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Short Communication

Basal Media for *In Vitro* Germination of Red-Purple Dragon Fruit *Hylocereus polyrhizus*

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ABSTRACT

Five types of basal media, DM 94 (a modified MS), Gamborg B5 (1968), Murashige and Skoog (1962, MS), Lloyd and McCown (1980, LM), and Chinese A (a modified MS) were screened for germination of dragon fruit seeds obtained from the local supermarket. Based on preliminary experiments to determine the potential hormone combinations giving high germination percentage, three combinations were selected. The treatments consisted of 0.8 ppm IBA, 0.5 ppm IBA + 1.0 ppm BAP, 0.5 ppm IBA + 1.0 ppm kinetin and control. Germination of seeds, after three weeks in the media, varied between 62.8% and 89.3%. The highest and the lowest percentages came from similar treatment consisting of 0.5 ppm IBA + 1.0 ppm kinetin but different basal media, Chinese A and DM 94, respectively. The best medium for germination and subsequent growth was a modified MS called Chinese A, which gave 100% transplanting success. This screening experiment showed that this medium can be used for future seed germination of red-purple dragon fruit *Hylocereus polyrhizus*.

Keywords: *In vitro, Hylocereus polyrhizus*, transplanting

ABSTRAK

Lima jenis media iaitu DM 94 (ubahsuai MS), Gamborg B5 (1968), Murashige and Skoog (1962, MS), Lloyd & McCown (1980, LM), dan Chinese A (MS diubahsuai) telah dipilih untuk percambahan biji benih buah naga yang diperolehi dari pasaraya tempatan. Berdasarkan keputusan eksperimen untuk menentukan kombinasi hormon yang terbaik untuk percambahan, tiga kombinasi telah dipilih. Kombinasi yang dipilih adalah terdiri daripada 0.8 bpj IBA, 0.5 bpj IBA + 1.0 bpj BAP, 0.5 bpj IBA + 1.0 bpj kinetin, dan juga media tanpa hormon sebagai kawalan. Selepas 3 minggu, percambahan biji benih di atas media adalah di antara 62.8% hingga 89.3%. Peratusan tertinggi dan terendah diperolehi dari rawatan yang sama iaitu 0.5 bpj IBA + 1.0 bpj kinetin, tetapi daripada media yang berbeza iaitu Chinese A dan DM94. Media terbaik untuk percambahan dan pertumbuhan adalah media Chinese A yang mana diperolehi berjaya hidup 100% selepas dipindahkan ke tanah. Keputusan yang diperolehi ini boleh digunakan pada masa akan datang untuk percambahan biji benih *Hylocereus polyrhizus*.

Kata kunci: *In vitro*, *Hylocereus polyrhizus*, pemindahan ke tanah

INTRODUCTION

Most of the varieties of dragon fruits from Asia are predominantly *Hylocereus undatus* that are self-compatible, while some of these are autogamous and will set fruit without the involvement of a pollen vector. Several of these clones produce 350 gm fruit on the average. The only disadvantage to autogamous varieties is that the fruit is often smaller than if the flowers were cross-pollinated with pollen from a different cultivar or different species (Nerd and Mizrahi, 1997; Lichtenzveig *et al*., 2000). Fruit weight is positively correlated with the number of viable seeds (Weiss *et al.,* 1994). The fruit can weigh up to 900 gm but the average weight is between 350-450 gm.

Dragon fruit is known to be a long day plant (Lauders, 1999). The flowering season in California is from May through November (Raveh *et al*., 1993; Mizrahi and Nerd, 1999; Thomson, 2002). Drew and Azimi (2002) have developed a protocol for the micropropagation of this cactus species. If this protocol can be adapted to a large-scale operation, it will enable the production of a large numbers of plants from a relatively small stock. However, *in vitro* passage may take quite a long time before the plants reach maturity and bear fruits. The basis for *in vitro* germination as compared to *in situ* germination is to save space and to enable selection of elite mother plants.

Planting materials obtained from cuttings are totally unknown until they bear fruits. Thus to ensure the quality of the planting materials, seeds from selected fruits can be used as a known explant source. Although we know that seeds are not true-to-type, there is a probability that the selected seedlings will be like the mother in their fruit quality. Anyone in Malaysia wanting to plant commercial acreage of dragon fruit has to propagate them themselves or must import cuttings, which can be costly.

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. Initial capital for starting this industry was found to be higher than any other food crops. In Thailand, the crop is grown in areas with temperatures which are between 20-30 °C and 500-1500 mm rainfall. It thrives on sandy or sandy-loam soil rich in organic matter.

MATERIALS AND METHODS

Media Composition

The following five basal media contained components that can be categorized into four groups: mineral elements, organic compounds, plant growth regulators, and support systems (Table 1).

Experimental Design

The treatments were summarized in the matrix in Table 2. Each treatment consisted of 250-300 seeds each. Since the seeds of dragon fruits are very tiny, only half of the whole fruit was sufficient for use in a single experiment.

Methods

Explant materials such as the whole dragon fruit were sterilized with 5% Clorox® (2-3 drops of Teepol® added) for 5 minutes. After sterilization, the explants should be thoroughly rinsed with sterile distilled water. The fruit was cut and the seeds, which were naturally sterile, were directly inoculated into each petri dish labeled as shown in Table 2. Ten seeds were placed into each petri dish, after which they were sealed and incubated in a dark room at 25 ± 2 °C for several weeks.

A preliminary experiment was carried out to determine a few promising hormone combinations. Using the results of this experiment, a few combinations were selected and used for the experiment proper.

Table 1. Composition of the five types of media for seed germination test.

Component	DM 94	B5	MS	LM	Chinese A
Macronutrients (mg/L)					
KNO_3	1520	2500	1900	-	1900
NH_4NO_3	1320	-	1650	400	1650
$MgSO_4.7H_2O$	444	250	370	370	555
$CaCl_2.2H_2O$	264	150	440	96	330
KH_2PO_4	360	-	170	170	425
$(NH_4)_2SO_4$	-	134	-	-	-
$Ca(NO_3)_2.4H_2O$	-	-	-	556	-
$NaH_2PO_4.H_2O$	-	150	-	-	-
Iron salts (mg/L)					
Na_2EDTA	37.3	37.3	37.3	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8	27.8
Micro nutrients (mg/L)					
$MnSO_4.4H_2O$	33.8	-	22.3	22.3	-
$ZnSO_4.7H_2O$	17.2	2	8.6	8.6	10
H_3BO_3	12.4	3	6.2	6.2	10
$CuSO_4.5H_2O$	0.05	0.025	0.025	0.25	0.025
$Na_2MoO_4.2H_2O$	0.5	0.25	0.25	0.25	0.25
KI	1.66	0.75	0.83	-	-
$CoCl_2.6H_2O$	0.05	0.025	0.025	-	-
$MnSO_4.H_2O$	-	10	-	-	18.9
Vitamin and supplements (mg/L)					
Thiamine	0.4	10	0.1	0.1	0.4
Pyridoxine	0.5	1	0.5	-	0.5
Nicotinic acid	0.5	1	0.5	-	0.5
Glycine	2	-	2	-	2
Myo -inositol (mg/L)	100	100	100	-	100
Sucrose (mg/L)	50000	20000	30000	30000	30000
Agar (gm/L)	7.5	7.5	7.5	7.5	7.5

Table 2. Effect of selected combinations in five types of basal media.

Basal culture media	Control (no growth regulators)	0.8 ppm IBA	0.5 ppm IBA + 1.0 ppm BA	0.5 ppm IBA + 1.0 ppm Kinetin
DM 94	DM 1	DM 2	DM 3	DM 4
B5	B5 1	B5 2	B5 3	B5 4
MS	MS 1	MS 2	MS 3	MS 4
LM	LM 1	LM 2	LM 3	LM 4
Chinese A	CA 1	CA 2	CA 3	CA 4

Each treatment consisted of about 250-300 seeds initially.

RESULTS AND DISCUSSION

Preliminary Results

All the hormone combinations gave a high percentage germination, however only three were selected and they were B, E and J (Table 3). Most of the treatments gave > 80% germination, but treatments B and E were selected because BAP and Kinetin are cheaper than Zeatin. Secondly, while IBA concentration of 0.5 ppm is lower than 0.8 ppm, the percentage of germination is higher, thus it would be cheaper to use the lower concentration for large scale preparation. Treatments J and A gave the same percentage of germination, but J was selected because more roots are desirable, thus auxins are preferred to cytokinins. Subsequently, the same three combinations were used in the Experiment 1.

Table 3: Percentage germination of *Hylocereus polyrhizus* after 3 weeks.

Auxin / Cytokinin	IBA 0	IBA 0.5 ppm	IBA 0.8 ppm
BAP	A	B	C
1.0 ppm	88%	89%	82%
Kinetin	D	E	F
1.0 ppm	81%	80%	81%
Zeatin	G	H	I
1.0 ppm	73%	84%	86%
			J
			88%

Each treatment consisted of 100 seeds.

Most of the seeds germinated after two weeks in the petri dishes containing approximately 10 mL of the basal media. However, a visual observation was carried out regularly with one set of petri dish per treatment for a few months. These observations showed that after about 3-7 weeks, the growth rate was obviously better for three of the media tested. The stems of seedlings from Chinese A were observed to be bigger and fatter than the rest. Media MS and LM also gave good stem growth like the Chinese A, and all three continued to give better growth and vigor than the rest. The hormone combinations did not seem to have any significant effect on the growth of these stems. The germination after three weeks was scored and shown in Table 4. Basal media DM 4 and CA 4 gave 89.3% and 62.8% germination, respectively; and in Table 5 their transplanting success was 60.9% and 50.8%, respectively. These results indicated that there may be no relationship between a good germination and transplanting success, because the best transplanting success was obtained with treatment CA 1 and not DM 4.

Experiment 1

Table 4. Percentage germination after 3 weeks.

Basal media	Control (no hormone)	0.8 ppm IBA (J)	0.5 ppm IBA + 1.0 ppm BA (B)	0.5 ppm IBA + 1.0 ppm kinetin (E)
DM 94	245/300 = 81 .0%	213/260 = 81.9%	213/280 = 76.1%	241/270 = 89.3%
B5	228/300 = 76 .0%	213/300 = 71.0%	216/260 = 83.8%	239/270 = 88.5%
MS	261/300 = 87 .0%	235/300 = 79.3%	245/300 = 81.7%	248/300 = 82.7%
LM	224/300 = 74.7%	241/300 = 80.3%	230/300 = 76.7%	241/300 = 80.3%
Chinese A	231/300 = 77 .0%	229/270 = 84.8%	222/300 = 74 .0%	182/290 = 62.8%

Table 5. Transplanting success into soil in polybags after 5 months.

Basal media	Control (no hormone)	0.8 ppm IBA	0.5 ppm IBA + 1.0 ppm BA	0.5 ppm IBA + 1.0 ppm kinetin
DM 94	43.9%	31.0%	-	60.9%
B5	48.6%	34.1%	-	-
MS	42.9%	92.3%	-	-
LM	60.4%	67.3%	-	-
Chinese A	100%	27.7%	-	50.8%

Discussion

Since germination success for dragon fruit used in these experiments was fairly good (> 70%) for all treatments, it may be assumed that *in vitro* germination could be useful and valuable. However, a good germination success did not lead correspondingly to a good transplanting success. The germination media may have an immediate effect on the other parameters such as girth and length of the stems, which were not measured in this experiment, but a small sample from each treatment was visually observed (Figure 1a).

Three of the media did show promising effects, they were Chinese A, MS and LM. The advantage of *in vitro* selection is space saving and culling can be carried out at an early stage. Those seedlings that are more vigorous can be selected and used for further multiplication either *in vitro* or in the nursery. From other experiments we have seen that this species could be readily multiplied *in vitro* using most media with low auxin concentration.

After six months in Jiffy 7 medium, these plantlets were transferred together with Jiffy 7 into polybags consisting of sand and soil mixture, and they were placed inside a greenhouse with 30% shading. Subsequent growth of the seedlings was found to be quite slow. Photo (Figure 1b) was taken five months after they were transplanted into the soil. The plants were watered on alternate days and liquid fertilizers ("Serbajadi" and "Nutrimulti") were applied once every two months. The growth of these seedlings showed multiple branching habits in most of the population (> 200 polybags), indicating that if they were further cut we could get thousands of cuttings derived from each polybag. Each polybag has > 10 large stems, which means that the multiplication rate is most encouraging. However, flowering and fruit set patterns are still to be determined. Pruning technique and systematic pruning should be developed to determine potential flowering stems.

(a) Root and shoot growth.

(b) Five months after transplanting into soil.

Fig. 1. Germination and growth of dragon fruit seedlings *in vitro* and after transplanting.

Similar germination media were repeated on two types of dragon fruits; red and yellow fruits, *Selenicereus megalanthus*. The average germination for the red was found to be 83.2% and for the yellow it was 77.0%. Germination without continuous supply of the medium and not done *in vitro* was observed to be less vigorous. This showed that it is quite useful to germinate dragon fruit seeds by *in vitro* method as it ensures a good growth of hard-to-get and more delicious yellow dragon fruits. However, subsequent growth and flowering of this genus has yet to be determined.

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