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ASSESSMENT OF HORMONAL EFFECT ON *IN VITRO* RESPONSE OF DRAGON FRUIT (HYLECEREUS UNDATUS)

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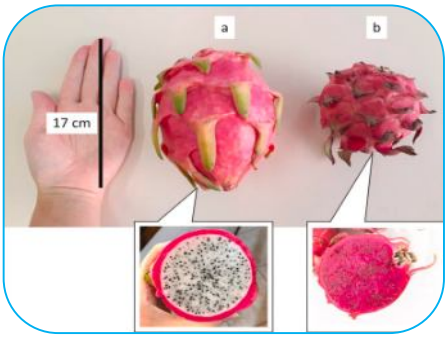
ABSTRACT

This study was carried out to find out the effect of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l). The shoot tips of Dragon plants were used as explants which were sterilized with 70% ethanol and 0.1% HgCl2. The highest percentage of shoot induction (76.00%) was observed in MS medium with BA 2.0 mg/l in minimum 12.2 days. The maximum number of shoot (3.20) and the highest shoot length (4.02 cm) were obtained at 42 days with the treatment BA 2.0 mg/l and BA 2.50 mg/l respectively. In this study, feasible protocol has been developed for in vitro rapid regeneration of Dragon fruit having potential application in large scale multiplication for commercial purpose.



KEYWORDS: *BA, dragon fruit, in vitro culture.*

INTRODUCTION

Dragon fruit belongs to family Cactacea and grows best in dry, tropical or subtropical climates. The fruit of this crop is popular and eaten chilled. It is also used to flavor drinks and pastries. Unopened flower buds are cooked and eaten as vegetables (www.tradewindsfruit.com)

Dragon fruit, a recently introduced super fruit in India, is considered a promising, profitable fruit crop. The fruit has a very attractive color and a soft, melt-in-your-mouth pulp with black edible seeds embedded in the pulp along with immense nutritional value that attracts growers from different parts of India to cultivate this fruit crop that is native to Mexico. and Central and South America. (Britton and Rose, 1963; Morton, 1987 and Mizrahi et al., 1997). It is a long-day plant with a beautiful night-blooming flower that is nicknamed "Noble Woman" or "Queen of the Night". The fruit is also known as strawberry pear, dragon fruit, pitaya, night blooming cereus, night bell, Cinderella herb and Jesus in the cradle. The fruit is called pitaya because of the bracts or scales on the rind of the fruit and hence the name pitaya which means "scaly fruit". It has an ornamental value due to the beauty of the large flowers (25 cm) that bloom at night; they are creamy white in color. It is considered as a fruit crop

for the future (Gunasena and pushpakumara, 2006 and Gunasena et al., 2006). The fruit comes in three types, all with slightly leafy, leathery skin: Hylocereus undatus—white flesh with pink skin, Hylocereus polyrhizus—red flesh with pink skin, Hylocereus costaricencis—with violet-red flesh and pink skin, and Hylocereus (Selenicerus) megalanthus —White meat with yellow skin. The biggest advantage of this crop is that once planted, it will grow for about 20 years, and 1 hectare could house about 800 pitahaya plants. It is grown commercially in Israel, Vietnam, Taiwan, Nicaragua, Australia, and the United States (Merten, 2003). It produces fruits in the second year after planting and reaches full production in five years.

The use of in vitro culture techniques can ensure the rapid growth of valuable species (Sunandakumari et al., 2004) and the possibility of obtaining biological materials free of pathogens on a large scale (Kane, 2014). The balance between auxin and cytokinin determines the in vitro regeneration of plants grown in artificial medium. In general, cytokinin helps in shoot proliferation and auxin helps in callus formation and rooting of extended shoots. The presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to achieve shoot formation (Caboni and Tonelli, 2009). However, cytokinin and auxin requirements depend on plant species, genotype, cultivars and culture conditions. The requirement of growth regulator depends on the type of specification and also its physical condition.

Optimum growth regulators, growth conditions and optimal media with appropriate specifications are required to become standard for large-scale production of plants. Therefore, this study was conducted to examine the effect of different levels of plant growth regulators on the in vitro regeneration of dragon fruit.

MATERIAL AND METHODS

The experiment was carried out in 2022 at the Biotechnology Laboratory of the Department of Biotechnology, Shridhar University, Pilani to select the most suitable explant and medium for callus production from dragon fruit explants.

Plant Material

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

Sterilization of explants

The newly formed shoots were collected from the healthy mother plants of dragon fruit. The axillary buds and stem segments were excised from the shoots. They were rinsed thoroughly in running water and were then washed in distilled water. Subsequently they were dipped in 70% ethanol for 1 min and surface sterilized using 30% of Clorox™ (Sodium hypochlorite, 5.25% active ingredient) with 1 ml of tween 20 for 20 min. Thereafter they were washed four times thoroughly in sterilized distilled water to avoid presentence of Clorox™ residues.

Culture Medium

In this experiment, MS media containing two different concentrations (2.0mg/l, 3.0 mg/l.) of BAP (6- benzyl – amino- purine) or TDZ (thidiazuron) with 0.5mg/l. NAA (α- naphthalene acetic acid) were prepared for culture established. Agar (8 g/L.) was added to solidify the medium and the pH was adjusted to 5.8 with 1N HCL or 1 N NAOH. Ten ml of medium was poured into each culture vessel (125 ml capacity) and they were covered by using plastic lid. These culture bottles were autoclaved at 121^{o}C for 20 min at 15 psi subsequently they were kept to cool before the inoculation of explants.

Inoculation of Explant

The sterilized axillary buds from shoots of dragon fruit as well as stem segments (0.5 cm long) from the middle portion of the stem were excised under sterile laminar flow with the sterilized scalpel and forceps. Subsequently those excised explants were cultured on MS media containing and then once in two days for in vitro response. This experiment was repeated thrice.

Culture Environment

The cultured bottles containing explants were incubated at 25± 2 ^{o}C under white fluorescent light in photoperiod of 16 hrs light and 8 hrs dark. In first weeks, the cultures were observed daily for contamination and then once in two days for in vitro response. This experiment was repeated thrice.

Data Collection

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

Result and Discussion

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

Percent of explants showing Shoot induction

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

Days to shoot induction:

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

Number of Shoots Per Explant

There was a significant influence of different concentrations of BA on the number of shoot at 5% level of significance. The highest number of shoot (1.80, 2.40 and 3.20) at 28 DAI, 35 DAI and 42 DAI respectively was noticed from the treatment 2.0 mg/l BA followed by shoot numbers 1.00, 2.00 and 2.60 at 28 DAI, 35 DAI and 42 DAI respectively from 2.5 mg/L BA treatment (Plate 5). Whereas the lowest number of shoots 0.80, 1.00 and 1.20 at 28 DAI, 35 DAI and 42 DAI, respectively were noticed in

control treatment (Table 1). Soe (2019) reported that proximal portion of shoot explants of Dragon fruits produced the maximum number of shoot (4.42 per plant) supplemented with the 10 µM BAP.

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

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

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

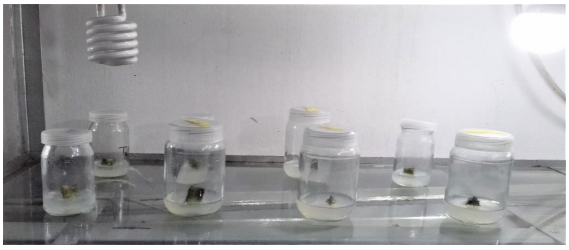
Average length of shoot (cm)

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

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

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

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



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

CONCLUSION

The results could be concluded that the most responsible plant part for the friable callus formation was the stem segment cultured on MS medium supplemented with the effect of BA (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l). The treatment BA 2.0 mg/l gave the highest percentage of shoot (76.00%) in 12.2 days and the maximum number of shoot (1.80, 2.40 and 3.20) at 28, 35 and 42 DAI respectively where the control treatment found the lowest percentage of shoot (24.00%) in 21.6 days and the minimum number of shoot (0.80, 1.00 and 1.20) at 28, 35 and 42 DAI respectively. The maximum length of shoot (1.68cm, 2.78 cm and 4.02 cm) at 28, 35 and 42 DAI respectively was recorded with BA 2.5 mg/l where control treatment showed the minimum length of shoots. Uniform good quality callus is required for a large scale production of planting materials through organogenesis or somatic embryogenesis in dragon fruit.

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