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In vitro regeneration of exotic fruit dragon (*Hylocereus undatus*) from stem fraction

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Abstract

Hylocereus undatus, commonly known as dragon fruit, is one of the most popular exotic fruits due to its high quality and market value. This fruit is facing different constraints, such as loss of seed viability and various diseases that affect production because the seed and cutting methods are followed to create a new generation. *In vitro* plant regeneration is a promising technique to overcome these barriers with explants. An efficiency of this protocol was established in dragon fruit using MS + 2.0 mg/L BA + 2.0 mg/L Kin. with explants, stem fraction. Eighty percent explants were responded for shoot initiation treated with 2.0 mg/L BAP in combination with 2 mg/L Kin. The highest number of shoots and the maximum shoot lengths were observed in this medium inoculation after 90 days. Shoots were transferred to media containing ½ strength of MS with root induced PGRs. Ninety percent of the *in vitro* raised shoots were responded for rooting treated with 2.5 mg/L IBA inoculation after 30 days. The highest number of roots and the maximum root lengths were recorded at 2.5 mg/L IBA. The response of inoculated explants or *in vitro* raised shoots were positively correlated with the number of shoots and roots respectively. The average shoot and root lengths were negatively correlated with each other resulting in root lengths increasing by decrease of shoot lengths. The healthy shoots with well rooted plantlets were transferred to natural condition where about 80% plantlets were found to be acclimatized.

Keywords: Dragon fruit; Stem fraction; Regeneration; Pitaya; PGR

1. Introduction

Hylocereus undatus, is ranked second position after *Opuntia ficus-indica* with respect to fruit production commercially, a climbing vine cactus species belongs to the family of cactaceae and known as “dragon fruit” or “pithaya” or “pitaya” which is originated from Central and South America [1]. Now a days its grown all over the world especially tropical and subtropical region and become popularity in many countries for its own qualities such as highly decorative, unique appearance, refreshing taste and potential health benefit [2,3,4]. It is an exotic crop in Bangladesh and has started for cultivation commercially due to its high marketing value. The fruit is a bright red skin with green scales and white or red flesh with tiny black seeds. The juicy flesh of the fruit is delicious in taste and eaten chilled. The fruit is rich in vitamin C, B_2, E, Fe, Ca and P [2,5,6,7,8]. Previous research reported that this fruit do not contain cholesterol and saturated fats as well as responsible to control high sugar, manage blood pressure and cholesterol levels, prevent cancer and promote dental health [2,9]. Unsaturated fatty acids are highly present in seeds, reduce triglycerides and lower the risk of cardiovascular disorder [10,11,12,13]. The pulp of the fruit has antioxidant and polyphenol properties which could inhibit cancer cell growth [9, 14,15,16]. It is considered as night-flowering ornamental plants as well as a fruit crop, it will grow for about 20 years with a density of about 800 plants/ha [17,2]. It is mainly propagated by seeds or cuttings [18]. This traditional method of propagation damaged mother plant, slow release of propagules, create seasonal barrier, seeds produced are not true to type due to cross pollination and propagules carries insect-pest-diseases [2, 19, 18].

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Seeds are stored only for 28 days without losing viability and productivity and quality of the crop are also suffer due to lack of elite propagules and damping off disease [20]. *In vitro* mass propagation would be a viable and alternative technique to generate disease free propagules from elite clone. It is also useful for genetic transformation, creation of somaclonal variation, *in vitro* mutagenesis, conservation and international germplasm exchange. There are several reports available on *in vitro* regeneration of dragon fruit [21,22,23,3]. But most of them are lack of comprehensive information on large scale production of dragon fruit propagules.

Red pitaya also contains high amounts of flavonoids and phenolic acids from both flesh and peels, where these properties have healthy benefits for human diet and could be exploited for the formulation of nutraceuticals and food applications [24]. Pitaya seeds were contained high amount of essential fatty acids such as linoleic and linolenic which could be used as a new source of essential oil [25,26]. The fruit is also helps to boost up the immune system, promoting quicker recovery from wounds and bruises, and reducing the risk for respiratory problems [27]. Thus, the present investigation was conducted to develop an efficient *in vitro* regeneration protocol of dragon fruit using stem fraction as explant which will facilitate this valuable nutritious fruit cultivation in the country and future research and development as well.

2. Materials ad Methods

2.1. Plant materials collection and surface sterilization

Young stems, explants about 2 to 2.5 cm long and 1.0 cm diameter, were collected from the experimental field of plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh. The explants were washed thoroughly with liquid detergent trix. After that it was put under running tap water to eliminate the dust and surface contaminants. These were then transferred to sterile conical flask and surface sterilized with 0.1% $HgCl_2$ for 10 minutes under laminar air flow cabinet. The explants were then washed with sterile distilled water for 3 times.

2.2. Plant culture and growth condition

Sterilized explants were excised and cultured on Murashige and Skoog (MS) media supplemented with different concentration of cytokine such as Benzylaminopurine (BAP), Kinetin (Kin), Zeatin and Thidiazuron (TDZ) alone and combination with auxin like 1-Naphthaleneacetic acid (NAA) to observe the shoot initiation. Plants were grown in culture bottles at 25±2° C under white fluorescent light with 70-80% relative humidity under a 16 hrs light and 8 hrs dark photoperiod. Subcultures were done on each respective medium at 30 days interval for promoting strong and healthy multiple shoots. Good and healthy shoots were excised individually and transferred to ½ strength of MS media supplemented with different concentrations of Indole-3-butyric acid (IBA), NAA and indole-3-acetic acid (IAA) for root induction. The media contained 3% of sucrose (table sugar), 0.7% agar and the pH of the media adjusted to 5.8 prior to autoclaving.

2.3. Data collected and analysis

This experiment was carried in completely randomized design (CRD) with 10 replications and the experiment was repeated thrice. Data was recorded at regular intervals. Data were collected on different characters at day 90 for multiple shooting and at day 30 for rooting of *in vitro* raised shoots. All data were collected in this experiment for multi-way analysis of variance (ANOVA) was analyzed by Duncun`s Multiple Range Test (DMRT) using the statistical software SPSS ver.23. and Pearson's correlation coefficient test was calculated using Microsoft Excel 365. Observations on cultures were carried out daily. Each value represents mean with standard errors (SE).

3. Results and Discussion

In this investigation, different concentrations or in combinations of plant growth regulators (PGRs) were added to cultural media for the effectiveness of *in-vitro* mass propagation. Because the PGRs [28,29] with different concentrations and combinations as well as endogenous hormones [30] in the culture medium were affected on growth qualities of micro-propagules. Collected explants, stem fraction, 80% of inoculant were responded to initiate shoots on MS supplemented with 2.0 mg/L BAP in combination with 2.0 mg/L Kin comparing other concentrations or in combinations of PGRs (Fig.1 & Table1). The highest (8.2 ± 0.46) number of shoots were showed on MS media supplemented with 2.0 mg/L BAP in combination with 2.0 mg/L Kin where the lowest (1.5) shoots number was found in 1.5 mg/L BAP and 0.5 mg/L NAA suggesting shoot regeneration numbers of explants were 5.47-fold higher on former media combinations (Fig.1 & 2). These obtained results are partially consistent with the results of Feria *et. al*., 2012 demonstrated that 5.3-

fold more shoots were produced on MS medium supplemented with 0.2 mg/L NAA and 2.0 mg/L BAP in red/purple-fleshed pitaya *H. purpusii* refer to different genotypes or explants were used as an inoculant. The maximum (2.44 cm) shoot length was obtained on MS media supplemented with 2.0 mg/L BAP in combination with 2 mg/L Kin whilst it was lowest (0.93 cm) by supplemented with 2.0 mg/L Kin only. The shoot length (0.98 cm) was almost similar with last one added with 1.5 mg/L BAP and 0.5 mg/L NAA suggesting NAA could be suppressed the shoot lengths supporting the previous results of [30] Mauseth and Halperin, 1975 showed that explants organogenesis affected by types and concentrations of PGRs during tissue culture. On the other hand, numerous studies are documented on *in vitro* shooting of dragon fruit by BA + NAA of PGRs where they were used different parts as explants [3,31,19,32]. PGRs, in combinations with BA and Kin were found more effective for multiple shoot induction comparing to BA, Kin, TDZ and Zeatin alone. These obtained results indicate that the competence of media composition by PGRs along with explants and genotype triggers explants for maximum proliferation. Moreover, although [20,33,34,35] achieved better results using TDZ and Zeatin alone or in combinations with NAA or IBA those are partially in agreement with the present investigations.

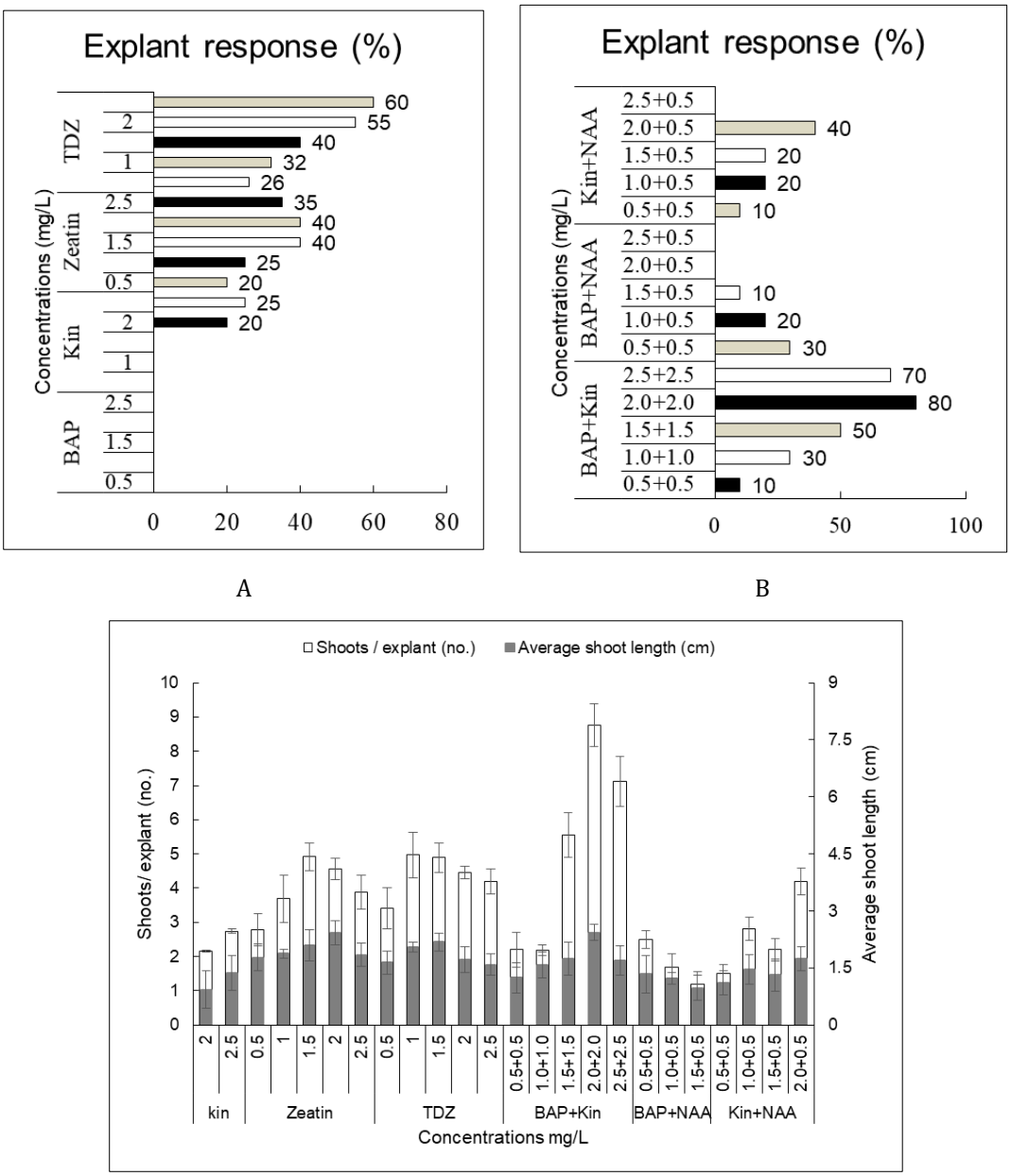


Figure 1 Effects of different concentrations and combinations of plant growth regulators (PGRs) on *in vitro* shoot induction from stem fraction explants inoculation after 90 days. Response of PGRs on inoculated explants (A & B) and effects of responded PGRs on inoculated explants (C).

Table 1 Effect of different concentrations and combinations of plant growth regulators (PGRs) on MS supplemented media for *in vitro* multiple shoot induction from stem fraction explants inoculation after 90 days

PGRs (mg/l)		**Shoot induced/explant (no.) (Mean ± SE)**	**Average shoot length (cm) (Mean ± SE)**
Kin	2.0	2.16±0.15^{d}	0.93±0.03^{e}
	2.5	2.74±0.23^{cd}	1.37±0.07^{d}
Zeatin	0.5	2.78±0.09^{cd}	1.77±0.90^{c}
	1.0	3.69±0.06^{c}	1.88±0.70^{c}
	1.5	4.92±0.16^{bc}	2.10±0.40^{b}
	2.0	4.56±0.07^{bc}	2.42±0.32^{ab}
	2.5	3.88±0.02^{c}	1.84±0.49^{c}
TDZ	0.5	3.42±0.12^{c}	1.64±0.80^{c}
	1.0	4.97±1.20^{bc}	2.05±0.67^{b}
	1.5	4.89±0.90^{bc}	2.18±0.44^{b}
	2.0	4.46±0.70^{bc}	1.73±0.19^{c}
	2.5	4.19±0.65^{bc}	1.59±0.36^{c}
BA + Kin	0.5+ 0.5	2.20±0.92^{d}	1.24±0.72^{d}
	1.0+ 1.0	2.19±0.22^{d}	1.58±0.16^{c}
	1.5+ 1.5	5.56±1.29^{b}	1.74±0.65^{c}
	2.0+2.0	8.76±1.34^{a}	2.43±0.62^{a}
	2.5+ 2.5	7.12±1.20^{ab}	1.69±0.72^{c}
BA+ NAA	0.5+ 0.5	2.5±0.56^{cd}	1.34±0.25^{d}
	1.0+ 0.5	1.7±0.32^{de}	1.23±0.39^{d}
	1.5+ 0.5	1.2±0.l4^{e}	1.41±0.36^{e}
Kin +NAA	0.5+ 0.5	1.5±0.21^{e}	1.11±0.26^{de}
	1.0+ 0.5	2.8±0.36^{cd}	1.46±0.34^{c}
	1.5+ 0.5	2.2±0.34^{de}	1.31±0.52^{c}
	2.0+ 0.5	4.2±0.56^{bc}	1.74±0.69^{bc}

Different alphabets indicate the significance calculated by DMRT at P≤ 0.05. Data were shown Mean ± SE, n=10.

Auxins such as NAA, IBA and IAA plays an important role for the initiation of adventitious root by *in vitro* raised shoots. The concentrations and combinations of these PGRs for root induction are basically dependent on the source of explants and genotypes as well. The maximum number (90%) of *in vitro* raised shoots with rooted were responded in ½ strength MS and 2.5 mg/L IBA 30 days after transferred to rooting media. The highest number of roots per *in-vitro* raised shoot was 6.9 found in ½ strength MS and 2.5 mg/L IBA whilst the lowest value was 2.58 in ½ strength MS and 1.0 mg/L IBA (Fig3 & Table2). These results suggested that shoots number were 2.67-fold higher in 2.5 mg/L IBA to 1.0 mg/L IBA by increasing concentration. And the maximum (6.36 cm) root length was observed in ½ strength MS and 2.5 mg/L IBA which was 2.9-fold higher (2.18 cm) compared to 1.0 mg/L NAA. Rooting response was found poor on NAA as well as IAA supplemented media and media with devoid of plant growth regulators. These findings are consistent with previous reported by [3,19] showed that IBA was more functioned with effectively than NAA for root induction. Besides, the superiority of IBA for *in vitro* rooting over other auxins has also been reported [36,37,38,39].

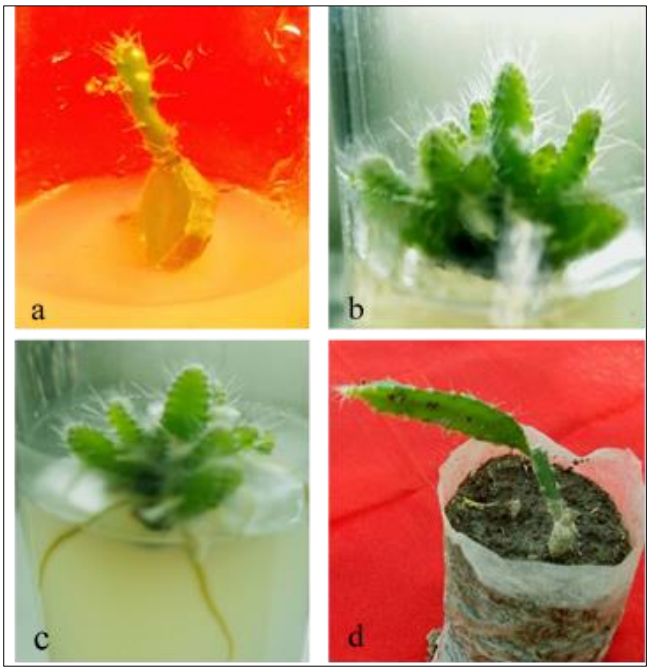
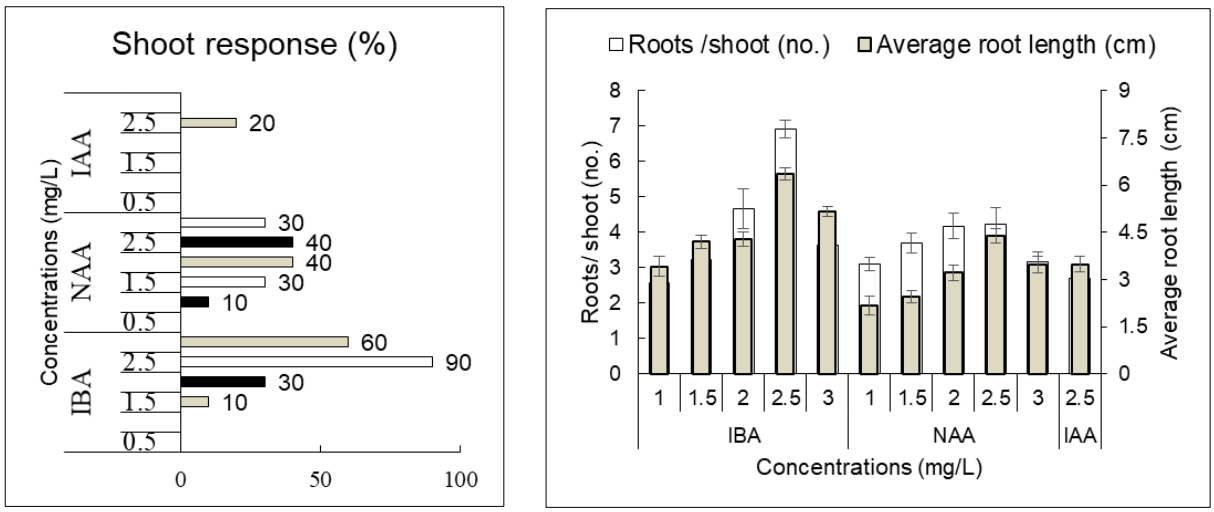


Figure 2 *In vitro* shoot initiation and plant regeneration from stem fraction explants of dragon fruit. Shoot initiation and multiple shoot proliferation on MS + 2.0 mg/l BA + 2.0 mg/l Kin after 1 and 3 months of culture medium respectively (a & b). Root induction on MS + 2.5 mg/l IBA after 1 month of culture medium (c). *In vitro* raised plant resumed new growth in the polybag (d).

The number of shoots and roots were positively correlated with shoot and root lengths respectively whereas the shoot length and root lengths were negatively correlated with each other (Table 3). These results suggested that roots were elongated by decreasing shoot length which is partially similar with the results of [40] demonstrated total shoot length was very poorly positive correlated with root length. This inconsistent was happened due to different types of PGRs such as activated charcoal. For the acclimatization, comparatively healthy rooted shoots were taken out from the culture vessels and washed the roots with gently under running tap water to get rid of agar. Then they were transferred to earthen pots containing a mixture of soil and compost (2:1) and covered with plastic films to maintain humidity for 5-7 days. The plantlets were kept in a shade and misted twice a day to adapt at the natural environment. About 90% plantlets were survived in outdoor condition. The protocol for *in vitro* regeneration method was developed in this study which will be useful for commercial cultivation as well as genetic improvement of this valuable exotic fruit plant.



A B

Figure 3 Effects of auxins for root induction by *in vitro* raised shoots on 1/2 strength of MS media inoculation after 30 days. Response of shoots by auxins (A) and effects of auxins on roots growth (B)

Table 2 Effect of different auxins and their concentrations for root induction of *in vitro* raised shoots of dragon fruit on half strength of MS supplemented media inoculation after 30 days

Auxins (mg/l)		Roots/shoot (no.) (Mean ± SE)	Average root length (cm) (Mean ± SE)
IBA	1.0	2.58±0.28^{d}	3.42±0.21^{bc}
	1.5	3.22±0.32^{c}	4.21±0.33^{b}
	2.0	4.67±0.39^{b}	4.29±0.56^{b}
	2.5	6.93±0.23^{a}	6.36±0.25^{a}
	3.0	3.65±0.46^{bc}	5.18±0.19^{ab}
NAA	1.0	3.12±0.26^{c}	2.18±0.19^{c}
	1.5	3.69±0.39^{bc}	2.46±0.28^{c}
	2.0	4.18±0.33^{b}	3.22±0.35^{bc}
	2.5	4.22±0.23^{b}	4.39±0.49^{b}
	3.0	3.16±0.43^{c}	3.47±0.28^{bc}
IAA	2.5	2.69±0.21^{d}	3.49±0.23^{bc}

Different alphabets indicate the significance calculated by DMRT at P≤ 0.05. Data were shown Mean ± SE, n=10.

Table 3 Pearson's correlation coefficients for *in vitro* plantlet growth characteristics at P≤ 0.05

	Shoots / explant (no.)	Average shoot length (cm)	Roots /shoot (no.)	Average root length (cm)
Shoots / explant (no.)	1			
Average shoot length (cm)	0.7843	1		
Roots /shoot (no.)	-0.0342	0.1905	1	
Average root length (cm)	-0.0078	-0.0370*	0.7061	1

*Indicate significantly different between the parameters.

4. Conclusion

In this study, we reported a protocol for mass regeneration of exotic fruit dragon. Adding different PGRs to media and evaluated the effectiveness on micropropagation in term of growth factors such as shoot length, root length of explants. We proved that MS medium supplemented with 2.0 mg/L BAP + 2.0 mg/L Kin. as well as ½ strength MS and 2.5 mg/L IBA were the best combinations and concentrations for shoot and root regeneration respectively. We also observed that spontaneous shoot development was decreased by root increasing on rooting media. These obtained results will be provided a new information as an efficient reference protocol for micropropagation of dragon fruits.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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