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Clonal Micropropagation of Purplish-Red Dragon Fruit (*Hylocereus costaricensis*) Newly Introduced To Egypt

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ABSTRACT

In the present study experiments were carried out to micropropagate *Hylocereus costaricencis* which is newly introduced to Egypt. Surface sterilized areole's sections (as a starting material) were subjected to various factorial concentrations of benzyladenine, kinetin and thidiazuron at 0.5, 1.0 and 1.5 mg/l added to MS culture media to induce shoot morphogenesis. The obtained shoots were rooted using indole butyric acid and/or naphthalene acetic acid individually, combined and with or without addition of activated charcoal. The results obtained revealed that cytokinins could successfully induce multiple shoot (mini-joints) morphogenesis and the response depended on their types and concentrations. After 12 weeks of treatment the number of shoots obtained using BA, Kin and TDZ at 1.5 mg/l reached up 13.67±2.03, 8.67±1.2 and 8.67±1.2 respectively in comparison with 1.0±0.58 shoots per control explant. The maximum number of rootlets 4.00±0.58 per shoot was obtained in response to the combination of IBA/NAA at 0.25mg/l for 12 weeks. The addition of charcoal (0.1%) reduced the number of rootlets from 4.00±0.58 to 3.00±0.58 with concomitant increase in the root length from 0.83±0.17 to 4.17±0.44. The rooted regenerants were transferred to pots containing a mixture of peat moss: sand: clay in the ratio of 1:1:1 with a 100% survival regardless of the type of treatments used for either shoot or root induction. The obtained results indicate that *in vitro* micropropagation of *H. costaricensis* can be used as a modern biotechnological tool to meet with the growing local market demand and help the spread of this newly introduced plant in Egypt.

Keywords:
Pitaya, Biotechnology, Tissue culture, *In vitro*.

1. INTRODUCTION

Dragon fruit plant (*Hylocereus spp.*) is generally a perennial climbing cactus species **[1].** the most common pitayos that being cultivated in many countries nowadays are the white dragon fruit (*Hylocereus undatus*); the pink dragon fruit (*Hylocereus sp*.); the red dragon fruit (*Hylocereus polyrhizus*); the purplish-red dragon fruit (*Hylocereus costaricensis*); and the yellow dragon fruit (*Hylocereus megalanthus*). Countries of the tropical climates of the North, South and Central America are the native motherlands of Pitayos **[2].**

Cultivation of the dragon fruit plants has spread over many countries in the world. According to Le Bellec **[3].** The European Union and Asia "especially China" are the largest import markets of pitaya). It is cultivated also in Vietnam, Colombia, Mexico, Costa Rica, and Nicaragua and to a lesser degree in Australia, Israel Reunion Island and Indonesia **[4].** For the last few years, it has been introduced to Egypt too.

The fruits of pitaya "dragon fruit" exhibit marked decorative, nutritional, health benefits, biological and therapeutic effects. **Bozkurt et al., (2020) [5]** mentioned that Pitaya attracts attention because of its uses as a fruit color, a source of valuable minerals, powerful antioxidant properties and valuable nutritional contents. Dragon fruits contain glucose, vitamins, organic acids, soluble dietary fibers, phyto albumins and minerals **[6, 7]** reported that betalain pigments which are abundant in dragon fruits are known to exhibit many bioactive effects including anti-inflammatory, hepatoprotective, hypoglycemic, cardiovascular protective effects, and anticancer properties. The antimicrobial and prebiotic potential of the fruits of pitaya were also reported by **[8,9]** who stated also that betacyanins which are natural food pigments rarely found in edible products were recorded to have antioxidant and anti-inflammatory properties, which may be helpful in preventing some human oxidative stress-related disorders. **Quiroz-Gonzalez [10] and Hussein [11**] additionally explained that the natural betalain pigments (nitrogen-rich group of pigments derived from tyrosine) found in pitaya are considered advantageous to anthocyanins. It is worthy to mention that the biological and therapeutic effects of pitaya fruits are not only associated with its famous and important secondary metabolite betalain but also may come back to the fruit contents of other flavonoids and phenolic acids **[12].**

Pitaya was regarded as a suitable candidate to Egypt's climate and has become one of the most important plants that introduced to Egypt. It is mainly cultivated by traditional means for commercial purposes. However, **Jeronimo [13]** stated that these edible plants are very promising for horticulture in different regions of the world because many representatives of that genus "*Hylocereus"* can tolerate extreme environmental factors like cold, heat, dryness and deficiency in soil essential nutritional compounds. The success of introducing pitaya to Egypt's climate may come back to these unique characteristics.

The growth of pitayos is influenced by temperature, humidity, soil conditions and rainfall and that they are known to grow well in arid and semi-arid regions and can withstand long periods of drought because they can use water five to ten times more efficient than regular crops. This characteristic is due to CAM

metabolism (Crassulaceae-type metabolism) of dragon fruit plants which tend to open its stoma (overnight) for CO_2 fixation to avoid water loss by the day. Thickness of the plant skin (14 mm) is another adaptation too **[14].**

There are some reports that traditional propagation of pitaya is not commercially feasible **[3,15].** Traditionally, Dragon fruit plants are propagated sexually by seeds or by vegetative cuttings. Unfortunately the propagation by seeds results in plants that have long juvenile periods and delayed fruit production for several years and the propagation of pitaya by cuttings from field plants results in low multiplication rates due to the deficiency of starting mother plant material (about 50 cm length cuttings) **[3]** and this is why declared that such propagation is not commercially feasible while biotechnological tools like plant tissue culture (which is adopted in this study) is considered as a promising tool to overcome the many disadvantages of traditional propagation. Plant tissue culture is regarded generally advantageous to traditional breeding in creating new cultivars because it may avoid many of the problems associated with traditional breeding like exhausting hybridization processes, continuous selection, limited gene pool and long juvenile phase before reproductive inflorescences emerge, which finally result in a slow pace of new cultivar development **[16].**

The present work may represent, at least, one of the earlier publications on micropropagation of dragon fruit in Egypt and an additional source of information that may facilitate spreading its cultivation by providing cultivable plant material for the marked growing local market.

2. Materials and methods

This study was conducted in Tissue Culture Res. Lab., Botany and Microbiology department, Faculty of science, Al-Azhar University, Cairo, Egypt.

1- Plant material: The mother Dragon fruit plant (*Hylocereus costaricensis*), from which explants were taken was obtained from the green house (about 1 meter length), Agricultural Research Institute, Dokky, Giza, Egypt.

2- Surface sterilization of explants: Areoles segments of 5 cm length were surface sterilized according to Chawla **[17]** by submerging the explants into a solution of 70% ethanol with continuous and gentle stirring for one min, transferring them to 100 ml conical flask containing 20% solution of commercial sodium hypochlorite with continuous gentle stirring for seven min. The sterilant was decanted and the explants were washed with three successive rinses of sterile distilled water under aseptic conditions. The explants were then dried between two layers of sterile filter papers in a Petri dish. Using sterile scalpel, the explants were cut into smaller pieces (2.5 cm length) containing buds.

3- Culture medium: The basal salts mixture of MS medium containing 30 g L^{-1} sucrose, solidified with 7.0 g L^{-1} agar and pH adjusted to 5.7 **[18]**.

4- Treatments: For *in vitro* shoot (mini or micro-joints) induction, each of benzyladenine (BA),

kinetin (Kin) and thidiazuron (TDZ) was added to MS culture media at 3 concentrations (0.5, 1.0 and 1.5 mg/l) to induce multiple shoot morphogenesis from explants. Explants were subjected to cytokinin treatments for 12 weeks. Percentage of response and the number of shoots per explant was counted after 2, 4, 6, 8, 10 and 12 weeks. After that individual mini-joints were excised and transferred under aseptic conditions to the root inducing media. For *in vitro* root morphogenesis of the obtained mini-joints, MS culture media was impregnated with indole butyric acid (IBA) at a concentration of 0.5 mg/l, naphthalene acetic acid (NAA) at a concentration of 0.5 mg/l and a mixture of both IBA and NAA (0.25mg/l each) with and without the addition of charcoal at 1, 2 and 3%. The rooting treatment lasted for 8 weeks and after that regenerants were taken out of jars, washed and transferred to pots for acclimatization after counting the number of rootlets obtained and measuring the average root length.

5- Cultural conditions: Cultures were generally incubated in growth room under controlled conditions, where temperature was maintained at 25 ± 1 °C, day/night schedules 16/8 at and low light intensity of 1500 lux using white cool fluorescent lamp 120 cm long 40 watts.

6- Acclimatization: Rooted plantlets were taken out of the growth jars, transferred to pots containing (peat moss: sand: clay in a ratio of (1: 1:1 v/v). No any special measures were needed for acclimatization which reached almost 100% success.

Statistical analysis: Data was analyzed using Minitab 20 and Excel 365. Descriptive statistics including mean, Standard error (SE) have been calculated from three replicates for each level. Inferential statistics have been used to compare results of different groups. Different comparisons were done using one-way and Two-way analysis of variance (ANOVA) under fit General linear model. P value were considered significant at $\alpha < 0.05$. Post hoc analyses of the interactions among all groups were done using Tukey test for pairwise comparisons. Results of the post hoc analyses are represented as letters where groups that share same letters are non-significantly different while different letters express significant differences among different groups

3- Results and Discussion

1- Effect of different cytokinins on shoot proliferation of pitaya *in vitro*

This part of the work aimed at detecting the effect of different cytokinins (Benzyladenine, Kinetin and Thiadiazhuron) individually on inducing or proliferation of New mini-joints from pitaya explants *in vitro*. Results were recorded for 12 weeks.

With respect to benzyladenine (BA), the results presented in table (1) and Plate (1) revealed that BA accelerated and increased the percentage of response of the explants and increased the number of induced New mini-joints. The results depended also on the concentration of BA used. Beside acceleration of response (which started after just two weeks of incubation), BA at a concentration of 0.5 mg/l resulted in a statistically significant increase in the number of induced New mini-joints which reached up to 7.67±0.88 per explant. Increasing BA concentration to 1.0 mg/l increased (at the end of incubation period) the number of proliferated New mini-joints to 4.33±0.88. On the other hand, the

increase in BA in the culture media up to 1.5 mg/l was accompanied by an increase in the number of New mini-joints up to 13.67±2.03 per explant. This value is probably 13 folds the corresponding control and was statistically significant over almost all the other obtained values.

Regarding the effects of kinetin, the results given in table (2) and Plate (2) may show that all this cytokinin concentrations used (0.5, 1.0 and 1.5 mg/l) exhibited some positive results with respect to both the percentage of response of explants and the number of New mini-joints obtained per single mother joint section explant. The maximum percentage of response obtained by control explants was 66.6% recorded after 8 weeks of incubation while all the used kinetin concentrations could increase that percentage up to 100 from the 10^{th} week of incubation. By the end of the incubation period (12 weeks), the number of proliferated of New mini-joints reached up 9.00 ± 1.15, 10.33 ± 1.45 and 8.67 ± 1.20 per explant in response to treatment with 0.5, 1.0 and 1.5 mg/l kin respectively. These results although statistically significant over the corresponding control, they are insignificant if compared to each other.

With respect to thidiazuron (TDZ), the results illustrated in table (3) and Plate (3) may show that TDZ exhibited positive results on both the percentage of response and proliferation of New mini-joints. Beginning of proliferation in control explants started only after 8 weeks of incubation and reached up 66.6%. Incorporation of TDZ in culture media accelerated response of explants (which from the second week instead of the 8^{th} week) and increased the percentage of response from 66.6% to 100%. It could be observed also from the same table and the same Plate that the explants treated with 1.5 mg/l of the tested cytokinin gradually, with passage of time, proliferated new mini or micro-joints. By the end of the incubation period, the number of proliferated New mini-joints increased from 1.00 ±0.58 per explant to 8.67±1.2. Statistical analysis proved that the obtained numbers are statistically significant over the corresponding control. Although the number of newly formed New mini-joints (5.00 ±1.15 per explant) using 0.5 mg/l TDZ is smaller than the number obtained with the higher concentration used it is still considered statistically also significant over the corresponding control too.

The exogenous application of different cytokinins such as BA and TDZ has become obligatory for induction of multiple shoot in many plants **[19]**. The results obtained in this study strongly agree with that concept and also what was previously stated **[20]** that the addition of plant growth regulators to the culture media individually or combined results in maintenance of specific and balanced organic and inorganic components in the cultured tissue leading to subsequent development of shoots, roots or even death of the explant. The general observations made in this study "as whole" may also agree and confirm what was stated by **[21, 22]** that micropropagation can be a useful tool that may replace traditional propagation to avoid limitation of starting mother plant material, to accelerate propagation cycles, to maintain the genetic fidelity and shorten the propagation periods from several months to a few weeks and to obtain disease-free plants. **Vinas** [**23]**, in particular, mentioned that Limitations to large-scale propagation of purple pitahaya (*Hylocereus costaricensis* [F.A.C. Weber] Britton & Rose) can be overcome through utilization of *in vitro* culture technologies. Regardless of some differences, the results obtained in this study may be more or less similar to some earlier successful trials to micropropagate

Cactus - in general- like **[24, 25, 26]** and different Dragon fruit species - in particular- like (white-fleshed pitaya "*H. undatus"* **[27, 28],** purple pitaya "*H. costaricensis"* **[23],** *Hylocereus undatus* **[29],** genetically diverse pitaya **[30]**, red pitaya "*Hylocereus polyrhizus*" by **[31, 32]** and different pitaya cultivars **[5].** Working, for example, with cactus (Opuntia *Ficus indica***) [24],** The MS medium supplemented with plant growth regulators (benzyladenine, kinetin, and naphthalene acetic acid NAA) exhibited some positive effects on the shoot development and that the highest shoot multiplication rate was recorded in MS medium supplemented with 0.5 mg/l BA. The results and observations of this study may generally agree with **[25, 26]** that a number of cactus species have been found to regenerate entire plants by activating meristematic cells in the areoles via cytokinins alone or in combination with low levels of auxins **[26].** explained that an areole is a highly specialized axillary bud that contains meristematic tissues and that new shoots and lateral branches in cactus originate from these structures. Areoles are unique structures in these plants that also produce spines, trichomes and flowers. When cactus tissues are cultured *in vitro* in the presence of cytokinins, occasionally in combination with low concentrations of auxins, areoles may sprout and form one or multiple new shoots. After being separated from the original tissue, these shoots may be rooted and grown into new plants, which is a simple and fast system for propagating cactus species *in vitro.*

Working with the red fleshed pitaya *"Hylocereus undatus"* **[27]**, reported that only intact areoles produced axillary shoots. **[28],** working with the white fleshed pitaya, successfully induced axillary shoots using cytokinins too. Although **[23]** failed to obtain axillary shoots using TZD, this was possible to obtain in the present study successfully using different TDZ concentrations from 0.5 to 1.5 mg/l as described before. However, a number of reports have shown that different genotypes (varieties) require different specific media compositions to support optimal growth **[31].** In a more or less similar experiment to the present study could obtain a maximum of 4.31 shoots per explant after 12 weeks of treatment with 20 µM BA compared to 7.67 shoots per explant using 0.5 mg/l BA and 5 shoots per explant using 0.5 mg/l TDZ and 10.33 shoots per explant using 1 mg/l Kin. However, this difference may be explained on the lights of what **[5]** concluded after their experiments with different pitaya cultivars where they found out that the highest value of the multiplication coefficient (5.41) was observed in (Halley's Comet) variety cultivated in MS medium supplemented with 2.0 mg/l BAP while the lowest multiplication coefficient value (1.84) was recorded in "Bloody Mary" cultivar growing in MS medium supplemented with the same cytokinin type and concentration. Actually, beside the cultivar-dependent effects, the type and concentration of the cytokinin applied may have played a great role.

Table (1): Effect of BA on pitaya cultured *in vitro* on MS culture media with respect to percentage of response of explants (%) and the mean number of newly proliferated mini -joints per single explant. Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at $p \geq 05$. All treatments are compared to each other in both columns and rows.

BA mg/l	Weeks of incubation											
	2		4		6		8		10		12	
	%	M	%	M	%	M	%	M	%	M	%	M
0	0 %	0.00 ± 0.00^{G}	0 %	0.00 ± 0.00^{G}	0 %	0.00 ± 0.00^{G}	66.6%	1.00 ± 0.58^{EFG}	66.6%	1.00 ± 0.58 ^{EFG}	66.6%	1.00 ± 0.58 ^{EFG}
0.5	100%	2.00 ± 1.00^{EFG}	100%	2.67 ± 0.88^{EFG}	100%	3.33 ± 0.88^{DEFG}	100%	3.33 ± 0.88^{DEFG}	100%	5.33 ± 0.33^{BCDEF}	100%	7.67 ± 0.88^{BCD}
1.0	66.66%	0.67 ± 0.33 ^{FG}	100%	1.00 ± 0.57^{EFG}	100%	2.67 ± 0.88^{EFG}	100%	3.00 ± 0.58^{DEFG}	100%	3.00 ± 0.58 ^{DEFG}	100%	4.33 ± 0.88^{CDEFG}
1.5	100%	1.67 ± 0.6^{EFG}	100%	4.00 .± 1.15^{CDEF}	100%	5.67 ± 1.2^{BCDE}	100%	8.67 ± 1.45 ^{BC}	100%	10.00 ± 1.73^{AB}	100%	13.67 ± 2.03^{A}

Weeks	BA mg/l			
	0.0	0.5	1.0	1.5
2				
4				
6				

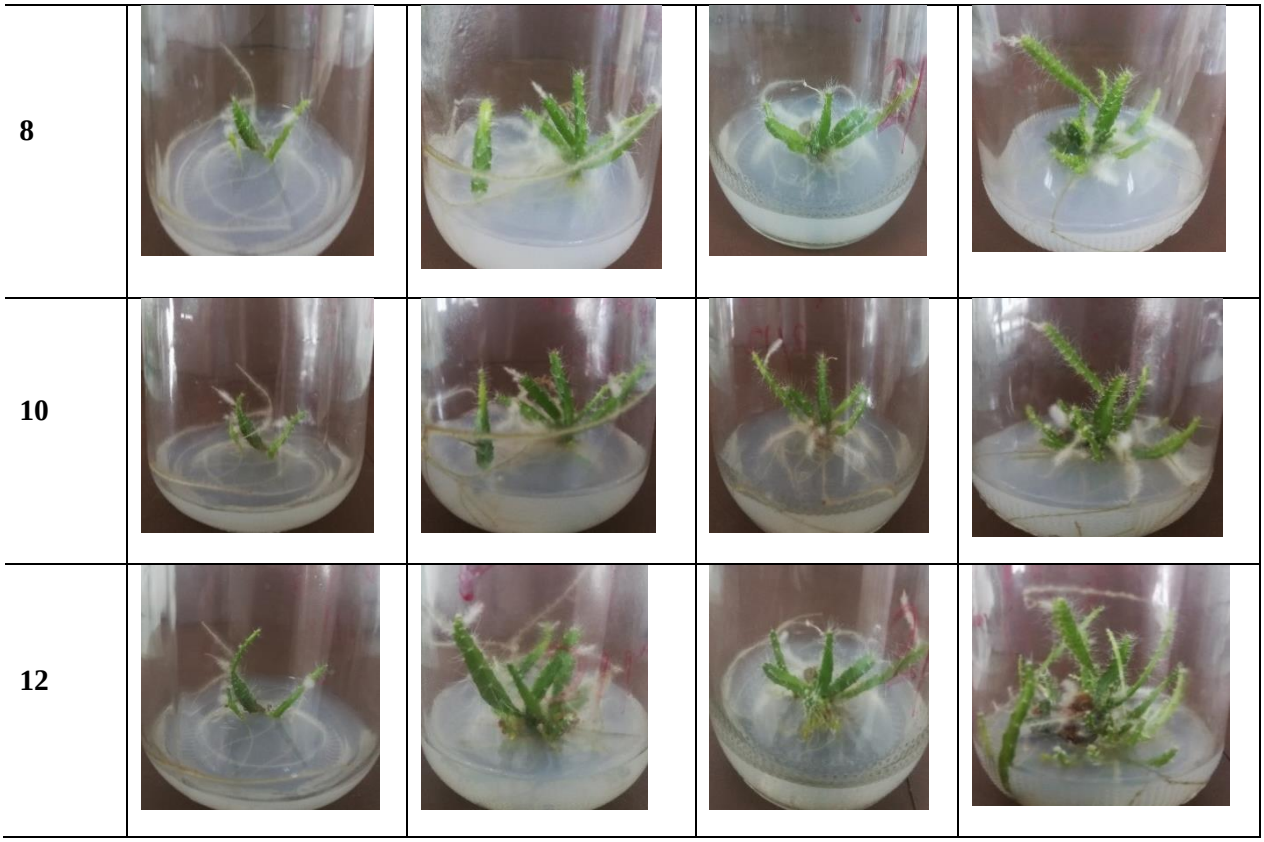


Plate (1): Effect of different BA concentrations on explants of pitaya over a period of 12 weeks

Table (2): Effect of Kinetin "Kin" on percentage of response of explants "%" and the mean number of newly proliferated mini -joints per single explant of pitaya cultured in vitro on MS culture media.

Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at p ≥05. All treatments are compared to each other in both columns and rows.

Kin mg/l	Weeks of incubation											
	2		4		6		8		10		12	
	%	M	%	M	%	M	%	M	%	M	%	M
0	0 %	0.00 ± 0.00^{F}	0 %	0.00 ± 0.00^{F}	0 %	0.00 ± 0.00^{F}	66.6%	1.00 ± 0.58^{EF}	66.6%	1.00 ± 0.58 ^{EF}	66.6%	1.00 ± 0.58^{EF}
0.5	33.33%	0.67 ± 0.6^{EF}	33.33%	2.00 ± 1.00^{DEF}	66.66%	2.67 ± 1.20^{DEF}	66.66%	4.33 ± 1.45^{BCDEF}	100%	6.67 ± 1.20^{ABCD}	100%	9.00 ± 1.15^{AB}
1.0	66.66%	1.00 ± 0.58^{EF}	100%	2.33 ± 0.88^{DEF}	100%	4.00 ± 1.00^{BCDEF}	100%	5.67 ± 1.20^{ABCDE}	100%	8.67 ± 1.45^{ABC}	100%	10.33 ± 1.45^{A}

1.5	**33.33%**	**0.33 ± 0.33^{F}**	**66.66%**	**1.00 ± 0.58^{EF}**	**66.66%**	**2.00 ± 0.58^{DEF}**	**66.66%**	**3.67 ± 1.20 ^{CDEF}**	**100%**	**5.67 ± 1.20^{ABCD}**	**100%**	**8.67 ± 1.20^{ABC}**

Weeks	**kin mg/l**			
	0.0	**0.5**	**1.0**	**1.5**
2				
4				
6				
8				
10				

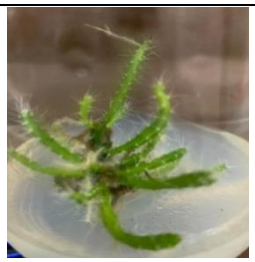
12				

Plate (2). Effect of different kin concentrations on explants of pitaya

Table (3): Effect of TDZ on percentage of response of explants "%" and the mean number of newly proliferated mini or micro-joints per single explant of pitaya cultured in vitro on MS culture media

Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at p ≥05. All treatments are compared to each other in both columns and rows.

TDZ mg/l	Weeks of incubation											
	2		4		6		8		10		12	
	%	M	%	M	%	M	%	M	%	M	%	M
0	0 %	0.00 ± 0.00^{E}	0 %	0.00 ± 0.00^{E}	0 %	0.00 ± 0.00^{E}	66.6%	1.00 ± 0.58^{DE}	66.6%	1.00 ± 0.58^{DE}	66.6%	1.00 ± 0.58^{DE}
0.5	100%	3.33 ± 0.88^{BCDE}	100%	3.33 ± 0.88^{BCDE}	100%	3.33 ± 0.88^{BCDE}	100%	4.00 ± 1.15^{BCD}	100%	4.33 ± 0.88^{BCD}	100%	5.00 ± 1.15^{ABC}
1.0	100%	1.00 ± 0.00^{DE}	100%	1.33 ± 0.33^{CDE}	100%	1.33 ± 0.33 ^{CDE}	100%	1.67 ± 0.67^{CDE}	100%	1.67 ± 0.67^{CDE}	100%	2.00 ± 0.58^{CDE}
1.5	100%	2.67 ± 0.88^{BCDE}	100%	4.00 ± 0.58^{BCD}	100%	5.00 ± 0.58^{ABC}	100%	6.00 ± 0.58^{AB}	100%	6.00 ± 0.58^{AB}	100%	8.67 ± 1.2^{A}

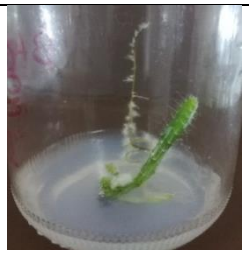
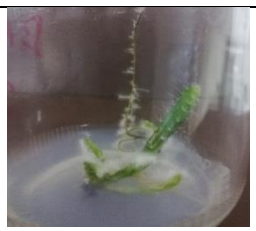
Weeks	TDZ mg/l			
	0.0	0.5	1.0	1.5
2				
4				
6				
8				
10				
12				

Plate (3): Effect of treatment of pitaya explants with different TDZ concentration

The results illustrated in table (4) and Plate (4), represent a comparison of the effects of the adopted cytokinins and their different concentrations on the number of newly induced mini or micro-joints per single mother explant at the end of the incubation period (12 weeks). It may be concluded that using 1.5 mg/l of BA is probably the best concentration in inducing mini or micro-joints (13.67±2.03). This value is significant over the control group and most of the applied cytokinin concentrations. However it should be taken into consideration that not only the number of microjoints obtained is important but also their subsequent survival matters.

Table (4): Comparison between the effects of different concentrations of TDZ, BA and Kin on induction of mini or micro-joints from pitaya explants at the end of treatment period (12 weeks). Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at p ≥05. All treatments are compared to each other in both columns and rows.

Concentration	Plant growth substances		
	BA	Kin	TDZ
0.0	**1.00±0.58 ^{E}**	**1.00±0.58 ^{E}**	**1.00±0.58 ^{E}**
0.5mg/l	**7.67±0.88 ^{BCD}**	**9.00±1.15 ^{ABC}**	**5.00±1.15 ^{BCDE}**
1.0 mg/l	**4.33±0.88 ^{CDE}**	**10.33±1.45 ^{AB}**	**2.00±0.58 ^{DE}**
1.5 mg/l	**13.67±2.03 ^{A}**	**8.67±1.2 ^{ABC}**	**8.67±1.2 ^{ABC}**

	Plant growth substances		
	BA	**Kin**	**TDZ**
0.0 mg/l			
0.5 mg/l			
1.0 mg/l			
1.5 mg/l			

Plate (4): Comparison between the effects of different concentrations of BA, Kin and TDZ on induction of mini -joints from pitaya explants at the end of treatment period (12 weeks).

2-Rooting of the shoots of pitaya obtained *in vitro*

For this purpose, the clusters of shoots were cut into individual ones and transferred under aseptic conditions to Murashige and Skoog's culture media **[18]** that was impregnated with 0.5 mg/l IBA, 0.5 mg/l NAA and a mixture of them at 0.25 mg/l each with or without the addition of activated charcoal from 0.1% to 0.3%. Regenerants with their obtainable roots were detached from jars after 12 weeks of treatment and transferred to acclimatization pots after the required measurements were taken.

First of all, it has been observed that although shoots were excised as individual ones for rooting, shoot formation continued to appear parallel to root formation. This is probably due to the residual amounts of cytokinins present in the shoots (mini-joints) from the previous cytokinin treatments or due to the new hormonal balance created between residual cytokinins and the freshly applied auxins. This means that the omission of cytokinins and replacing them with auxin did not hinder further shoots proliferation. It is to be taken into consideration that [**25,26]** declared that cytokinins alone or in combination with low levels of auxins can trigger meristematic cells of the areoles to regenerate shoots. However, the results obtained show that the number of rootlets produced by pitaya regenerants obtained *in vitro* ranged between 0.33±0.33 and 4.00±0.58. In the absence of charcoal, it was observed that IBA (0.5 mg/l) and NAA (0.5 mg/l) although resulted in an increase in the number of rootlets obtained, this increase was statistically insignificant while on the other hand the number of rootlets obtained when the two auxins (IBA and NAA) were combined, the increase in the number of rootlets obtained was statistically significant (table 5 and Plate 5).The average root length under the same treatments ranged between (1.17±0.17 cm and 0.83±0.17 cm) did not show any significant difference from the corresponding control group regenerants as illustrated in table (6) and Plate (5). In case of the addition of charcoal to the culture media and except for the treatment with IBA/NAA 0.25 mg/l each in addition to 0.1% activated charcoal, all the other treatments exhibited insignificant effects on the number of rootlets obtained by regenerants (table 5 and plate 5). However, it has been observed that the increase in the number of rootlets induced was accompanied by a decrease in the average root length and vice versa (table 5, 6). From the results obtained, it may be understood that the combination of IBA/NAA 0.25 mg/l each with or without the presence of activated charcoal (0.1%) is the appropriate treatment for rooting of pitaya mini-joints obtained *in vitro*.

The success of a micropropagation protocol depends strongly on the rooting efficiency of regenerated shoots and their subsequent acclimatization to the field condition **[34].** Rooting without exogenous application of PGRs to the culture media is sometimes much more preferable over auxin-induced rooting of the obtainable shoots because spontaneous rooting reduces the time needed for the rooting stage of the *in vitro* life **[23].** The roots induced in vitro under the effect of auxin application, although sometimes are not functional but they are needed to allow regenerants to survive the transplantation process and they usually recover faster than plants that still have to form roots **[5 ,35]** after their studies on pitaya came to the conclusion that the best medium for rooting was MS medium supplemented with 1 mg/l IBA.

Table (5): Effect of IBA, NAA alone or combined together or with charcoal on the number of roots pitaya regenerants. Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at p ≥05. All treatments are compared to each other in both columns and rows.

PGRs				
Charcoal percentage	**No PGRs**	**IBA 0.5 mg/l**	**NAA 0.5 mg/l**	**IBA/NAA 0.25 mg/l each**
0.0	**0.33±0.33 ^{C}**	**1.67±0.67 ^{BC}**	**1.33±0.33 ^{BC}**	**4.00±0.58 ^{A}**
0.1%	**1.67±0.67 ^{BC}**	**1.67±0.67 ^{BC}**	**2.00±0.58 ^{ABC}**	**3.00±0.58 ^{AB}**
0.2%	**1.00±0.00 ^{BC}**	**1.33±0.33 ^{BC}**	**2.67±0.33 ^{ABC}**	**2.33±0.33 ^{ABC}**
0.3%	**2.00±0.58^{ABC}**	**1.33±0.33 ^{BC}**	**2.00±0.00 ^{ABC}**	**2.33±0.33 ^{ABC}**

Table (6): Effect of IBA, NAA alone or combined with charcoal on average root length of pitaya regenerants. Each value is a mean of 3 determinations ± SE. Dissimilar letter (s) means a significant value at p ≥05. All treatments are compared to each other in both columns and rows.

PGRs				
Charcoal Percentage	**No PGRs**	**IBA 0.5 mg/l**	**NAA 0.5 mg/l**	**IBA/NAA each 0.25 mg/l**
0.0	**1.17±0.17 ^{C}**	**1.33±0.33^{C}**	**1.17±0.17^{C}**	**0.83±0.17^{C}**
0.1%	**1.17±0.17 ^{C}**	**1.00±0.00^{C}**	**1.00±0.00^{C}**	**4.17±0.44^{A}**
0.2%	**1.33±0.33^{C}**	**1.08±0.22^{C}**	**0.91±0.17^{C}**	**1.33±0.33^{C}**
0.3%	**0.67±0.17 ^{C}**	**1.50±0.29^{BC}**	**1.08±0.08^{C}**	**2.83±0.60^{AB}**

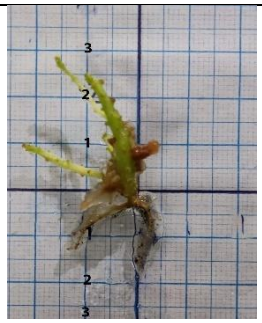
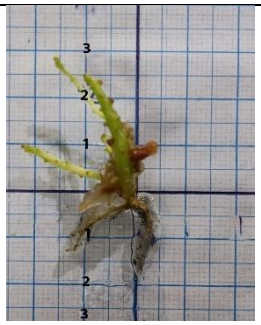
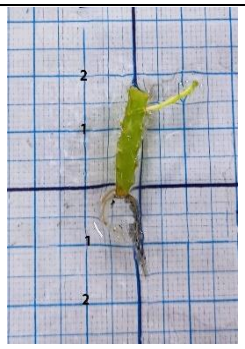
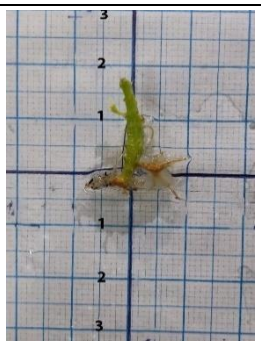
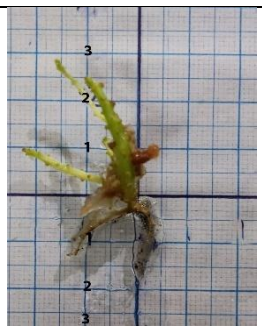
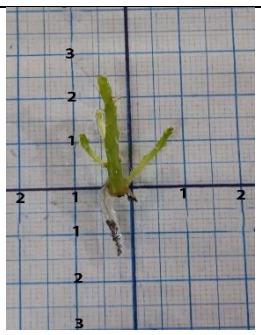
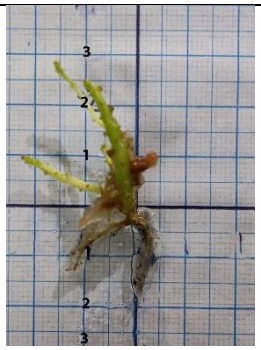
Charcoal	0	IBA 0.5mg/l	NAA 0.5mg/l	IBA 0.25mg/l + NAA0.25mg/l
Zero				
1%				
2%				
3%				

Plate . (5): Effect of different rooting treatments on pitaya shoots obtained *in vitro*

3-Acclimatization

The obtained plantlets with their developed roots were transferred to pots containing peat moss /sand/clay in the ratio of 1:1:1. All plantlets were successfully acclimatized easily. No further specific treatments were needed, when these plantlets reached 7-9 centimeters, they were transferred to larger pots (25 cm diameter) where they continued to grow for about 6 month and reached about 30 centimeters (Plate 6). **[36]** reported that the acclimatization of both red and yellow pitayos obtained *in vitro* by organogenesis reached up 100%.

February April June

Plate 6: Acclimatized pitaya plants

4. CONCLUSION

The present study indicates that purplish red Dragon fruit (*Hylocereus costaricensis*)which introduced to Egypt can be micro-propagated succefully *in virto* using different cytokinins for new mini joint proliferation and rooted using Auxins in combination with activated charcoal. The obtained propagules could succefully be acclimatized and transferred to pots to continue growth.

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