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Studies on the effect of different concentrations of kinetin on callus induction of dragon fruit plant (Hylocereus undatus)

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Abstract

Dragon fruit (*Hylocereus undatus*) belong to family Cactacea, originated in Vietnam requires dry, tropical or subtropical climates and annual rainfall of 20- 50". The plant bears high nutritional and pharmaceutical qualities so it can be a great option to the farmer in tropical region to cultivate the crop. To provide tissue culture technology can produce high quality and large scale plantlets. The present investigation was carried to optimize the concentrations of kinetin for induction callus in dragon fruit (*Hylocereus undatus*). The result reveled that Maximum fresh weight was produced in media supplemented with 2.5mg/l Kinetin and 0.01 mg/l constant NAA (T2). Whereas, least callus production was observed in T1 (1.0mg/l) Kinetin and 0,01mg/l NAA. At the concentration of kinetin (2.5 mg/l kinetin), the 15 days required after inoculation for callus initiation, the fresh weight observed 2.8 gm and the diameter of the callus was 15.86 mm which was recorded highest among all in *Hylocereus undatus*.

Keywords: Hylocereus undatus, kinetin, callus induction

Introduction

Dragon fruit (Hylocereus undatus), is a tropical fruit, also known as pitahaya, Buah naga or strawberry pear belong to the family, Cactacea originated in Vietnam (Mertens, 2003) [8]. The plant require dry, tropical or subtropical climates and annual rainfall of 20-50". The name Dragon fruit might have given because of vibrant red skin and scale-like leaves like the dragon. It is climbing vine initially utilized as a ornamental plant but later as a fruit crop. The fruit have immense nutritional value and it can be utilized for various processed food products. Several researchers found importance of dragon fruit as food additive and in pharmaceutical industries (Harivaindaran et al., 2008; Rebecca et al., 2010; Wu et al., 2006; Wichienchot et al., 2010) [3, 10, 12, 11]. Because of low seed viability of the fruit the plant propagation is done by stem cuttings. The demand of fruit has increased drastically in recent years so it is very important to meet the dragon fruit production. Tissue culture technology could help to produce high quality and large scale plantlets. The perusal of literature regarding tissue culture in dragon fruit has revealed that very few researcher (Lichtenzveig, et al. 2000; Castillo, et al. 2003; Le Bellec, et al. 2004) [6, 1, 5] have developed the plant micro-propagation for dragon fruit. This study was conducted to establish an efficient In vitro Shoot Initiation protocol for Dragon fruit. The objective of our investigation was to study the effect on callus induction of dragon plant (Hylocereus undatus) by using different concentrations of kinetin.

Materials and Methods

Sample Collection, Preparation and Disinfection of Explants

The Leaves of red dragon plant (*Hylocereus undatus*) was collected from cultivated fields in Shendra village, nearby Aurangabad (MS). Leaves were soaked overnight separately and surface-sterilized by spraying 70% ethanol for 2 minutes and dipping in 1% sodium hypochlorite solution containing 6% Tween 20 for 10 minutes. Sterilized leaf were then properly washed out with autoclaved distilled water. The explants was further rinsed five times with sterile double distilled water and then inoculated on a Murashige and Skoog basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar and different concentration of kinetin.

Preparation of media

Murashige and Skoog (MS) medium was commonly used for all the experiments. Murashige and Skoog basal medium was supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar with different concentration of kinetin. The pH adjusted at 5.6 to 5.8 before autoclaving.

Inoculation on micro propagation media: Sterilized leaf explants were inoculated on micro propagation media supplemented with different concentrations of kinetin (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) Add 3% sucrose and 0.8% agar and adjust the pH 5.6 to 5.8 before autoclaving. Those were incubated at 250 c temperature with 16/8 hour photoperiod at 3000 lux light intensity

Treatment Details

Concentrations of kinetin

Effect of different concentration of kinetin growth hormone on shoot initiation of Dragon Plant.

Show Concentration of Kinetin

Treatments	Concentration of Kinetin (mg/L)	
T_1	1.0	
T_2	2.0	
T ₃	3.0	
T_4	4.0	
T ₅	5.0	

Explants were observed periodically

- a. Days for callus initiation. (Fifteen days interval).
- b. Fresh weight of callus. (After 4 weeks).
- c. Diameter of callus. (After 4 weeks).

Data collection: Explants were observed after weekly intervals, in respect to

Culture free from contamination: After inoculation of explants in test tubes, it was ensured to free from fungus, bacteria, browning etc.

Days to callus initiation: No. of days for callus initiation from leaf explant was expressed as mean number of days per explant.

Colour of callus: After 4 weeks of inoculation, the colour of the callus was observed visually and were recorded as 3 for green, 2 for creamy and 1 for white.

Nature of callus: After 4 weeks of inoculation, nature of callus was recorded and graded as 3 for compact, 2 for friable and 1 for loose of its texture.

Fresh weight of callus: After 4 weeks of inoculation, the weight of callus was measured in gram (g) with the help of electrical balance. Fresh weight of callus expressed as mean weight of callus induced. For fresh weight of callus was transferred to pre-weighed foil boat (W1) and quickly take the weight (W2). Fresh weight=W2-W1.

Diameter of callus: After 4 weeks of inoculation, the diameter of callus was measured in mm with the help of scale. Analysis of Data the data obtained on various observations was analyzed by "Analysis of Variance" method (Panse and Sukhatme 1967) ^[9].

Result

The results obtained in the present investigation on "In vitro Shoot Initiation of Dragon Plant by Tissue Culture techniques" are presented under the following headings.

Table 1: Biometric observations of *Hylocereus undatus* for callus initiation (40 DAI)

Sr. No.	Treatment	A. Days required for callus initiation	B. Fresh weight of callus (mm)	C. Diameter of callus (gm)
1	T_1	19.50	0.504	7.087
2	T_2	19.95	0.820	9.387
3	T ₃	17.50	1.618	11.811
4	T_4	15.50	2.888	15.861
5	T_5	16.25	2.046	13.988
		17.7	1.587	11.63
		0.801	0.129	1.07
		2.414	0.39	3.226

The leaf explant produced different callus per explant of *Hylocereus undatus* as enriched by different level of Kinetin with constant NAA at (30 DAI) as per data presented in Table 1. Maximum fresh weight was produced in media

supplemented with 2.5mg/l Kinetin and 0.01 mg/l constant NAA (T2). Whereas, least callus production was observed in T1 1.0mg/l Kinetin and 0,01mg/l NAA (Table 1).

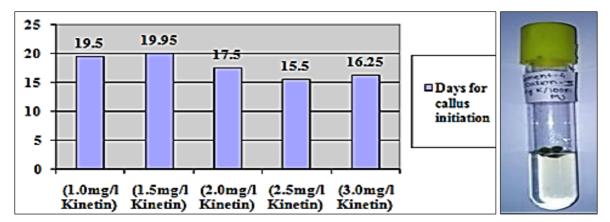


Fig 1: Initiations of callus from leaf segment of dragon plant in T₄

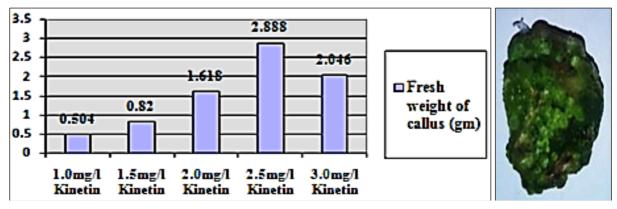


Fig 2: Fresh weight in T₄

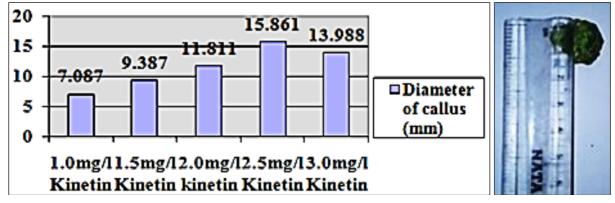


Fig 3: Diameter of callus in T₄

Discussion

The effects of kinetin on callus induction were corroborated in earlier studies in various plants. In the present investigation the Kinetin concentration was optimized fro callus induction which was also studied in *Barringtonia racemosa* (Dalila, *et al.* 2013) ^[2], *Medicago sativa* L (Wan, *et al.* 1988) ^[11]. Physalis angulata (Mastuti, *et al.* 2019) ^[7] and *Taxus b revifolia* (Karimian *et al.*) ^[4]. At the concentration of kinetin (2.5 mg/l kinetin), the 15 days required after inoculation for callus initiation, the fresh weight observed 2.8 gm and the diameter of the callus was 15.86 mm which was recorded highest among all in *Hylocereus undatus*.

Conclusion

On the basis of results obtained in the present investigation, It could be finally concluded callus of dragon fruit (*Hylocereus* spp.) can be maintained upon MS supplemented with kinetin concentration range from 1 to 3 (mg/L) but the MS supplemented with concentration 2.5 (mg/L) optimum induction and growth. These finding will be useful for further micropropagation research in *Hylocereus undatus*.

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