EFFECTS OF EXPLANTS AND GROWTH REGULATORS ON *IN VITRO* REGENERATION OF DRAGON FRUIT (*Hylocereus undatus* Haworth)

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Department of Horticulture
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The thesis attached here to, entitled **"Effects of Explants and Growth Regulators on *In Vitro* Regeneration of Dragon Fruit *(Hylocereus undatus* Haworth*)"*** was prepared under the direction of the chairperson of the candidate supervisory committee and has been approved by all members of that committee and the board of examiners as a partial fulfillment of the requirements for the degree of **Master of Agricultural Science (Horticulture)**.

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DECLARATION OF ORIGINALITY

This thesis represents the original work of the author, except where otherwise stated. It has not been submitted previously for a degree or to any other University.

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DEDICATED TO MY BELOVED PARENTS

U BA CHIT AND DAW SAW TIN

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ABSTRACT

The experiment was conducted at Plant Tissue Culture Laboratory, Experiment and Lecture Building - 2 (ELB-2), Department of Horticulture, Yezin Agricultural University (YAU) from 2016 to 2018. This study was carried out to investigate effects of explant types and to examine effects of different levels of plant growth regulators on *in vitro* regeneration of dragon fruit. The experiment was arranged in factorial RCB design with four replications and factor A was two kinds of explants; distal and proximal portions (7-10 mm) of shoot developed *in vitro* and factor B was five different levels of N-6 benzyl aminopurine (BAP) for shoot multiplication. For root induction, factor A was two types of explants; distal and proximal portions (7-10 mm) and factor B was three different levels of naphthalene acetic acid (NAA).

According to the experimental results, in shoot multiplication, the maximum number of shoots per explant (4.42) and the longest shoot (1.39 cm) was obtained from proximal portion of shoot explants. The medium supplemented with 10 μM BAP was observed production of the highest number of shoots, shoot length and shoot fresh weight. In root induction, there was no significant effect of different explant types on number of roots, root length and fresh weight of plantlets. However, the maximum number of roots, root length and fresh weight of plantlets were found at 0.3 μM NAA containing media. By the results, it can be concluded that the proximal portion of shoot explant should be used in shoot multiplication and the medium containing 10 μM BAP is recommended. For root induction, the explants cultured on 0.3μM NAA containing medium is recommended for *in vitro* root induction stage.

Key words: BAP, dragon fruit, explants, in vitro regeneration, NAA

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CHAPTER I
INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is the tropical fruit and belongs to the Cactaceae family. It is also known as pitaya or pitahaya or strawberry pear or night blooming cereus. 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 *Hylocereus megalanthus* (yellow peel and white pulp) (Nerd et al. 2002).

Dragon fruit is native to Mexico, Central America and South America; but now widely cultivated as fruit crops in Southeast Asian countries (Haber 1983; Mizrahi et al. 1997). 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. Zainudin (2006) has reported that hectarage in Vietnam, Taiwan, Thailand, China and Malaysia are 10000 ha, 4000 ha, 1000 ha, 200 ha and 280 ha, respectively.

In Myanmar, dragon fruit plants grow well and become a promising new crop. Growing areas of this fruit in Myanmar are found in Popa, Nyaung Oo, Kyaukpadaung, Pakokku, Meiktila, Heho, Naungcho, Aungban, Kyaukmae, Taunggyi, Nay Pyi Taw. It is commercially produced in other areas by private growers. 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 (Nwe 2014). 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).

Plant tissue culture became a tool to propagate desire elite plants into planting materials for commercial production. Therefore, determination on governing factors of *in vitro* propagation is still needed. 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). Explant derivation can influence growth and morphogenetic potential. Moreover, the maximum rate of micropropagation depends on the selection of the most suitable explants (Gunasena et al. 2006).

Use of plant growth regulators (PGRs) in cactus tissue culture is of fundamental importance. Cytokinins and auxins are regarded to be the two most important growth regulators utilized to control organogenesis and embryogenesis. 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).

Therefore, this study was conducted to investigate effect of different explant types and to examine effect of different levels of plant growth regulators on *in vitro* regeneration of dragon fruit.

CHAPTER II
LITERATURE REVIEW

2.1 Description of Dragon Fruit

Dragon fruit (*Hylocereus undatus* Haworth) 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 to rapidly multiplicate 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 insures 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 Type of explant

Explants show different capacity in initial cultured *in vitro* depending on their location on donor plant. For example, survival and growth of terminal bud explants are normally greater than lateral bud explants. Similar lateral meristem explants from the

distal and proximal of a single shoot may respond differently *in vitro*. There is a suitable size for explants used to initiate tissue cultures. Very small, explants, whether they are shoot or meristem tips, fragments of whole plant tissues, or pieces of callus, do not survive well in culture, but large explants may be difficult to decontaminate effectively or are less easily manipulated (Roberta 2013).

2.3.2 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 et al. l99l). 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).

2.3.3 Subculturing

The rate of *in vitro* shoot multiplication depends greatly on subculturing of proliferating shoot cultures. Apostolo et al. (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 (Debnath 2004).

2.3.4 *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 et al. (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 et al. (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 2003, Gaba 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 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 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 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 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 of tissue culture plantlets

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 non functional 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

The experiment was conducted at Plant Tissue Culture Laboratory, Experiment and Lecture Building - 2, Department of Horticulture, Yezin Agricultural University (YAU) from 2016 to 2018. Murashige and Skoog (1962) medium was used as a basal medium and *in vitro* shoot explants of *Hylocereus undatus* Haworth (red skin and white pulp) were used as a source of explants in this study.

3.1 Effects of Different Explants and Different Levels of N-6 Benzyl Aminopurine (BAP) on *In Vitro* Shoot Multiplication of Dragon Fruit

3.1.1 Experimental procedure

Distal and proximal portions of *in vitro* shoot explants developed in shoot multiplication (approximately 7-10 mm in length) were used as explants for *in vitro* shoot multiplication (Plate 1). Shoot multiplication medium was composed of MS medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose, and 0.6% (w/v) agar and different levels of BAP (0, 10, 20, 30 and 40 μM) (Table 3.1). The pH of the medium was adjusted to 5.7. Then the culture vessels were incubated at 25 ± 2º C with 16 hr photoperiod under light intensity of approximately 30 μmol $m^{-2}s^{-1}$ for 12 weeks.

In this experiment, four explants were inoculated in each culture vessel and five culture vessels were involved for each treatment. The experimental design was set up in factorial Randomized Complete Block Design (RCB) with 4 replications.

3.2 Effects of Different Explant types and Different Levels of Naphthalene Acetic Acid (NAA) on *In Vitro* Root Induction of Dragon Fruit

3.2.1 Experimental procedure

Distal and proximal portions of *in vitro* shoot explants developed in shoot multiplication (approximately 7-10 mm in length) were also used as explants for root induction. Root induction medium was composed of MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose, and 0.6% (w/v) agar and three different levels of NAA (0.0, 0.3, and 0.5 μM) were given as treatments (Table 3.2). The pH of the medium was adjusted to 5.7. Then the culture vessels were incubated as in shoot multiplication stage.

In this experiment, four explants were cultured in each culture vessel and each treatment had five culture vessels. The experiment was set up in factorial Randomized Complete Block Design (RCB) with 4 replications.

Table 3.1 Different levels of N-6 Benzyl aminopurine (BAP) used to culture two explant types for shoot multiplication of dragon fruit crop

Explant Types	BAP[x] (µM)
Distal	0
	10
	20
	30
	40
Proximal	0
	10
	20
	30
	40

[x], N-6 Benzyl aminopurine

Table 3.2 Different levels of Naphthalene acetic acid (NAA) used to culture two explant types for root induction of dragon fruit crop

Explant Types	NAA[y] (µM)
Distal	0.0
	0.3
	0.5
Proximal	0.0
	0.3
	0.5

[y], Naphthalene acetic acid

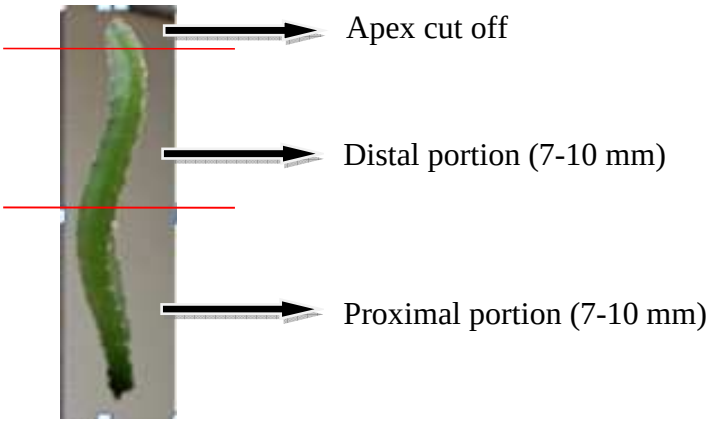


Plate 1. Distal and proximal portions of *in vitro* shoot explants for shoot multiplication of dragon fruit crop

3.3 Data Collection

3.3.1 Shoot culture

Survival percent, days to induce first shoot and shoot formation percent were recorded within four weeks after culture during *in vitro* shoot multiplication. Number of shoots per explant was collected by counting the shoot producing from each treatment at twelve weeks after culture. Average shoot length (cm) was calculated by measuring three shoots (nearly equal length). Shoot fresh weight (g) was measured by using digital balance at twelve weeks after culture.

3.3.2 Root induction culture

Days to induce first root and root formation percent were collected within four weeks after culture and number of roots per plantlet was recorded at twelve weeks after culture. Root length (cm) and fresh weight of plantlet (g) were also measured at twelve weeks after culture on in vitro root induction.

3.4 Acclimatization of I*n Vitro* Raised Plantlets

After root induction, plantlets were taken out from culture vessels. Agar was carefully removed from the roots by washing with tap water. Then the plantlets were cultured in 2:1 sand and burnt rice husk substrates and survival percent were collected.

3.5 Data Analysis

The statistical analysis performed by using CropStat (version 7.2) package least significant difference (LSD) at 0.05% level was used to compare treatment means.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of Different Explant Types and Different Levels of N-6 Benzyl Aminopurine (BAP) on Shoot Multiplication of Dragon Fruit

4.1.1 Survival percent

Different levels of BAP influenced the survival percent of explant types (Figure 4.1). In this experiment, the 100 % survival rate was observed in the medium containing hormone free, 10 and 20 µM BAP followed by 98.75 % survival rate at 30 µM BAP level in both explant types.

On the other hand, minimum survival percent of explants taken from distal and proximal portions (98.75% and 95% respectively) were found at 40 µM BAP containing medium. Therefore, it may be assumed that the higher levels of BAP decreased survival percent in initial culture. Strosse et al. (2008) reported a decrease in survival percent with increasing cytokinins concentrations in banana.

4.1.2 Days to induce first shoot

Different levels of BAP were found to influence days to induce first shoot (Table 4.1). For distal portion, explants cultured in MS medium with BAP levels below and above 30 µM showed later days to induce first shoot. On the other hand explants cultured in MS media supplemented 20 µM BAP were found to be induced shoot earlier than those from other treatments. Jafari et al (2011) stated that benzyl aminopurine (BAP) reduce the apical dominance and induce both axillary and adventitious shoot initiation from meristematic explants in banana. Therefore, it may be assumed that BAP levels influence first shoot initiation culture for both explant types.

4.1.3 Shoot formation percent

Effect of different levels of BAP on shoot formation percent from different kinds of explants is shown in Table 4.1. All of the explant types were responded to the different levels of BAP in all treatments.

It was found that 100 % shoot formation from distal portion was recorded from hormone free and 10 µM BAP containing medium which was followed by 99.25 % from 20 µM BAP. For proximal portion, the 100 % shoot formation was found from the medium with hormone free, 10 and 20 µM BAP levels. Minimum shoot formation percent was recorded on 40 µM BAP level from both explant types. 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*).

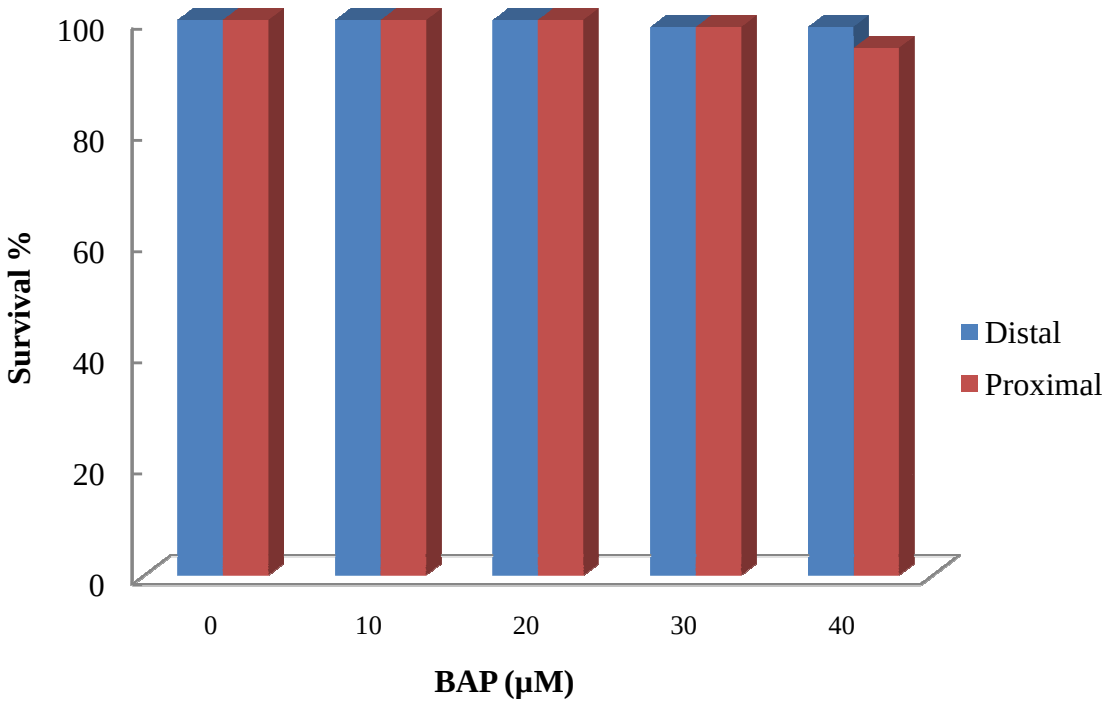


Figure 4.1 Effect of different levels of N-6 benzyl aminopurine (BAP) on survival percent of different explant types of dragon fruit crop cultured *in vitro*

Table 4.1 Effect of explant types and N-6 benzyl aminopurine (BAP) levels on days to induce first shoot and shoot formation percent of dragon fruit crop

Explants	BAP (µM)	Days to induce first shoot	Shoot formation %
Distal	0	16	100.00
	10	18	100.00
	20	23	99.25
	30	15	96.25
	40	24	93.75
Proximal	0	18	100.00
	10	16	100.00
	20	13	100.00
	30	15	98.75
	40	17	97.50

4.1.4 Number of shoots per explant, shoot length (cm) and shoot fresh weight (g)

The effect of different explant types and different levels of BAP on number of shoots per explant is shown in Table 4.2, 4.3, Plate 2 and 3. These results showed that the effects of explant types and BAP levels on number of shoots per explant were significantly different in shoot multiplication. However, there was no interaction effect between type of *in vitro* shoot explants and different levels of BAP on number of shoots per explant, shoot length and shoot fresh weight (Table 4.4).

The maximum number of shoots per explant (4.42) was obtained from proximal portion of *in vitro* shoot and the minimum number of shoots (3.61) was resulted from distal portion (Table 4.2). The results showed that the proximal portion of *in vitro* shoot was higher potential on number of shoots per explants than the distal portion. Pickens et al. (2006) that shoot regeneration rate depends on the types of explants (leaf and stem) in *in vitro* plant production.

The highest number of shoots per explant (5.55) was found in the medium containing BAP 10 µM which was followed by 20 µM BAP containing medium at 4.31. The lowest number of shoots (3.13) was observed at 40 µM BAP medium (Table 4.3). Mohamed et al. (2007) stated that the medium supplemented with more than 6.5 µM benzyladeenine (BA) decreased the number of shoots per explant for *in vitro* propagation of *Opuntia* spp. The current result was not in line with Vinas et al. (2012) who observed that BAP levels of 5, 15, or 30 µM in the media produced only one shoot per areole and 45 or 60 µM BAP in the media gave two to three shoots per areole in dragon fruit (*Hylocereus costaricensis*).

The effect of different explant types and BAP levels on shoot length is described in Table 4.2, 4.3 and Plate 4. The results showed that the effect of explant types and different levels of BAP on shoot length were significantly different. The longest shoot (1.39 cm) was obtained from the proximal portion of explants and the shortest one (1.18 cm) was observed in the distal portion.

The explants grown in the medium containing hormone free and 10 µM BAP produced the longest shoot 2.05 cm and 1.66 cm, respectively while that in the medium containing 40 µM BAP gave the shortest shoot (0.66 cm). This result agreed with the finding of Castro-Concha *et al*. (1990), who observed that hyperhydric shoots with a low ability to growth and develop were normally produced by using high concentrations of cytokinin (40 µM BAP) during the multiplication stage along with high concentrations of

ammonium and nitrate ions in *Agave* species. It may be assumed that the higher the levels of BAP, the lower the length of the shoots.

Fresh weight of shoots per explant as affected by different explant types and BAP levels is shown in Table 4.2 and 4.3. In both explant types, there was no significant effect on shoot fresh weight, however highly significant difference in the medium containing different BAP levels. The maximum fresh weight (1.97 g) was recorded in 10 and 20 µM BAP containing medium which was followed by (0.67 g) in 30 µM BAP involved. The minimum fresh weight (0.29 g) was obtained in 40 µM BAP containing medium.

The medium supplemented with 10 µM BAP gave the highest number of shoots. According to the results, the higher the levels of BAP, the lower the number of shoots, shoot length (cm) and shoot fresh weight (g).

Table 4.2 Effect of different explant types on number of shoots per explant, shoot length and shoot fresh weight of dragon fruit crop cultured *in vitro* at 12 weeks after culture period

Explant types	No. of shoots per explant	Shoot length (cm)	Shoot fresh weight (g)
Distal	3.61 b	1.18 b	0.81
Proximal	4.42 a	1.39 a	0.91
Pr ≥ F	**	*	Ns
LSD $_{(0.05)}$	0.49	0.17	0.33
CV %	14.34	11.64	7.75

Means within each column followed by the same letters are not significantly different at 5 % level

ns: Not significant * Significant at 5 % level ** Significant at 1 % level

Table 4.3 Effect of different levels of N-6 benzyl aminopurine (BAP) on number of shoots per explant, shoot length and shoot fresh weight of dragon fruit crop cultured *in vitro* at 12 weeks after culture

BAP (µM)	No. of shoots per explant	Shoot length (cm)	Shoot fresh weight (g)
0	3.33 b	2.05 a	0.45 b
10	5.55 a	1.66 a	1.97 a
20	4.31 ab	1.13 b	1.97 a
30	3.75 b	0.91 b	0.67 b
40	3.13 b	0.66 b	0.29 b
Pr ≥ F	*	**	**
LSD $_{(0.05)}$	1.41	0.48	0.64
CV %	24.24	44.07	76.90

Means within each column followed by the same letters are not significantly different at 5 % level

* Significant at 5 % level ** Significant at 1 % level

Table 4.4 Effect of different explant types and N-6 benzyl aminopurine (BAP) levels on number of shoot per explant, shoot length and shoot fresh weight of dragon fruit crop cultured *in vitro* at 12 weeks after culture

Treatments	No. of shoots per explants	Shoot length (cm)	Shoot fresh weight (g)
Explants	6.62 **	0.44 *	0.09 ns
BAP levels	7.57 *	2.56 **	3.51**
Explants × BAP	0.77 ns	0.10 ns	0.48 ns
CV %	17.9	18.9	55.8

Means within each column followed by the same letters are not significantly different at 5 % level

ns: Not significant * Significant at 5 % level ** Significant at 1 % level

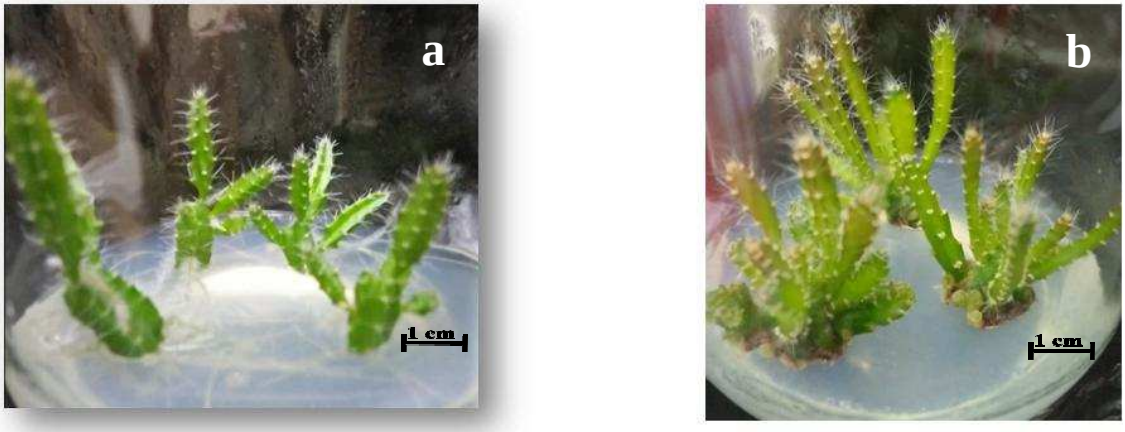


Plate 2. Effect of explant types on shoot proliferation of dragon fruit crop cultured *in vitro* at 6 weeks after culture (a) distal portion and (b) proximal portion

Plate 3. Effect of different levels of N-6 benzyl aminopurine (BAP) on shoot proliferation of dragon fruit crop at 12 weeks after culture (a) control, (b) 10 µM BAP, (c) 20 µM BAP, (d) 30 µM BAP and (e) 40 µM BAP

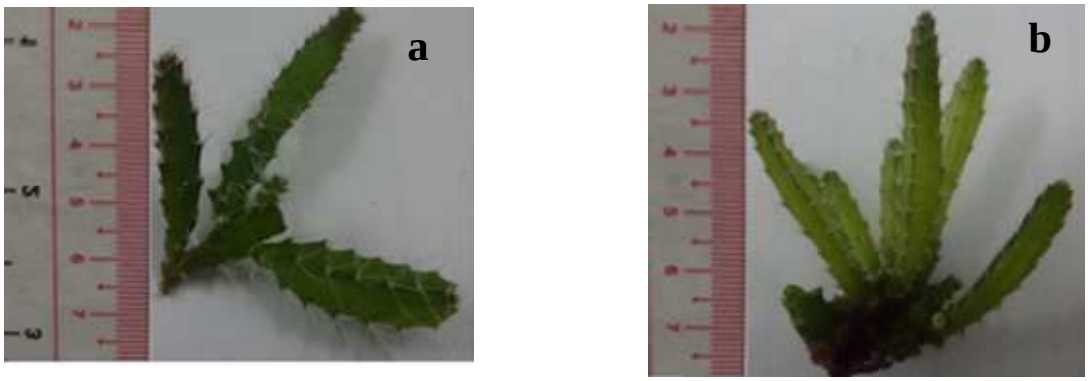


Plate 4. Effect of different explant types on shoot length of dragon fruit crop at 12 weeks after culture (a) distal portion and (b) proximal portion

4.2 Effects of Different Explant Types and Different Levels of Naphthalene Acetic Acid (NAA) on Root Induction of Dragon Fruit

4.2.1 Days to induce first root

Different levels of naphthalene acetic acid (NAA) influenced days to induce first root (Table 4.5). According to the table, the earliest days to induce first root (9 days in distal portion and 11 days in proximal portion) were observed in 0.3 µM NAA containing medium. It was in line with the finding of Seyyedyousefi et al. (2013) who stated that apical and lateral bud explants cultured in MS media with 5 µM NAA gave the highest root proliferation on *Alstroemeria* cv. "Fuego".

4.2.2 Root formation percent

Effect of different levels of NAA on root formation percent of different kinds of explants is shown in Table 4.5. All of the explant types responded to different levels of NAA.

It was found that 100 % root formation was recorded from 0.3 µM NAA containing medium from both explant types which was followed by 98.75 % at 0.5 µM NAA from proximal portion. In addition, minimum root formation percent was recorded in hormone free medium from both explant types. Dahanayake and Ranawake (2011) observed root induction of proliferated shoots on MS medium supplemented with 0.05 µMNAA regardless of the shoot induction medium in dragon fruit crop.

4.2.3 Number of roots per plantlet, root length (cm) and fresh weight of plantlet (g)

Effect of explant types and different levels of NAA on number of roots per plantlet in root induction stage is shown in Table 4.6, 4.7, 4.8 and Plate 5. There was no significant effect of explant types on number of roots per plantlet, root length and fresh weight of plantlet (Table 4.6). However, NAA levels had highly significant effect on number of roots per plantlet, root length and fresh weight of plantlet.

The maximum root number per plantlet (3.63) was produced in the medium supplemented with 0.3 µM NAA which was followed by 0.5 µM NAA containing medium at 3.20. The minimum number of roots (2.70) was observed at hormone free medium (Table 4.7).

The explants grown in the medium containing 0.3 and 0.5 µM NAA produced the longest shoot at 9.32 cm and 8.23 cm, respectively, while that in the medium without hormone gave the shortest shoot (6.58 cm).

The maximum fresh weight of plantlet (0.60 g) was recorded in 0.3 µM NAA containing medium which was followed by 0.53 g in MS medium with 0.5 µM NAA. The minimum fresh weight of plantlet (0.46 g) was obtained in hormone free medium.

After twelve weeks of culture under different NAA treatments, various responses of *in vitro* rooting were observed. Some of the shoots produced shoots without rooting, drying and callus formation during root induction particularly at the highest NAA level (Plate 6). Therefore, it may be assumed that drying and callus formation occurs due to the use of high levels of NAA for root induction.

On the other hand, there was no interaction effect between explant types and NAA levels on number of roots per plantlet, root length and fresh weight of plantlets (Table 4.8). It means that the effect of NAA levels on root induction was not influenced by types of explants in all parameters.

Table 4.5 Effect of explant types and naphthalene acetic acid (NAA) levels on days to induce first root and root formation percent of dragon fruit crop in root induction

Explants	NAA (µM)	Days to induce first root	Root formation %
Distal	0.0	11	96.25
	0.3	9	100.00
	0.5	12	96.50
Proximal	0.0	13	96.50
	0.3	11	100.00
	0.5	15	98.75

Table 4.6 Effect of different explant types on number of roots per plantlet, root length and fresh weight of plantlet of dragon fruit crop in root induction at 12 weeks after culture

Explant types	No. of roots per plantlet	Root length (cm)	Fresh weight of plantlet (g)
Distal	3.09	7.81	0.51
Proximal	3.36	8.28	0.54
Pr ≥ F	ns	ns	Ns
LSD $_{(0.05)}$	0.84	1.50	0.06
CV %	3.96	4.18	4.17

Means within each column followed by the same letters are not significantly different at 5 % level

ns: Not significant

Table 4.7 Effect of naphthalene acetic acid (NAA) levels on number of roots per plantlet, root length and fresh weight of plantlet of dragon fruit crop in root induction at 12 weeks after culture

NAA (µM)	No. of roots per plantlet	Root length (cm)	Fresh weight of plantlet (g)
0.0	2.70 c	6.58 b	0.46 c
0.3	3.63 a	9.32 a	0.60 a
0.5	3.20 b	8.23 a	0.53 b
Pr ≥ F	**	**	**
LSD $_{(0.05)}$	0.42	1.34	0.06
CV %	14.70	17.13	12.63

Means within each column followed by the same letters are not significantly different at 5 % level

** Significant at 1 % level

Table 4.8 Effect of different explant types and naphthalene acetic acid (NAA) levels on number of roots per plantlet, root length and fresh weight of plantlet of *in vitro* cultured dragon fruit crop at 12 weeks after culture

Treatments	No. of roots per plantlet	Root length (cm)	Fresh weight of plantlet (g)
Explants	1.018 ^{ns}	0.142 ^{ns}	0.004 ^{ns}
NAA levels	11.389 **	1.309 **	0.027 **
Explants × NAA	2.273 ^{ns}	0.082 ^{ns}	0.002 ^{ns}
CV %	20.300	14.200	9.000

Means within each column followed by the same letters are not significantly different at 5 % level

ns: Not significant ** Significant at 1 % level

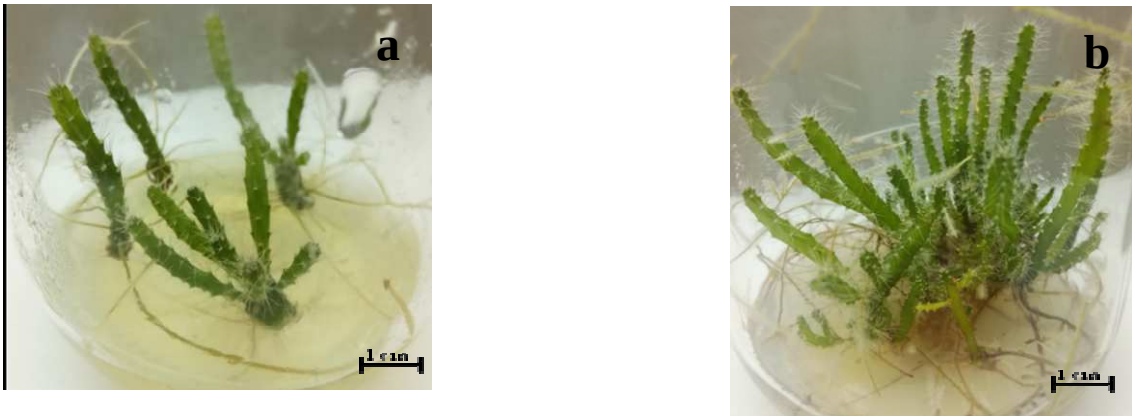


Plate 5. Effect of naphthalene acetic acid (NAA) levels on root formation of shoot developed from shoot culture of dragon fruit crop at 12 weeks after culture (a) distal portion and (b) proximal portion

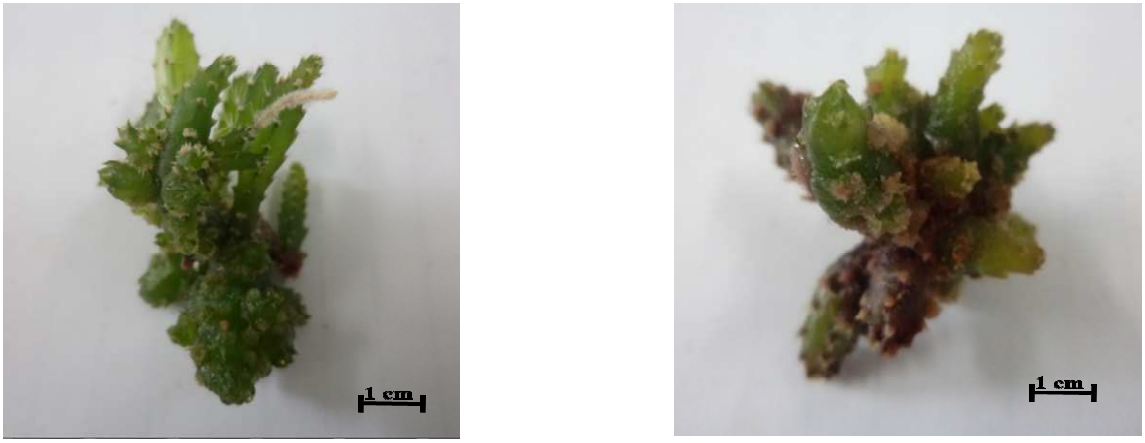


Plate 6. Drying and callus formation from some explants of dragon fruit crop in 0.5 µM naphthalene acetic acid (NAA) containing medium at 12 weeks after culture

4.3 Acclimatization of *In Vitro* Plantlets of Dragon Fruit

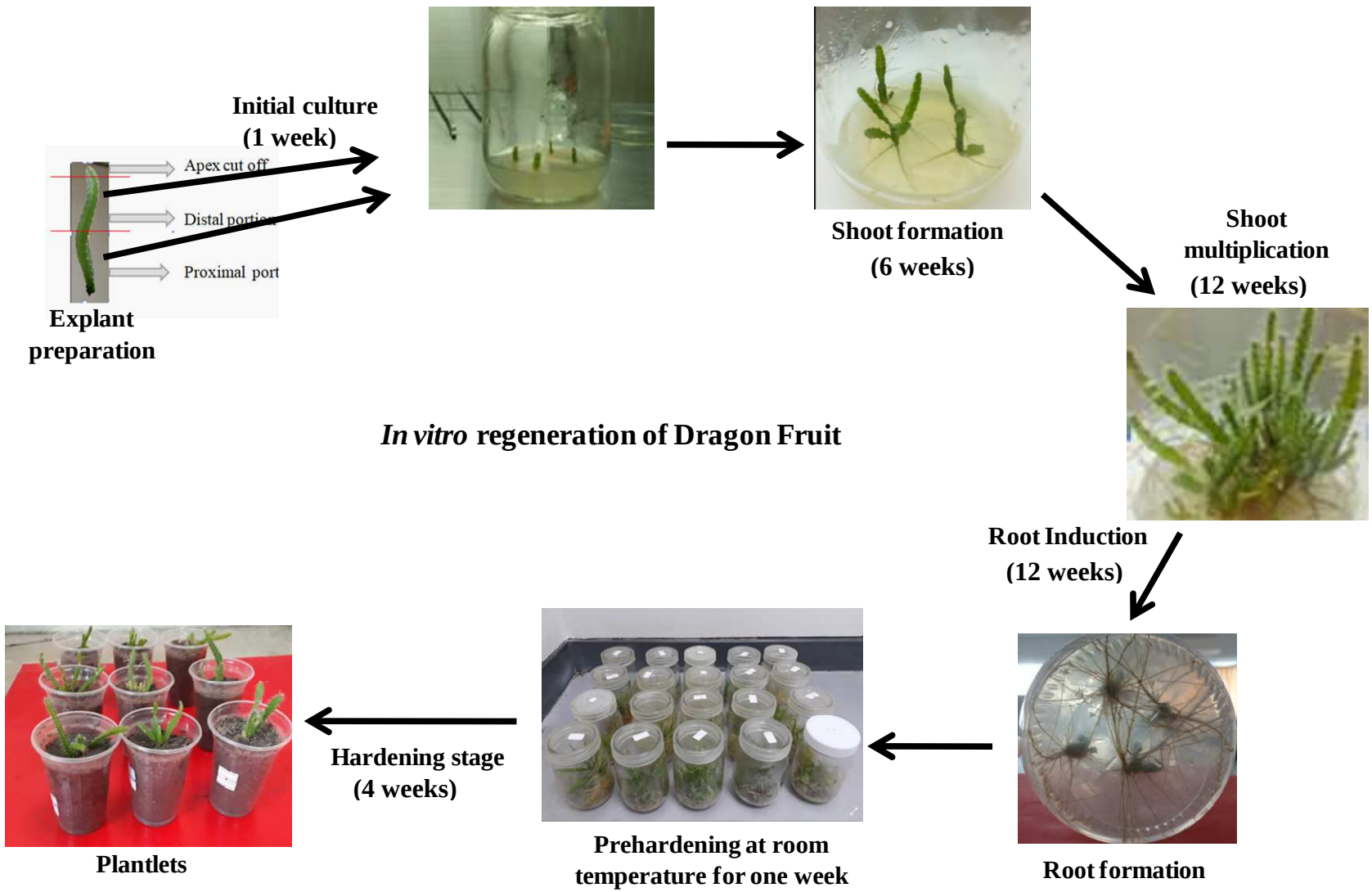
The plantlets derived from both explant types cultured on media with 0.3 and 0.5 µM naphthalene acetic acid (NAA) survived 100 % after root induction at 4 weeks after transplanting (Table 4.9).The plantlets cultured on hormone free media resulted in 98.25 % survival in distal portion and 98 % in proximal portion. Vinas et al. (2012) observed survival of more than 75 % of the *in vitro* derived *Hylocereus costaricensis* plants after 60 days in the greenhouse.

Although the plantlets derived from hormone free, 0.3 and 0.5 µM NAA did not differ on survival percent for both explant types, the medium with 0.3 µM NAA gave higher number of roots per plantlet, root length and fresh weight of plantlet than other treatments. It may be assumed that the plantlets cultured on 0.3 µM NAA containing medium gave higher performances in acclimatization of *in vitro* plantlets of dragon fruit.

According to Malda et al. (1999), plants with succulent stems and crassulacean acid metabolism (CAM) have positive characteristics to minimize water stress during acclimatization. Kluge et al. (2001) suggested that CAM metabolism after the *in vitro* phase might have afforded an advantage due to the high plasticity of CAM plants for carbon assimilation, which was reflected by better acclimatization performances.

Table 4.9 Survival percent of plantlets of dragon fruit crop derived from different root induction media containing 3 levels of naphthalene acetic acid (NAA) at 4 weeks after transplanting

NAA (µM) applied in root induction culture	Survival %	
	Distal portion	Proximal portion
0.0	98.25	98.00
0.3	100.00	100.00
0.5	100.00	100.00



***In vitro* regeneration of Dragon Fruit**

Figure 4.2 Establishment of *in vitro* regeneration of dragon fruit crop by shoot culture

CHAPTER V
CONCLUSION

For shoot multiplication, the proximal portion of *in vitro* shoot explants has more potential to produce shoot through *in vitro* multiplication than that of distal portion. On the other hand, N-6 benzyl aminopurine (BAP) level plays an important role in the number of shoots per explant and shoot length. It is recommended that 10 µM BAP containing medium is the best for the number of shoots per explant and shoot length in the present study.

For root induction, the explant types were not governed by the naphthalene acetic acid (NAA) levels in all parameters. The medium with 0.3 µM NAA gave higher performances in number of roots per explant, root length and plantlet fresh weight, whereas higher NAA level than 0.3 µM NAA tends to induce abnormal shoots and callus.

In addition, it can be suggested that further experiments should be carried out by using different kinds of explants such as a thin layer of young *in vitro* shoots and areoles and different genotypes of dragon fruit such as *Hylocereus polyrhizus* and *Selenicereus megalanthus*.

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APPENDIX

Appendix 1. Composition of Murashige and Skoog's medium (1962)

Components	Concentrations in medium ($mg.L^{-1}$)
NH_4NO_3	1650.00
KNO_3	1900.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.2H_2O$	370.00
KH_2PO_4	170.00
$FeSO_4.7H_2O$	27.80
NA_2EDTA	37.30
H_3BO_4	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.4H_2O$	8.60
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.03
$CoCl_2.6H_2O$	0.03
Nicotinic acid	0.50
Pyridoxin.HCl	0.50
Thiamine.HCl	0.10
Glycine	2.00
Myoinositol	100.00