg/l + 1.0 mg/l NAA

T_{17}= BA 3.0 mg/l + 0.25 mg/l NAA

T_{18} = BA 3.0 mg/l + 0.50 mg/l NAA

T_{19}= BA 3.0 mg/l + 0.75 mg/l NAA

T_{20}= BA 3.0 mg/l + 1.0 mg/l NAA

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

3.5 Preparation of culture media

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

Step-1: 700 ml of sterile distilled water was poured into 1000 ml beaker.

Step-2: 5 gm of MS media (Readymade, Duchefa, Netherland) and 30 gm of sucrose were added and gently stirred to dissolve these ingredients completely with the help of a Hot Plate magnetic stirrer.

Step-3: Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well.

Step-4: The volume was made up to 1000 ml with addition of sterile distilled water.

Step-5: The pH was adjusted at 5.8.

Step-6: Finally, 8 gm agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar.

Step-7: 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 explant.

3.7 Sterilization of culture room and transfer area

In the beginning, the culture room was sprayed with formaldehyde and then the room was kept closed for 3 days. After that the room was cleaned through gently washing the floors, walls and rakes with detergent. This is followed by careful wiping them with 70% ethanol. This process of sterilization of culture room was repeated at regular intervals. The transfer area was also cleaned with detergent and also sterilized twice in a month by 70% ethanol. Laminar airflow cabinet was usually sterilized by switching UV ray to kills the microbes inside the laminar airflow. It switches on 30 minutes before working in empty condition and for 20 minutes with all the instruments. The working surface was wiping with 70% ethanol before starting the transfer work.

3.8 Preparation of explants

The trimmed shoot tips were washed thoroughly under running tap water and then with autoclaved distilled water for several times. Subsequently the explant were transferred to laminar airflow cabinet and kept in a 250 ml sterilized beaker. The beaker with explant 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 explant were immersed in 0.1% $HgCl_2$ within a beaker and added 3-4 drops of Tween-20 for about 4-5 minutes with constant shaking in clockwise and anticlockwise direction. Then explant were washed 3-4 times with autoclaved distilled water

to make the material free from chemical and ready for inoculation in culture media.

3.9 Inoculation of explants in culture media

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 hands and forearms were washed thoroughly with soap and water and repeatedly sprayed with 70% alcohol during the period of work. The mouth of culture vial was flamed before and after positioning of the explant on the medium. For inoculation, explants were transferred to large sterile glass petridish or glass plate with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer leaves were removed with sterile scalpel blade to make suitable size. After cutting explant into suitable size (1.5-2 cm), explants are transferred to culture bottles containing MS medium with plant growth regulator (Plate 3). After vertically inoculating the explant singly in culture bottle, the mouth of bottle was quickly flamed and capped tightly. After proper labeling, mentioning media code, date of inoculation etc. the bottles was transferred to growth room.

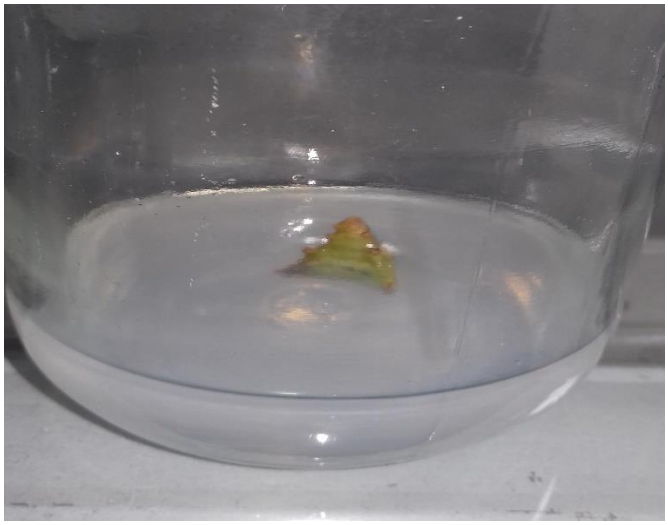

Plate 3. Inoculation of explant into culture media

3.10 Incubation

The bottles were kept on the culture racks and allowed to grow in controlled environment (Plate 3). 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).

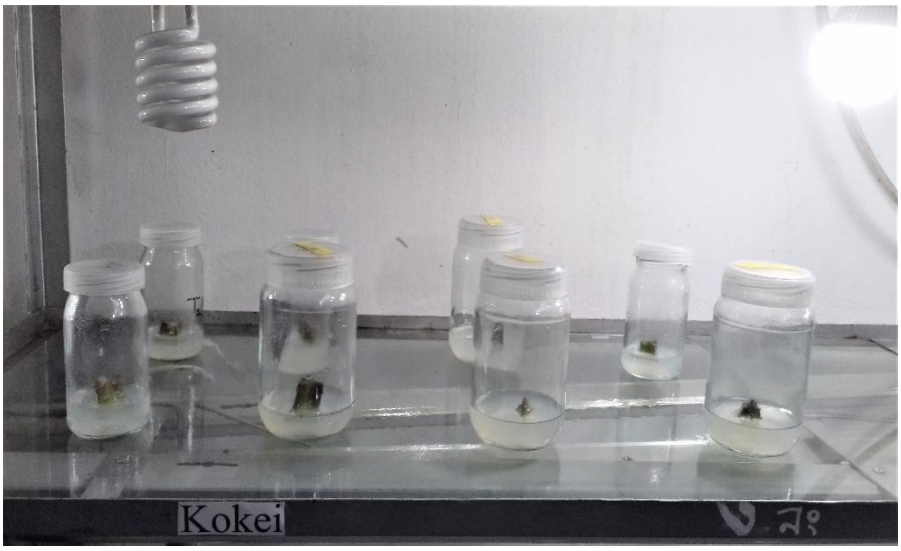

Plate 4. Incubation of inoculated culture vial

3.11 Maintenance of proliferating shoots

The explants were cultured on MS nutrient medium supplemented with different concentrations of BA alone or in combination of NAA. After successful shoot proliferation, subculture was done with newly formed shoot. Shoot were excised in aseptic condition with the 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 shoot were made throughout the entire culture period. Data recording was started after 2 weeks from inoculation.

3.12 Root formation of regenerated shoot

Newly formed shoot with adequate length were excised individually from the culture vial and transferred to rooting media. One type of growth regulators (NAA) was used separately in different concentration (0.25, 0.5, 0.75 and 1.0 mg/L) along with MS media. The observations on development pattern of root were made throughout the entire culture period. Data were recorded from 3rd week of inoculation.

3.13 Acclimatization

Acclimatization or "hardening-off' is a process by which *in vitro* propagated plants are made to adapt to an *in vivo* environment.

Step-1: After 60 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 root 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 root, the plantlets were then transplanted into the pots with special care.

Step-2: After planting, the plantlets were thoroughly watered and were kept at 25±2 °C with light intensity varied from 2000–3000 lux. The photoperiod was generally 14 hours light and 10 hours dark and 70% RH for 7 days with consecutive irrigation.

Step-3: 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 (2 days interval).

Step-4: After 3 weeks, the plants were transferred to the soil following depoting and poting into different pot having bigger size. The plants were watered periodically and upper layer of the soil was mulched occasionally whenever necessary.

3.14 Data recording

The observation on development pattern of shoot and root were made throughout the culture period. Five replicates each of them containing 4 bottles (single shoot per culture bottle) were used per treatment. Data were recorded after 4, 5 and 6 weeks of culture, starting from day of inoculation on culture media in case of shoot proliferation. In the event of root formation, it was also done after 4, 5 and 6 weeks of culture. The following observations were recorded in cases of shoot and root formation under *in vitro* condition:

1. Days to shoot induction

2. No. of shoots per explant

3. Average length of shoot (cm)

4. Days to root induction

5. No. of roots per explant

6. Average length of root (cm)

7. Percent of explants showing shoot induction

8. Percent of explants showing root induction

3.14.1 Percent of explants showing shoot induction

The number of shoots were produced per explant were recorded and the percentage of soot regeneration was calculated as

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

3.14.2 Days to shoot induction

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

3.14.3 Number of shoots per explant

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

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

3.14.4 Average length of shoot (cm)

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

3.14.5 Percent of explants showing root induction

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

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

3.14.6 Days to root induction

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

3.14.7 Number of roots per explant

Average number of roots per explant was calculated by using formula:

$$\text{Number of root per explant} = \frac{\text{Number of roots per explant}}{\text{Number of observation}}$$

3.14.8 Average length of root (cm)

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

3.14.9 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:

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

3.15 Statistical analysis

The experiment was one factorial set up in a completely randomized design (CRD) with five replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Least Significant Difference (LSD) test at 5% probability level using Statistic-10 software.

CHAPTER-IV

RESULT AND DISCUSSION

CHAPTER IV

RESULT AND DISCUSSION

Different investigations were made on this experiment under laboratory condition to evaluate the effects of different plant growth regulators on multiple shoot and root induction in Dragon fruit. The overall objective of the present study is to develop a system of mass propagation of Dragon fruit. Optimizing the relative ratio of different plant growth regulators has been successfully used in the current investigation. The results of these experiments have been presented and discussed in this chapter with plate (5-9), figure (1-4) and tables (1-11). Analysis of variance in respect of all the parameters have been presented in Appendices (II-XXIX).

4.1 Sub-experiment 1: Effect of BA on multiple shoot proliferation in Dragon fruit (*Hylocereus undatus*)

This experiment was conducted under laboratory condition to evaluate the effects of cytokinine hormone on shoot proliferation. Manipulating the relative ratio of different growth regulators has been successfully used in the current investigation. Different levels of BA were used for direct multiple shoot proliferation. The results of the effect of different concentrations of BA have been presented under following headings with Figures 1-2 and Table 1-2

4.1.1 Percent of explants showing Shoot induction

There was a significant variation on percentage of explants showing shoot induction in presence of various concentrations of BA at 5% level of significance. The highest percentage (76.00%) of shoot induction was recorded at 2.0 mg/L BA. The lowest percentage (24.00%) was induced in hormone free media in Dragon fruit. (Figure 1). On the contrary, 40%, 44%, 60% and 48% shoot induction were observed respectively from 1.0 mg/L, 1.5 mg/L, 2.5 mg/L and 3.0 mg/L BA contained media. Giusti *et al*. (2002) and Khalafalla *et al*. (2007) stated that explants cultured in MS media with 30 µM BAP levels gave

the highest shoot growth and proliferation for other cactus species (*Escobaria minima, Mammillaria pectinifera, Pelecyphora aselliformis and Opuntia ficusindica*).

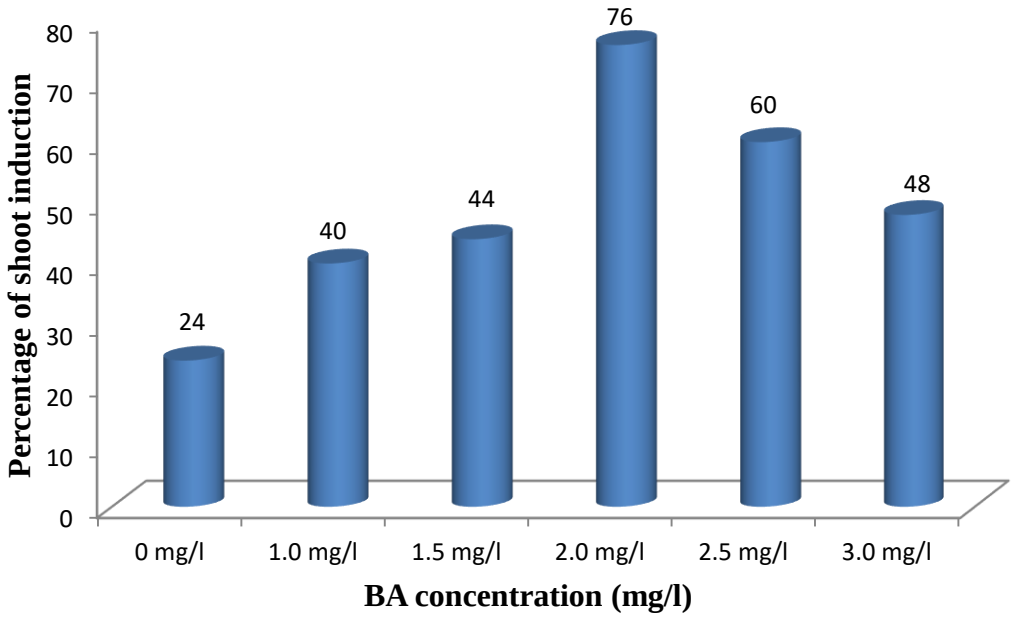

Figure 1. Effect of BA on percentage of shoot induction in Dragon fruit.

4.1.2 Days to shoot induction:

Significant variations were observed among different concentrations of BA on days to shoot induction. The maximum 21.6 days was recorded in control treatment for shoot induction followed by the treatment 1.00 mg/l BA (21 days). On the other hand, minimum 12.2 days was required in the treatment 2.0 mg/l BA followed by 2.5 mg/l of BA (15.2 days). Jafari *et al* (2011) stated that benzyl aminopurine (BAP) reduced the apical dominance and induced both axillary and adventitious shoot initiation from meristematic explants in banana. BAP has been considered to be most effective for the induction of shoot in plant tissue culture (Asamenew and Narayanaswamy, 2000; Baskaran and Jayabalan, 2005). Therefore, it may be assumed that BA levels influence first shoot initiation in culture.

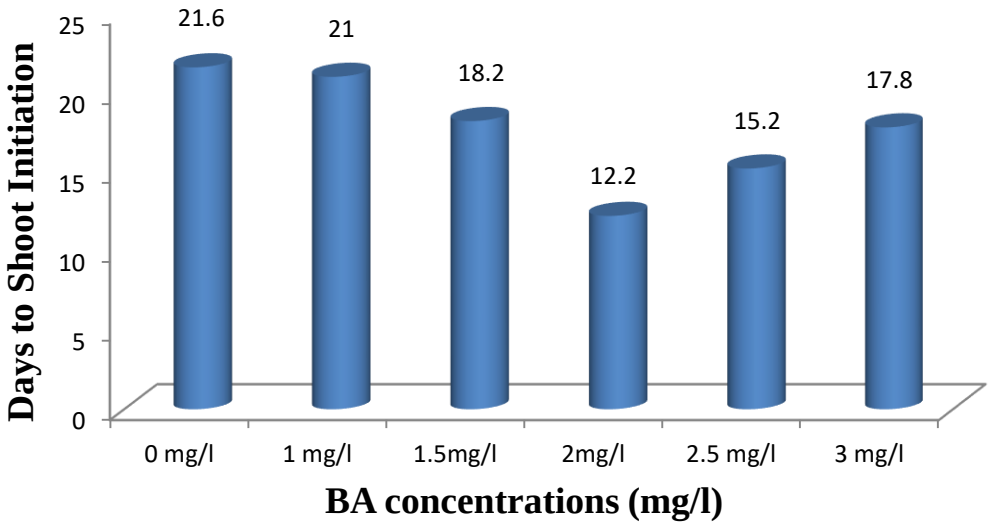

Figure 2. Effect of BA on days to shoot induction in Dragon fruit.

4.1.3 Number of shoots per explant

There was a significant influence of different concentrations of BA on the number of shoot at 5% level of significance. The highest number of shoot (1.80, 2.40 and 3.20) at 28 DAI, 35 DAI and 42 DAI respectively was noticed from the treatment 2.0 mg/l BA followed by shoot numbers 1.00, 2.00 and 2.60 at 28 DAI, 35 DAI and 42 DAI respectively from 2.5 mg/L BA treatment (Plate 5). Whereas the lowest number of shoots 0.80, 1.00 and 1.20 at 28 DAI, 35 DAI and 42 DAI, respectively were noticed in control treatment (Table 1). Soe (2019) reported that proximal portion of shoot explants of Dragon fruits produced the maximum number of shoot (4.42 per plant) supplemented with the 10 µM BAP.

Table1.Effect of different concentration of BA on number of shoot at different DAI.

Treatment BA (mg/L)	Number of shoot per explant		
	28 DAI	35 DAI	42 DAI
0	0.80 c	1.00 c	1.20 d
1.0	1.00 bc	1.40 bc	1.60 d
1.5	1.40 abc	2.00 ab	2.40 bc
2.0	1.80 a	2.40 a	3.20 a
2.5	1.60 ab	2.00 ab	2.60 ab
3.0	1.40 abc	1.60 bc	1.80 cd
CV (%)	41.08	32.47	27.07
$LSD_{(0.05)}$	0.71	0.73	0.75

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. DAI= Days after inoculation, CV=Coefficient of variation, LSD (0.05) = Least significant difference.

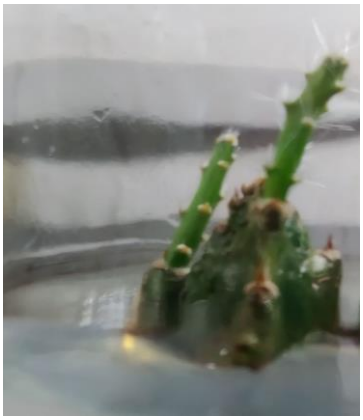

Plate 5. Shoot proliferation of Dragon fruit on MS media supplemented with BA 2.0 mg/l at 42 DAI

4.1.4 Average length of shoot (cm)

Significant variation of different concentrations of BA on average length of shoot was found. The highest length of shoot (1.68 cm, 2.78 cm and 4.02cm) at 28, 35 and 42 DAI respectively was noticed from the 2.50 mg/l BA which was statistically different from rest of the treatments. Whereas, the minimum length (0.48 cm, 0.72 cm and 1.42 cm) at 28, 35 and 42 DAI respectively were noticed in control treatment (Table 2).

Table 2. Effect of different concentration of BA on length of shoot at different DAI.

Treatment BA (mg/L)	Average length of shoot (cm)		
	28 DAI	35 DAI	42 DAI
0	0.48 d	0.72 e	1.42 f
1.0	0.82 c	1.22 d	2.12 e
1.5	1.08 b	1.56 c	2.48 d
2.0	1.18 b	2.02 b	3.72 b
2.5	1.68 a	2.78 a	4.02 a
3.0	0.76 c	1.58 c	2.72 c
CV (%)	8.47	5.97	4.12
$LSD_{(0.05)}$	0.1105	0.1283	0.1479

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

4.2 Sub-experiment 2. Effect of NAA on root induction potentiality in Dragon fruit (*Hylocereus undatus)*

The results of the effect of different concentrations of NAA have been presented under following headings with Figure (3-4) and Table (3-4).

4.2.1 Days to root induction

Significant variations were observed among different concentrations of NAA on days to root induction. The maximum days (22.20 days) to root induction were recorded in control followed by 1.00 mg/l (19.80 days). On the other

hand, minimum (12.40 days) was required in 0.25 mg/l NAA followed by 0.50 mg/l (15.60 days) (Figure 3).

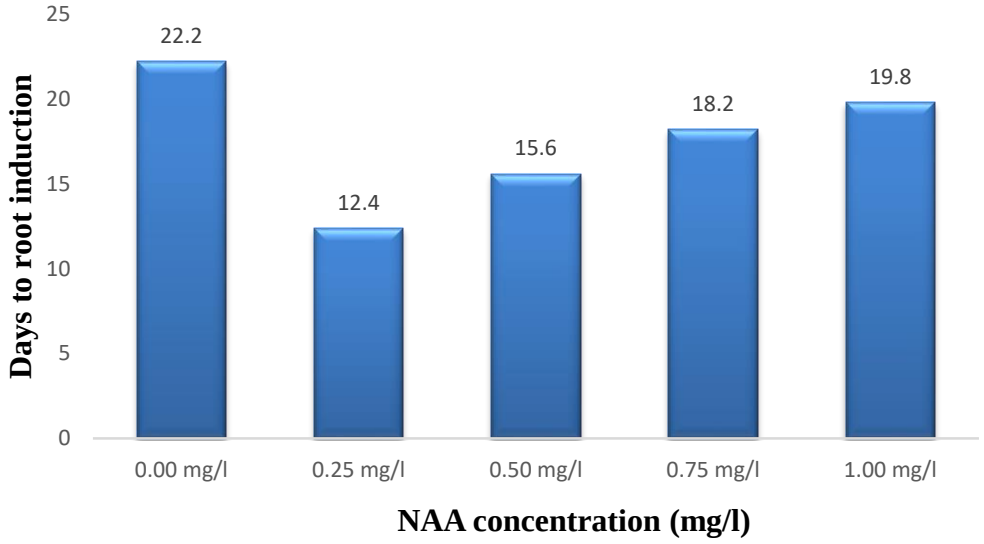

Figure 3. Effect of NAA on days to root induction in Dragon fruit.

4.2.2 Percent of explant showing root induction

There was a significant variation on percentage of proliferated shoot showing root induction in presence of various concentrations of NAA at 5% level of significance. The treatment NAA 0.50 mg/l had produced the highest percentage of root induction (68.00%), while the lowest percentage (20.00%) of root induction was produced in control treatment (Figure 4). On the other hand, 48%, 44%, and 36% root induction were observed respectively from 0.25 mg/L, 0.75 mg/L, and 1.00 mg/L NAA contained media. Dahanayake and Ranawake (2011) observed root induction of proliferated shoots on MS medium supplemented with 0.05 µM NAA regardless of the shoot induction medium in dragon fruit crop. This resulted variation might be due to the differences of genotype and culture environments. In addition, nature, origin and the physiological state of the explant and seasonal variation might have played a crucial role in the establishment of cultures and subsequent plant regeneration.

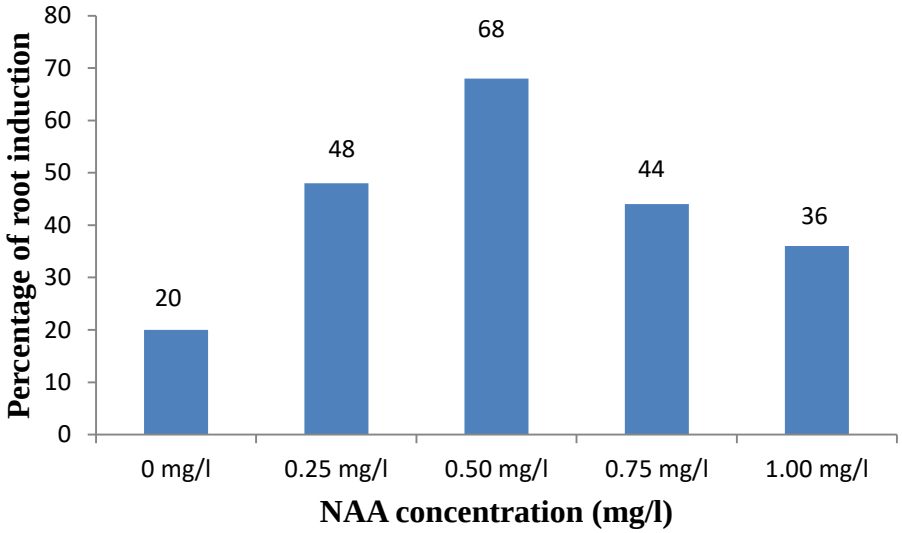

Figure 4. Effect of NAA on percentage of root induction in Dragon fruit.

4.2.3 Number of root per shoot

There was significant influence of different concentrations of NAA on the number of roots per shoot. The treatment NAA 0.50 mg/l gave the highest number of root (2.20, 3.60 and 5.40) at 28, 35 and 42 DAI and the second highest number of root (1.60, 2.40 and 3.80) was noticed at 28, 35 and 42 DAI respectively. The control treatment was found with the lowest number of root (1.00, 1.00 and 1.20) at 28, 35 and 42 DAI respectively (Table 3).

Table 3. Effect of different concentrations of NAA on number of root at different DAI

Treatment NAA (mg/L)	Number of root per explant		
	28 DAI	35 DAI	42 DAI
0	1.00 c	1.00 c	1.20 d
0.25	1.60 b	2.40 b	3.80 b
0.50	2.20 a	3.60 a	5.40 a
0.75	1.20 bc	1.60 c	2.40 c
1.00	1.00 c	1.20 c	1.80 cd
CV (%)	26.73	23.93	19.97
$LSD_{(0.05)}$	0.4936	0.6188	0.7693

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

4.2.4 Average length of root (cm)

There was a significant influence of different concentrations of NAA on the length of root at 5% level of significance in laboratory condition. The highest length of root (2.00 cm, 3.30 cm and 5.38 cm) at 28, 35 and 42 DAI respectively were noticed from the 0.25 mg/l NAA followed by 0.50 mg/l (1.46 cm, 2.42 cm and 4.20 cm) . On the other hand, the lowest length of root (0.44 cm, 0.94 cm, 1.82 cm) at 28, 35 and 49 DAI respectively were noticed in control treatment followed by 1.00 mg/l NAA (0.72 cm, 1.50 cm and 2.62 cm) respectively. (Table 4)

Table 4. Effect of different concentration of NAA on length of root at different DAI

Treatment NAA (mg/L)	Average length of root (cm)		
	28 DAI	35 DAI	42 DAI
0	0.44 e	0.94 e	1.82 e
0.25	2.00 a	3.30 a	5.38 a
0.50	1.46 b	2.42 b	4.20 b
0.75	1.30 c	2.02 c	3.24 c
1.00	0.72 d	1.50 d	2.62 d
CV (%)	9.10	6.48	3.15
$LSD_{(0.05)}$	0.14	0.17	0.14

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

4.3 Sub-experiment 3. Combined effect of BA and NAA on shoot and root induction potentiality in Dragon fruit (*Hylocereus undatus*)

The results of the combined effect of different concentrations of BA and NAA have been presented under following headings with Table (5-10) and Plate 6.

4.3.1 Days to shoot induction

Significant variations were observed among different concentrations of BA and NAA on days to shoot induction. The minimum duration (11.67 days) was obtained in the treatment BA 2.0 mg/l + NAA 0.50 mg/l over the rest of the treatments. On the other hand, the maximum days (23.33 days) to shoot

induction was recorded in the treatment BA 1.0 mg/l+ NAA 0.25 mg/l (Table 5). Daud *et al.* (2015) showed that the new plantlets were raised in a short period of time when explants were cultured on MS medium containing 1.0 mg/1 BAP and 0.5 mg/1 NAA. The variation may be due to influence of genetic and environmental factors.

4.3.2. Percent of explants showing shoot induction

There was a significant influence of different concentrations of BA and NAA on the percentage of shoot induction per explant. The highest percentage (86.67%) of shoot induction was noticed in the treatment BA 2.0 mg/l + NAA 0.50 mg/l and minimum percentage (33.33%) was noticed in the treatment BA 1.0 mg/l + NAA 0.50 mg/l (Table 5). Abbasi *et al.* (2016) observed that the treatment 5.0 mg/l BA and 1.0 mg/l NAA produced optimum percentage of shoot organogenesis after 4 weeks of subculturing. This variation might be due to growth regulators in the culture media, genetic, physiological and morphological change *in vitro* (Asamenew and Narayanswamy, 2000).

Table 05. Effect of different concentration of BA and NAA on days to shoot induction and percent of shoot induction

Treatments	Days to Shoot induction	Percentage of shoot induction
BA 1.0 mg/l + NAA 0.25 mg/l	23.33 a	33.33
BA 1.0 mg/l + NAA 0.50mg/l	20.33 b	40.00
BA 1.0 mg/l + NAA 0.75 mg/l	20.67 b	53.33
BA 1.0 mg/l + NAA 1.00 mg/l	20.33 b	46.67
BA 1.5 mg/l + NAA 0.25 mg/l	18.33 c	53.33
BA 1.5 mg/l + NAA 0.50 mg/l	15.67 fg	66.67
BA 1.5 mg/l + NAA 0.75 mg/l	20.67 b	53.33
BA 1.5 mg/l + NAA 1.00 mg/l	18.00 cd	60.00
BA 2.0 mg/l + NAA 0.25 mg/l	14.33 gh	73.33
BA 2.0 mg/l + NAA 0.50 mg/l	11.67 i	86.67
BA 2.0 mg/l + NAA 0.75 mg/l	17.33 cde	73.33
BA 2.0 mg/l + NAA 1.00 mg/l	20.00 b	66.67
BA 2.5 mg/l + NAA 0.25 mg/l	16.67 def	73.33
BA 2.5 mg/l + NAA 0.50 mg/l	13.33 h	80.00
BA 2.5 mg/l + NAA 0.75 mg/l	16.33 ef	66.67
BA 2.5 mg/l + NAA 1.00 mg/l	16.67 def	53.33
BA 3.0 mg/l + NAA 0.25 mg/l	15.67 fg	73.33
BA 3.0 mg/l + NAA 0.50 mg/l	15.33 fg	66.67
BA 3.0 mg/l + NAA 0.75 mg/l	21.33 b	46.67
BA 3.0 mg/l + NAA 1.00 mg/l	21.00 b	40.00
CV (%)	5.31	
$LSD_{(0.05)}$	1.57	

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 NAA showed significant variations on the number of shoot. The highest number of shoots 2.33, 3.33 and 3.67 were noticed from the BA 2.0 mg/l + NAA 0.5 mg/l combination and the second highest number 2.33, 2.67 and 3.00 at 28 DAI, 35 DAI and 42 DAI respectively, were observed at the treatment BA 2.5 mg/l + NAA 0.5 mg/l (Plate 6). Whereas the lowest average number of shoot (1.00, 1.00 and 1.00) at 28 DAI, 35 DAI and 42 DAI, respectively were noticed at the treatment BA 1.0 mg/l + NAA 0.25 mg/l (Table 6). Yaser *et al*. (2019) reported that fragmented shoots of Dragon tree cultured onto MS medium supplemented with 2 mg/L

BAP and 0.5 mg/L IBA treatment resulted in the highest amount of axillary shoots (7 shoots per explant) and the intact shoots had the highest axillary shoots (1.8 shoots per explant) when cultured onto a medium supplemented with a combination of 1 mg/L BAP and 0.5 mg/L IBA. Variation might be due to the age, nature, origin, and physiological state of the explant and seasonal variation play a crucial role in the establishment of culture and subsequent plant regeneration (Bajaj, Y. P. S. *et al.*, 1991)

Table 6. Effect of BA and NAA on number of shoot at different DAI

Treatments	Number of shoot per explant		
	28 DAI	**35 DAI**	**42 DAI**
BA 1.0 mg/l + NAA 0.25 mg/l	1.00 b	1.00 d	1.00 d
BA 1.0 mg/l + NAA 0.50mg/l	1.00 b	1.33 cd	1.33 cd
BA 1.0 mg/l + NAA 0.75 mg/l	1.33 b	1.33 cd	1.33 cd
BA 1.0 mg/l + NAA 1.00 mg/l	1.33 b	1.33 cd	1.67 cd
BA 1.5 mg/l + NAA 0.25 mg/l	1.33 b	1.33 cd	1.67 cd
BA 1.5 mg/l + NAA 0.50 mg/l	1.67 ab	2.00 bc	2.33 bc
BA 1.5 mg/l + NAA 0.75 mg/l	1.67 ab	1.67 cd	1.67 cd
BA 1.5 mg/l + NAA 1.00 mg/l	1.33 b	1.33 cd	1.67 cd
BA 2.0 mg/l + NAA 0.25 mg/l	1.67 ab	1.67 cd	2.00 bcd
BA 2.0 mg/l + NAA 0.50 mg/l	2.33 a	3.33 a	3.67 a
BA 2.0 mg/l + NAA 0.75 mg/l	1.33 b	1.67 cd	1.67 cd
BA 2.0 mg/l + NAA 1.00 mg/l	1.33 b	1.67 cd	1.67 cd
BA 2.5 mg/l + NAA 0.25 mg/l	1.33 b	1.67 cd	2.00 bcd
BA 2.5 mg/l + NAA 0.50 mg/l	2.33 a	2.67 ab	3.00 ab
BA 2.5 mg/l + NAA 0.75 mg/l	1.33 b	1.67 cd	1.67 cd
BA 2.5 mg/l + NAA 1.00 mg/l	1.00 b	1.67 cd	2.33 bc
BA 3.0 mg/l + NAA 0.25 mg/l	1.33 b	1.67 cd	1.67 cd
BA 3.0 mg/l + NAA 0.50 mg/l	1.67 ab	1.67 cd	2.00 bcd
BA 3.0 mg/l + NAA 0.75 mg/l	1.00 b	1.00 d	1.33 cd
BA 3.0 mg/l + NAA 1.00 mg/l	1.00 b	1.33 cd	1.33 d
CV (%)	34.52	32.31	34.11
$LSD_{(0.05)}$	0.79	0.87	1.03

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference

Plate 6. Shoot proliferation of Dragon fruit on MS media supplemented with BA 2.0 mg/l + NAA 0.50 mg/l at 42 DAI

4.3.4 Length of shoot (cm)

There was a variation in different concentrations of BA with NAA on the length of shoot at 5% level of significance. The highest length of shoot (2.16 cm, 3.03 cm and 4.36 cm) at 28, 35 and 49 DAI, respectively were noticed from the BA 2.0 mg/l + NAA 0.50 mg/l combination followed by BA 2.5 mg/l + NAA 0.50 mg/l (1.46 cm, 2.73 cm and 3.96 cm) at 28, 35 and 49 DAI respectively. Whereas the minimum length of shoot (0.33 cm, 0.56 cm and 1.23 cm) at 28, 35 and 42 DAI respectively were noticed in the treatment BA 1.0 mg/l + NAA 0.25 mg/l (Table 7)

Table 7. Effect of different concentration BA and NAA on length of shoot

Treatments	Average length of shoot (cm)		
	28 DAI	35 DAI	42 DAI
BA 1.0 mg/l + NAA 0.25 mg/l	0.33 h	0.57 j	1.23 l
BA 1.0 mg/l + NAA 0.50mg/l	0.57 g	0.83 i	1.67 k
BA 1.0 mg/l + NAA 0.75 mg/l	0.63 fg	1.17 h	2.03 j
BA 1.0 mg/l + NAA 1.00 mg/l	0.77 ef	1.07 h	2.07 j
BA 1.5 mg/l + NAA 0.25 mg/l	0.77 ef	1.47 ef	2.80 fg
BA 1.5 mg/l + NAA 0.50 mg/l	1.23 c	2.13 c	3.33 cd
BA 1.5 mg/l + NAA 0.75 mg/l	0.73 ef	1.37 fg	2.47 i
BA 1.5 mg/l + NAA 1.00 mg/l	0.93 d	1.63 de	2.67 ghi
BA 2.0 mg/l + NAA 0.25 mg/l	1.47 b	2.13 c	3.53 c
BA 2.0 mg/l + NAA 0.50 mg/l	2.17 a	3.03 a	4.37 a
BA 2.0 mg/l + NAA 0.75 mg/l	0.93 d	1.77 d	3.03 ef
BA 2.0 mg/l + NAA 1.00 mg/l	0.77 ef	1.47 ef	2.47 i
BA 2.5 mg/l + NAA 0.25 mg/l	1.13 c	2.07 c	3.53 c
BA 2.5 mg/l + NAA 0.50 mg/l	1.47 b	2.73 b	3.97 b
BA 2.5 mg/l + NAA 0.75 mg/l	0.57 g	1.23 gh	2.53 hi
BA 2.5 mg/l + NAA 1.00 mg/l	0.87 de	1.73 d	2.73 gh
BA 3.0 mg/l + NAA 0.25 mg/l	1.13 c	2.03 c	3.27 de
BA 3.0 mg/l + NAA 0.50 mg/l	0.83 de	1.47 ef	2.83 fg
BA 3.0 mg/l + NAA 0.75 mg/l	0.73 ef	1.10 h	2.03 j
BA 3.0 mg/l + NAA 1.00 mg/l	0.57 g	1.17 h	1.93 j
CV (%)	9.15	6.47	5.88
$LSD_{(0.05)}$	0.14	0.17	0.26

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference

Plate 7. Highest Shoot length of Dragon fruit on MS media supplemented with BA 2.0 mg/l + NAA 0.50 mg/l at 42 DAI

4.3.5 Days to root induction

Significant variation was observed among different concentrations of BA and NAA on days to root induction. The maximum (23.33 days) to root induction was recorded in the treatment BA 1.0 mg/l + NAA 0.25 mg/l and minimum (11.67 days) was required in BA 2.5 mg/l + NAA 0.50 mg/ which was statistically similar with 12.67 days in BA 2.0 mg/l + NAA 0.50 mg/l concentration (Table 8).

4.3.6 Percentage of root induction

Different concentrations of BA and NAA showed the significant variations on percent of explants showing root induction. The highest percentage (93.33%) of root induction was recorded with BA 2.0 mg/l + NAA 0.50 mg/l, whereas the lowest percentage (26.67%) of root induction was recorded in the treatment BA 1.0 mg/l + NAA 0.25 mg/l (Table 8)

Table 8. Effect of different concentration BA and NAA on days to root induction and percentage of root initiation

Treatments	Days to root induction	Percentage of root initiation (%)
BA 1.0 mg/l + NAA 0.25 mg/l	23.33 a	26.67
BA 1.0 mg/l + NAA 0.50mg/l	21.33 bc	33.33
BA 1.0 mg/l + NAA 0.75 mg/l	21.33 bc	53.33
BA 1.0 mg/l + NAA 1.00 mg/l	20.33 c	66.67
BA 1.5 mg/l + NAA 0.25 mg/l	17.33 de	66.67
BA 1.5 mg/l + NAA 0.50 mg/l	15.67 f	73.33
BA 1.5 mg/l + NAA 0.75 mg/l	18.67 d	60.00
BA 1.5 mg/l + NAA 1.00 mg/l	18.33 d	66.67
BA 2.0 mg/l + NAA 0.25 mg/l	16.33 ef	73.33
BA 2.0 mg/l + NAA 0.50 mg/l	12.67 g	93.33
BA 2.0 mg/l + NAA 0.75 mg/l	15.67 f	80.00
BA 2.0 mg/l + NAA 1.00 mg/l	17.67 de	73.33
BA 2.5 mg/l + NAA 0.25 mg/l	15.67 f	73.33
BA 2.5 mg/l + NAA 0.50 mg/l	11.67 g	86.67
BA 2.5 mg/l + NAA 0.75 mg/l	20.67 c	66.67
BA 2.5 mg/l + NAA 1.00 mg/l	20.33 c	60.00
BA 3.0 mg/l + NAA 0.25 mg/l	16.67 ef	73.33
BA 3.0 mg/l + NAA 0.50 mg/l	16.67 ef	73.33
BA 3.0 mg/l + NAA 0.75 mg/l	20.33 c	66.67
BA 3.0 mg/l + NAA 1.00 mg/l	22.33 ab	53.33
CV (%)	4.55	
$LSD_{(0.05)}$	1.14	

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference

4.3.7 Number of root per shoot

There was a significant influence of different concentrations of BA and NAA on the number of root per shoot. The treatment BA 2.0 mg/l + NAA 0.50 mg/ gave the highest number of root (2.67, 3.33 and 5.67) and second best result (2.33, 2.67 and 4.67) were noticed from BA 2.5 mg/l + NAA 0.50 mg/l at 28 DAI, 35 DAI and 42 DAI respectively whereas the lowest number of root (1.00, 1.00 and 1.00) at 28 DAI, 35 DAI and 42 DAI respectively were found in the treatment BA 1.0 mg/l + NAA 0.25 mg/l (Table 9 and plate 8). Pedda Kasim *et al* (2019) reported that transferred shoots to rooting medium supplemented with 3

mg/l BAP and 0.5 mg/l NAA which found to be effective concentrations for the growth and development of roots (4.8*) in vitro.*

Table 9. Effect of different concentration BA and NAA on number of roots at different DAI

Treatments	Number of roots per explant		
	28 DAI	**35 DAI**	**42 DAI**
BA 1.0 mg/l + NAA 0.25 mg/l	1.00 cd	1.00 e	1.00 g
BA 1.0 mg/l + NAA 0.50mg/l	1.00 cd	1.00 e	1.33 fg
BA 1.0 mg/l + NAA 0.75 mg/l	1.00 cd	1.00 e	1.67 efg
BA 1.0 mg/l + NAA 1.00 mg/l	1.00 cd	1.33 de	2.33 def
BA 1.5 mg/l + NAA 0.25 mg/l	1.33 cd	1.67 cde	2.67 cde
BA 1.5 mg/l + NAA 0.50 mg/l	1.33 cd	2.00 bcd	3.33 cd
BA 1.5 mg/l + NAA 0.75 mg/l	1.00 cd	1.33 de	2.33 def
BA 1.5 mg/l + NAA 1.00 mg/l	1.33 cd	1.33 de	2.00 efg
BA 2.0 mg/l + NAA 0.25 mg/l	1.67 bc	2.00 bcd	3.67 bc
BA 2.0 mg/l + NAA 0.50 mg/l	2.67 a	3.33 a	5.67 a
BA 2.0 mg/l + NAA 0.75 mg/l	1.67 bc	2.33 bc	3.33 cd
BA 2.0 mg/l + NAA 1.00 mg/l	1.33 cd	1.33 de	2.00 efg
BA 2.5 mg/l + NAA 0.25 mg/l	1.67 bc	2.00 bcd	3.67 bc
BA 2.5 mg/l + NAA 0.50 mg/l	2.33 ab	2.67 ab	4.67 ab
BA 2.5 mg/l + NAA 0.75 mg/l	1.67 bc	2.33 bc	3.33 cd
BA 2.5 mg/l + NAA 1.00 mg/l	1.00 cd	1.67 cde	2.33 def
BA 3.0 mg/l + NAA 0.25 mg/l	1.00 cd	1.33 de	2.33 def
BA 3.0 mg/l + NAA 0.50 mg/l	1.33 cd	1.67 cde	3.33 cd
BA 3.0 mg/l + NAA 0.75 mg/l	1.00 cd	1.33 de	2.33 def
BA 3.0 mg/l + NAA 1.00 mg/l	1.00 d	1.00 e	1.67 fg
CV (%)	30.61	30.17	25.27
$LSD_{(0.05)}$	0.68	0.82	1.12

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference

Plate 8. Root initiation of Dragon fruit on MS media supplemented with BA 2.0 mg/l + NAA 0.50 mg/l at 42 DAI

4.3.8 Length of root (cm)

There was significant influence of different combined concentrations of BA and NAA on the length of root. There was a significant variation at 28 DAI, 35 DAI and 42 DAI among different concentrations of BA and NAA. The highest length of root (3.03 cm, 4.07 cm and 6.27 cm) at 28 DAI, 35DAI and 42 DAI respectively were found in BA 2.0 mg/l + NAA 0.50 mg/l (Table 10). The treatment BA 1.0 mg/l + NAA 0.25 mg/l showed the lowest number of root (0.33 cm, 0.63 cm and 1.27 cm) at 28 DAI, 35 DAI and 42 DAI respectively.

Table 10. Effect of BA and NAA on length of root at different DAI

Treatments	Average length of root (cm)		
	28 DAI	35 DAI	42 DAI
BA 1.0 mg/l + NAA 0.25 mg/l	0.33 m	0.63 n	1.27 j
BA 1.0 mg/l + NAA 0.50mg/l	0.53 kl	0.93 m	1.93 i
BA 1.0 mg/l + NAA 0.75 mg/l	0.43 lm	1.07 lm	2.43 gh
BA 1.0 mg/l + NAA 1.00 mg/l	0.77 i	1.43 ij	2.93 ef
BA 1.5 mg/l + NAA 0.25 mg/l	0.97 h	1.67 hi	3.23 e
BA 1.5 mg/l + NAA 0.50 mg/l	1.77 d	2.67 c	4.03 d
BA 1.5 mg/l + NAA 0.75 mg/l	1.03 gh	1.87 gh	3.03 ef
BA 1.5 mg/l + NAA 1.00 mg/l	1.17 fg	1.93 fg	3.07 ef
BA 2.0 mg/l + NAA 0.25 mg/l	2.23 b	3.27 b	5.20 b
BA 2.0 mg/l + NAA 0.50 mg/l	3.03 a	4.07 a	6.27 a
BA 2.0 mg/l + NAA 0.75 mg/l	1.27 f	2.13 ef	3.90 d
BA 2.0 mg/l + NAA 1.00 mg/l	1.07 gh	1.67 hi	3.27 e
BA 2.5 mg/l + NAA 0.25 mg/l	1.57 e	2.33 de	4.53 c
BA 2.5 mg/l + NAA 0.50 mg/l	1.97 c	3.03 b	5.33 b
BA 2.5 mg/l + NAA 0.75 mg/l	0.73 ij	2.13 ef	3.87 d
BA 2.5 mg/l + NAA 1.00 mg/l	1.03 gh	1.67 hi	3.23 e
BA 3.0 mg/l + NAA 0.25 mg/l	1.27 f	2.13 ef	4.03 d
BA 3.0 mg/l + NAA 0.50 mg/l	1.63 de	2.43 cd	4.17 cd
BA 3.0 mg/l + NAA 0.75 mg/l	0.57 jkl	1.37 jk	2.77 fg
BA 3.0 mg/l + NAA 1.00 mg/l	0.63 ijk	1.17 kl	2.13 hi
CV (%)	10.24	7.53	7.58
$LSD_{(0.05}$	0.20	0.24	0.43

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. DAI= Days after inoculation, CV=Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference

Sub-experiment 4. Acclimatization and establishment of plantlets on soil

After a considerable shoots and roots were developed at 8-10 weeks of culture, the plantlets were removed from vial carefully without any root damage. The roots were washed with running tap water for removing media from plantlets. Then, the small plantlets were taken to growth cabinet for acclimatization and maintained for further observations under controlled conditions providing with light, temperature and relative humidity to make a favorable condition for plant establishment. In the meantime, the plantlets were transferred to vermiculite pot filled with sterilized soil: cowdung (1:1) and soil mixture provided with a

solution of 1% NAA and ultimately transferred to shade house for acclimatization. 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). After all, in the growth cabinet and in the shade house, plants were acclimatized and hardened before being transferred to the field conditions. At first 30 plants were transplanted and 23 survived in shade condition (76.67%). Finally, in normal atmospheric condition 25 plants were transplanted among them 21 survived and survival rate was 84% (Plate 9). Vinas *et al*. (2012) observed survival of more than 75 % of the *in vitro* derived *Hylocereus costaricensis* plants after 60 days in the greenhouse. So considering the survival rate it can be said that acclimatization potentiality of Dragon fruit was satisfactory.

Table 11. Survival rate of *in vitro* regenerated plantlets of Dragon fruit

Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate (%)
In shade area with controlled atmosphere	30	23	76.67
In natural conditions	25	21	84

Plate 9. Acclimatization and establishment of plantlets in natural condition

CHAPTER-V

SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSIONS

The present experiment was carried out in the Laboratory of Biotechnology, Department of Biotechnology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of February, 2020 to December, 2020 to study the effect of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), NAA (0.25, 0.50, 0.75 and 1.00 mg/l) individually or in combination of both for *in vitro* regeneration of Dragon fruit. The shoot tips of dragon fruits were used as explant materials which were sterilized with 70% ethanol and 0.1% $HgCl_2$. The experiments were arranged in Completely Randomized Design (CRD) with three replications.

The treatment BA 2.0 mg/l gave the highest percentage of shoot (76.00%) in 12.2 days and the maximum number of shoot (1.80, 2.40 and 3.20) at 28, 35 and 42 DAI respectively where the control treatment found the lowest percentage of shoot (24.00%) in 21.6 days and the minimum number of shoot (0.80, 1.00 and 1.20) at 28, 35 and 42 DAI respectively. The maximum length of shoot (1.68cm, 2.78 cm and 4.02 cm) at 28, 35 and 42 DAI respectively was recorded with BA 2.5 mg/l where control treatment showed the minimum length of shoots. The treatment 0.50 mg/l NAA had produced the highest percentage of root induction (68.00%) and minimum 12.4 days required to produce root with the treatment NAA 0.25 mg/l and minimum percentage (20.00%) was in 22.2 days in control treatment. The treatment 0.50 mg/l NAA gave the highest number of root (2.20, 3.60 and 5.40) at 28, 35 and 42 DAI and the control treatment found the lowest number of root at all DAI.

In combined effect, the maximum percentage (86.67%) of shoot induction was noticed at treatment BA 2.0 mg/l + 0.50 mg/l NAA in 11.67 days and the minimum percentage (33.33%) was found in the treatment BA 1.0 mg/l + NAA 0.25 mg/l in 23.33 days. The treatment BA 2.0 mg/l + 0.50 mg/l NAA gave the highest number of shoot (2.33, 3.33 and 3.67) at 28, 35 and 42 DAI respectively and the treatment BA 1.0 mg/l + NAA 0.25 mg/l showed the lowest number of shoot. The maximum length of shoot (2.17 cm, 3.03 cm and 4.37 cm) at 28, 35 and 42 DAI respectively were noticed from the combined dose of BA 2.0 mg/l + 0.50 mg/l NAA and the minimum length of shoot were noticed in control treatment. The treatment BA 2.0 mg/l + 0.50 mg/l NAA had showed the highest percentage of root induction (93.33 %) required minimum 12.67 days and minimum percentage (26.67%) was in the treatment BA 1.0 mg/l + NAA 0.25 mg/l in 23.33 days. The treatment BA 2.0 mg/l + 0.50 mg/l NAA gave the highest number of root (2.67, 3.33 and 5.67) at 28, 35 and 42 DAI and

the treatment BA 1.0 mg/l + NAA 0.25 mg/l showed the lowest number of root at all DAI.

A convenient protocol of Dragon fruit is established. The moderate dose of BA 2.0 mg/l gave the best results for shoot regeneration. In the combined dose BA 2.0 mg/l + 0.50 mg/l NAA gave the best result. Besides, 0.50 mg/l NAA gave the best performance in case of root regeneration. A protocol of *in vitro* rapid regeneration of Dragon fruit has been established which may contribute to large scale virus free seedlings production throughout the year.

RECOMMENDATIONS

RECOMMENDATIONS

Based on the summary and conclusion following recommendations can be made:

i. More explants such as meristem, leaf and root tip can be experimented for *in vitro* regeneration of Dragon fruit.

ii. Accurate and details investigation on influence of other factors such as different antioxidants and other auxin and cytokinin group of hormone should be considered.

iii. Further study can be done with different concentrations and combinations of auxins and cytokinins group of hormones for rapid proliferation of Dragon fruit.

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APPENDICES

APPENDICES

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

Components	Concentrations (mg/L)	Concentrations (µM)
Micro Elements		
$CoCl_2.6H_2O$	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		
$CaCl_2$	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		
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) on days to shoot induction with BA

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	314.267	62.8533	81.98	0.0000
Error	24	18.400	0.7667		
Total	29	332.667	-	-	-
CV (%)	4.96	-	-	-	-
LSD value	1.1429	-	-	-	-

Appendix III. Analysis of variance (ANOVA) on no. of shoots per explant with BA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	3.4667	0.69333	2.31	0.0755
Error	24	7.2000	0.30000	-	-
Total	29	10.6667	-	-	-
CV (%)	41.08	-	-	-	-
LSD value	0.7150	-	-	-	-

Appendix IV. Analysis of variance (ANOVA) on no. of shoots per explant with BA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	6.2667	1.25333	3.96	0.0093
Error	24	7.6000	0.31667	-	-
Total	29	13.8667	-	-	-
CV (%)	32.47	-	-	-	-
LSD value	0.7345	-	-	-	-

Appendix V. Analysis of variance (ANOVA) on no. of shoots per explant with BA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	13.4667	2.69333	8.08	0.0001
Error	24	8.0000	0.33333	-	-
Total	29	21.4667	-	-	-
CV (%)	27.06	-	-	-	-
LSD value	0.7536	-	-	-	-

Appendix VI. Analysis of variance (ANOVA) on length of shoot with BA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	4.30800	0.86160	120.22	0.0000
Error	24	0.17200	0.00717	-	-
Total	29	4.48000	-	-	-
CV (%)	8.47	-	-	-	-
LSD value	0.1105	-	-	-	-

Appendix VII. Analysis of variance (ANOVA) on length of shoot with BA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	12.3827	2.47653	256.19	0.0000
Error	24	0.2320	0.00967	-	-
Total	29	12.6147	-	-	-
CV (%)	5.97	-	-	-	-
LSD value	0.1283	-	-	-	-

Appendix VIII. Analysis of variance (ANOVA) on length of shoot with BA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	5	23.9667	4.79333	373.51	0.0000
Error	24	0.3080	0.01283	-	-
Total	29	24.2747	-	-	-
CV (%)	4.12	-	-	-	-
LSD value	0.1479	-	-	-	-

Appendix IX. Analysis of variance (ANOVA) on days to root induction with NAA

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	286.960	71.7400	132.85	0.0000
Error	20	10.800	0.5400	-	-
Total	24	297.760	-	-	-
CV (%)	4.17	-	-	-	-
LSD value	0.9695	-	-	-	-

Appendix X. Analysis of variance (ANOVA) on no. of roots per explant with NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	5.20000	1.30000	9.29	0.0002
Error	20	2.80000	0.14000	-	-
Total	24	8.00000	-	-	-
CV (%)	26.73	-	-	-	-
LSD value	0.4936	-	-	-	-

Appendix XI. Analysis of variance (ANOVA) on no. of roots per explant with NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	22.5600	5.64000	25.64	0.0000
Error	20	4.4000	0.22000	-	-
Total	24	26.9600	-	-	-
CV (%)	23.93	-	-	-	-
LSD value	0.6188	-	-	-	-

Appendix XII. Analysis of variance (ANOVA) on no. of roots per explant with NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	57.0400	14.2600	41.94	0.0000
Error	20	6.8000	0.3400	-	-
Total	24	63.8400	-	-	-
CV (%)	19.97	-	-	-	-
LSD value	0.7693	-	-	-	-

Appendix XIII. Analysis of variance (ANOVA) on length of root with NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	57.0400	14.2600	41.94	0.0000
Error	20	6.8000	0.3400	-	-
Total	24	63.8400	-	-	-
CV (%)	19.97	-	-	-	-
LSD value	0.7693	-	-	-	-

Appendix XIV. Analysis of variance (ANOVA) on length of root with NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	16.1696	4.04240	232.32	0.0000
Error	20	0.3480	0.01740	-	-
Total	24	16.5176	-	-	-
CV (%)	6.48	-	-	-	-
LSD value	0.1740	-	-	-	-

Appendix XV. Analysis of variance (ANOVA) on length of root with NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	4	16.1696	4.04240	232.32	0.0000
Error	20	0.3480	0.01740	-	-
Total	24	16.5176	-	-	-
CV (%)	6.48	-	-	-	-
LSD value	0.1740	-	-	-	-

Appendix XVI. Analysis of variance (ANOVA) on days to shoot induction with BA and NAA

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	560.667	29.5088	32.26	0.0000
Error	43	39.333	0.9147	-	-
Total	62	600.000	-	-	-
CV (%)	5.31	-	-	-	-
LSD value	1.57	-	-	-	-

Appendix XVII. Analysis of variance (ANOVA) on no. of shoots per explant with BA and NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	9.0794	0.47786	2.05	0.0254
Error	43	10.0000	0.23256	-	-
Total	62	19.0794	-	-	-
CV (%)	34.52	-	-	-	-
LSD value	0.79	-	-	-	-

Appendix XVIII. Analysis of variance (ANOVA) on no. of shoots per explant with BA and NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	16.6032	0.87385	3.13	0.0010
Error	43	12.0000	0.27907	-	-
Total	62	28.6032	-	-	-
CV (%)	32.31	-	-	-	-
LSD value	0.87	-	-	-	-

Appendix X IX. Analysis of variance (ANOVA) on no. of shoots per explant with BA and NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	22.4127	1.17962	3.04	0.0012
Error	43	16.6667	0.38760	-	-
Total	62	39.0794	-	-	-
CV (%)	34.11	-	-	-	-
LSD value	1.03	-	-	-	-

Appendix XX. Analysis of variance (ANOVA) on length of shoot with BA and NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	10.2498	0.53947	77.32	0.0000
Error	43	0.3000	0.00698	-	-
Total	62	10.5498	-	-	-
CV (%)	9.15	-	-	-	-
LSD value	0.14	-	-	-	-

Appendix XXI. Analysis of variance (ANOVA) on length of shoot with BA and NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	22.0565	1.16087	110.11	0.0000
Error	43	0.4533	0.01054	-	-
Total	62	22.5098	-	-	-
CV (%)	6.47	-	-	-	-
LSD value	0.17	-	-	-	-

Appendix XXII. Analysis of variance (ANOVA) on length of shoot with BA and NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	37.3565	1.96613	78.77	0.0000
Error	43	1.0733	0.02496	-	-
Total	62	38.4298	-	-	-
CV (%)	5.88	-	-	-	-
LSD value	0.26	-	-	-	-

Appendix XXIII. Analysis of variance (ANOVA) on days to root induction with BA and NAA

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	604.317	31.8062	45.59	0.0000
Error	43	30.000	0.6977	-	-
Total	62	634.317	-	-	-
CV (%)	4.55	-	-	-	-
LSD value	1.38	-	-	-	-

Appendix XXIV. Analysis of variance (ANOVA) on no. of roots per explant with BA and NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	12.9841	0.68338	4.01	0.0001
Error	43	7.3333	0.17054	-	-
Total	62	20.3175	-	-	-
CV (%)	30.61	-	-	-	-
LSD value	0.68	-	-	-	-

Appendix XXV. Analysis of variance (ANOVA) on no. of roots per explant with BA and NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	23.6508	1.24478	5.02	0.0000
Error	43	10.6667	0.24806	-	-
Total	62	34.3175	-	-	-
CV (%)	30.17	-	-	-	-
LSD value	0.82	-	-	-	-

Appendix X XVI. Analysis of variance (ANOVA) on no. of roots per explant with BA and NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	77.2698	4.06683	8.74	0.0000
Error	43	20.0000	0.46512	-	-
Total	62	97.2698	-	-	-
CV (%)	25.27	-	-	-	-
LSD value	1.12	-	-	-	-

Appendix X XVII. Analysis of variance (ANOVA) on length of root with BA and NAA at 28 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	27.0441	1.42338	98.72	0.0000
Error	43	0.6200	0.01442	-	-
Total	62	27.6641	-	-	-
CV (%)	10.24	-	-	-	-
LSD value	0.20	-	-	-	-

Appendix XXVIII. Analysis of variance (ANOVA) on length of root with BA and NAA at 35 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	42.0327	2.21225	103.40	0.0000
Error	43	0.9200	0.02140	-	-
Total	62	42.9527	-	-	-
CV (%)	7.53	-	-	-	-
LSD value	0.24	-	-	-	-

Appendix XX IX. Analysis of variance (ANOVA) on length of root with BA and NAA at 42 DAI

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Probability
Treatment	19	90.0965	4.74192	68.73	0.0000
Error	43	2.9667	0.06899	-	-
Total	62	93.0632	-	-	-
CV (%)	7.58	-	-	-	-
LSD value	0.43	-	-	-	-