

# Standardisation of Pre Hardening and Hardening Techniques for *In vitro* Derived Plantlets of Orchid and Anthurium

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## Research Article

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## Abstract

In laboratory for Orchid subcultures pre hardening treatments with ½ MS media + NAA 8 mg/l as standard media and growing media compositions like Leaf mould + Charcoal (1:1), Leaf mould + Charcoal + Brick (1:1:1), Leaf mould + Coco peat + Charcoal (1:1:1), Leaf mould + Brick + Coco peat (1:1:1) and Charcoal + Brick + Coco peat (1:1:1) as Control for prehardening and the same above growing media without sterilization had been practiced under shade net for hardening and in Anthurium laboratory treatments comprised of ½ MS media + NAA 2 mg/l as standard media and growing media compositions like Leaf mould + Saw dust (1:1), Leaf mould + Coco peat + Charcoal (1:1:1), Leaf mould + Coir pith (1:1), Leaf mould + Coir pith + Coco peat (1:1:1), Saw dust + Coir pith + Coco peat (1:1:1), Leaf mould + Coco peat (1:1) as Control for prehardening and the same above growing media without sterilization had been practiced under shade net for hardening Maximum survival percentage was observed in T<sub>2</sub> (Leaf mould + Charcoal + Brick (1:1:1) and Leaf mould + Coco peat + Charcoal (1:1:1) both in Orchid and Anthurium respectively. The number of leaves were highest in T<sub>5</sub> (5.81 and 26.03) and number of roots i.e., 10.77 and 23.9 / plant were higher in medium containing Charcoal + Brick + Coco peat (1:1:1) i.e., as control. The root and shoot length were recorded higher in T<sub>2</sub> (Leaf mould + Charcoal + Brick (1:1:1)) as 6.11 cm and 16.77 cm and 4.48 cm and 20.47 cm in laboratory and in shade net respectively. The survival percent was enhanced in T<sub>2</sub> as 92.43% and 91.53% in laboratory and field. Higher no. of leaves and plant height were observed in T<sub>2</sub> (Leaf mould + Coco peat + Charcoal (1:1:1)) followed by T<sub>6</sub> (Leaf mould + Coco peat (1:1)) [Control]. The plantlets in T<sub>2</sub> established well in terms of plant height in laboratory and in shade net (6.17 cm and 31.4 cm) and number of new shootlets (Plantlet (2.67 and 7.67)). The number of leaves were highest in T<sub>2</sub> (12.33 and 20.67) and number of roots i.e., 3.33 and 8.33 / plant were higher in medium containing Leaf mould + Coco peat + Charcoal (1:1:1) followed by control

i.e., T<sub>6</sub> (Leaf mould + Coco peat (1:1)). The root length was also recorded higher in T<sub>2</sub> (Leaf mould + Coco peat + Charcoal (1:1:1)) as 4.3 cm and 15.67 cm (Table 13) in laboratory and in shade net respectively.

**Keywords:** Shoot lets; MS media; IAA, NAA; Prehardening; Hardening; Shade net; Survival rate

## Introduction

Taxonomically orchids represent the most highly evolved family among monocotyledons with approximately 750 genera and 22,000 and 30,000 species and over 74,400 natural and manmade hybrids. And thus orchids had a potential growth in every day cut flower trade. In Thailand 92% of cut flowers comprises of *Dendrobium* orchids only. *Anthurium andreaeanum* Lind is cultivated for its colorful long lasting flowers, has gained importance as a major cut flower of the modern world. In this regard the media for orchids and anthurium is not being standardized precisely particularly before hardening chamber with different growing medias. Thus this study aims to develop the technique for pre hardening and hardening through media standardization of orchid and anthurium.

## Materials and Methods

### Technical programme

Crops: 1. Orchid (*Dendrobium*) hybrid Sonia-17  
2. Anthurium (*Anthurium andreaeanum*) var, Temptation

### Treatment Details

#### I. Pre hardening treatments

##### A. Orchid (In laboratory)

Media → ½ MS media + NAA 8 mg/l

T <sub>1</sub>	-	Leaf mould + Charcoal (1:1)
T <sub>2</sub>	-	Leaf mould + Charcoal + Brick (1:1:1)
T <sub>3</sub>	-	Leaf mould + Coco peat + Charcoal (1:1:1)
T <sub>4</sub>	-	Leaf mould + Brick + Coco peat (1:1:1)
T <sub>5</sub>	-	Charcoal + Brick + Coco peat (1:1:1)
		[Control]

##### B. Anthurium (In laboratory)

Media → ½ MS media + NAA 2 mg/l

T <sub>1</sub>	-	Leaf mould + Saw dust (1:1)
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T <sub>2</sub>	-	Leaf mould + Coco peat + Charcoal (1:1:1)
T <sub>3</sub>	-	Leaf mould + Coir pith (1:1)
T <sub>4</sub>	-	Leaf mould + Coir pith + Coco peat (1:1:1)
T <sub>5</sub>	-	Saw dust + Coir pith + Coco peat (1:1:1)
T <sub>6</sub>	-	Leaf mould + Coco peat (1:1) [Control]

#### II. Hardening treatments (Under shade net)

##### A. Orchid

T <sub>1</sub>	-	Leaf mould + Charcoal (1:1)
T <sub>2</sub>	-	Leaf mould + Charcoal + Brick (1:1:1)
T <sub>3</sub>	-	Leaf mould + Coco peat + Charcoal (1:1:1)
T <sub>4</sub>	-	Leaf mould + Brick + Coco peat (1:1:1)
T <sub>5</sub>	-	Charcoal + Brick + Coco peat (1:1:1)
		[Control]

##### B. Anthurium

T <sub>1</sub>	-	Leaf mould + Saw dust (1:1)
T <sub>2</sub>	-	Leaf mould + Coco peat + Charcoal (1:1:1)
T <sub>3</sub>	-	Leaf mould + Coir pith (1:1)
T <sub>4</sub>	-	Leaf mould + Coir pith + Coco peat (1:1:1)
T <sub>5</sub>	-	Saw dust + Coir pith + Coco peat (1:1:1)
T <sub>6</sub>	-	Leaf mould + Coco peat (1:1) [Control]

**Replication:** 3

**Design:** FCRD

## Results and Discussion

### Orchid

The survival percent was enhanced in T<sub>2</sub> followed by control (Table 1).

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	85.41	83.43	1.25	5.7
T <sub>2</sub>	92.93	91.27	1.78	7.4
T <sub>3</sub>	65.72	63.01	1.27	5.17
T <sub>4</sub>	58.13	56.17	0.96	4.57
T <sub>5</sub>	91.7	90.63	1.76	6.37
Mean	78.72	76.97	1.406	5.787
SE (d)	8.3544	8.179	0.1507	0.6125
CD (at 5%)	18.6149	18.2239	0.3358	1.3647

Table 1: Effect of prehardening and hardening of media on the survival percent and number of new shootlets *in vitro* derived plantlets of Orchid variety Sonia-17.

This may be due to the process of acclimatization of the plant lets to the hardening media in the *in vitro* condition itself. The transplantation shock was lesser in the hardening media for the prehardened plantlets that the control plants and hence they survived better [1]. The

plantlets in this media established well in terms of plant height in laboratory and in shade net (7.15 cm and 33.13 cm) (Table 2) and number of new shootlets (Plantlet (1.78 and 7.4)) at laboratory and shade net condition (Table 1) [2].

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	5.37	24.67	8.88	17
T <sub>2</sub>	7.15	33.13	10.77	23.9
T <sub>3</sub>	5.95	27.47	8.1	16.77
T <sub>4</sub>	4.56	22.7	6.58	13.4
T <sub>5</sub>	6.8	30.11	10.39	23.3
Mean	5.971	27.775	8.9533	18.892
SE (d)	0.6317	2.9288	0.9483	2.0187
CD (at 5%)	1.4074	6.5258	2.113	4.4979

Table 2: Effect of prehardening and hardening of media on the plant height and number of roots of *in vitro* derived plantlets of Orchid variety Sonia-17

However the number of leaves were highest in T<sub>5</sub> (5.81 and 26.03) and number of roots i.e., 10.77 and 23.9 /plant were higher in medium containing Charcoal + Brick + Coco peat (1:1:1) i.e., as control and it was closely followed by T<sub>2</sub> (Leaf mould + Charcoal + Brick (1:1:1)).

The root and shoot length were recorded higher in T<sub>2</sub> (Leaf mould + Charcoal + Brick (1:1:1)) a 6.11 cm and 16.77 cm and 4.48 cm and 20.47 cm in laboratory and in shade net respectively (Table 3).

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	4.57	20.81	4.68	12.73
T <sub>2</sub>	5.81	26.03	6.11	16.77
T <sub>3</sub>	5.18	22.7	4.11	13.5
T <sub>4</sub>	3.72	19.17	2.47	11.5
T <sub>5</sub>	5.45	24.8	5.86	15.6
Mean	4.95	22.7253	4.65	14.0327
SE (d)	0.5224	2.3877	0.5041	0.2239
CD (at 5%)	1.164	5.3201	1.1232	0.5164

Table 3: Effect of prehardening and hardening of media on number of leaves and root lengths of *in vitro* derived plantlets of Orchid variety Sonia-17.

Epiphytic orchids generally need good aeration this is because in their natural habitat they are tree dwellers with roots hanging in the air. In order to provide paper aeration than the potting mixture must be porous which allows excess water to drain off but at the same time must be able to soak up and the retain moisture without sogging. The better establishment of plantlets in leaf mould + charcoal + brick (1:1:1) followed by charcoal + brick + cocopeat (1:1:1) medium may be due to the maintenance of better micro environment with optimum

humidity and aeration compared to the other potting mixtures [3]. The reason for the maintenance of optimum humidity and moisture retention was due to the addition of leaf mould. In the present study the survival and establishment rate remained. Comparatively low in leaf mould + cocopeat + charcoal (1:1:1). However Sharma and Tandon (1992) [4] reported better survival of plantlets of *dendrobium* sp. In earthen pots containing charcoal + brick pits and coconut fibre (Table 4).

Treatments	Stage	
	Under laboratory condition	Under shade net
T <sub>1</sub>	4.68	12.73
T <sub>2</sub>	6.11	16.77
T <sub>3</sub>	4.11	13.5
T <sub>4</sub>	2.47	11.5
T <sub>5</sub>	5.86	15.6
Mean	4.65	14.0327
SE(d)	0.5041	0.2239
CD (at 5%)	1.1232	0.5164

Table 4: Effect of prehardening and hardening of media on root length of *in vitro* derived plantlets of Orchid variety Sonia – 17.

Spraying of ½ MS nutrient solution resulted in better establishment of plantlets. But there was a non-significant interaction between the pot mixture and nutrient solution spray on the establishment of plantlets. The reduced survival of the plantlets during hardening was resulted because of the shock due to the physiological changes during the sudden transition. However the solution of the medium in which the plantlets have been maintained,

have minimized the shock and supported the establishment of the plantlets.

### Anthurium

The survival percent was enhanced in T<sub>2</sub> as 92.43% and 91.53% in laboratory and in shade net followed by control (Table 5).

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	47.87	45.87	3.8	17.3
T <sub>2</sub>	92.43	91.53	6.17	31.4
T <sub>3</sub>	65.58	64.47	5.2	25.7
T <sub>4</sub>	87.48	85.43	4.8	20.5
T <sub>5</sub>	84.67	83	3.8	17.16
Mean	90.49	89.2	5.9	28.2
SE (d)	78.1644	76.709	4.9489	23.4017
CD (at 5%)	8.3333	8.1877	0.1065	0.6217

Table 5: Effect of prehardening and hardening of media on the survival percent and plant height of *in vitro* derived plantlets of Anthurium variety Temptation.

This may be due to the process of acclimatization of the plantlets to the hardening media and addition of NAA of 1 mg/l as the standard one which enhances the better rooting in the *in vitro* condition itself. The transplantation

shock was lesser in the hardening media for the prehardened plantlets that the control plants and hence they survived better [5]. The survival percent was higher in T<sub>2</sub> followed by T<sub>6</sub>. Higher no. of leaves and plant height

were observed in T<sub>2</sub> followed by T<sub>6</sub>. Jawaharlal et al. (2001) [6] reported from their studies with hardened *in vitro* plantlets on standardization of growing media, cocopeat applied alone and in combination with leaf mould and FYM produced the highest number of branches and suckers / plant.

The plantlets in this media established well in terms of plant height in laboratory and in shade net (6.17 and 31.4 cm) (Table 5) and number of new shootlets (Plantlet (2.67 and 7.67)) at laboratory and shade net condition (Table 6).

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	8.67	17.33	1.33	5
T <sub>2</sub>	12.33	20.67	2.67	7.67
T <sub>3</sub>	6.33	12.67	1.33	3.7
T <sub>4</sub>	6	10.67	1.31	3.4
T <sub>5</sub>	6.67	11	0.67	2.67
Mean	10.67	19	1.67	5.33
SE (d)	8.6572	15.2367	1.5056	4.6828
CD (at 5%)	1.6422	1.6462	0.0688	0.1841

Table 6: Effect of prehardening and hardening of media on number of leaves and number of shootlets of *in vitro* derived plantlets of Anthurium variety Temptation.

The number of leaves were highest in T<sub>2</sub> (12.33 and 20.67) Chandrappa (2002) [7] and number of roots i.e., 3.33 and 8.33 / plant were higher in medium containing Leaf mould + Coco peat + Charcoal (1:1:1) followed by control i.e., T<sub>6</sub> (Leaf mould + Coco peat (1:1)) (Table 6) [8].

The root length was also recorded higher in T<sub>2</sub> (Leaf mould + Coco peat + Charcoal (1:1:1)) as 4.3 cm and 15.67 cm (Table 7) in laboratory and in shade net respectively.

Treatments	Stages		Stages	
	Under laboratory condition	Under shade net	Under laboratory condition	Under shade net
T <sub>1</sub>	1.67	5	3.7	13.67
T <sub>2</sub>	3.33	8.33	4.3	15.67
T <sub>3</sub>	1.67	4.3	3.37	12.67
T <sub>4</sub>	1.33	3.3	2.93	10.33
T <sub>5</sub>	1.31	3.67	2.27	8.67
Mean	2	5.67	4	14.67
SE (d)	1.8917	5.0511	3.4328	12.6261
CD (at 5%)	0.0796	0.1912	0.3646	1.3416

Table 7: Effect of prehardening and hardening of media on number of roots and root length of *in vitro* derived plantlets of Anthurium variety Temptation.

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