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SHORT COMMUNICATION

USING BIOTECHNOLOGICAL APPROACHES FOR VANDA ORCHID IMPROVEMENT

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ABSTRACT

High frequency somatic embryogenesis and plant regeneration from callus cultures of vanda was established. Embryogenic calli from root tips and cut ends of stem and leaf segments on 1/4MS basal medium supplemented with 1-phenyl-3-(1,2,3-thiadiazol- 5yl) –urea (TDZ, 0,1-3mg/l), 2,4 D , 3-10mg/l) for 4 weeks. Embryogenic callus was maintained by subculture on the same medium for callus induction and proliferated 2 times in 1 month. The survival rate of plantlets under in vitro condition was 90% after 2 months. The high regeneration capacity may be due to the callus origin from meristematic cells of root tips. It was also found that very different ability of somatic embryogenesis between cell lines in the same callus origin.

Key words: callus, somatic embryogenesis

INTRODUCTION

Vanda coerulea Griff (Orchidaceae) has become economically important, mainly for use in cut-flower and potted plant industries. Plant tissue culture is a potentially useful technique in *ex situ* multiplication and restoration of ill-fated taxa (Wochok 1981). Successes achieved with the multiplication of tropical orchids of conservation and horticultural interest are generally through seed cultures (Fay 1988). There are some reports on the successful multiplication of species orchids using tissue cultures (Seeni and Latha 1992).The need for mass propagation of selected elite genotypes has led to the development of several *in vitro* methods. This work aims at developing efficient plant regeneration through somatic embryogenesis from callus cultures of vanda. This is also reproducible method for shoot initiation, rooting from stem, root and leave of vanda.

MATERIAL AND METHOD

Stem internodes of 5mm, leaves of 3-5cm and root tips of 5cm in length were used as explants for callusing. Explants were placed on the surface of basal medium with Murashige and Skoog (1962) supplemented with myoinositol (100mg/l) pyridoxine HCl(0,5) , thiamine HCl (0,1), glycine (2,0), Peptone(1000), Na_2HPO_4 (170), coconut milk (10%), sucrose 30000. The pH of the media was adjusted to 5.2 with 1N KOH or HCl prior to autoclaving for 15 min at 121^{o}C. Explants were incubated in two explants were planted in each culture tube Observation was made after one months of growth.

Callus lines were usually sub-cultured on the original media ever two months and were maintained in the same culture condition as mentioned above. Scanning electron micrographs were taken of tissues fixed in 5% stained.

RESULTS AND DISCUSSION

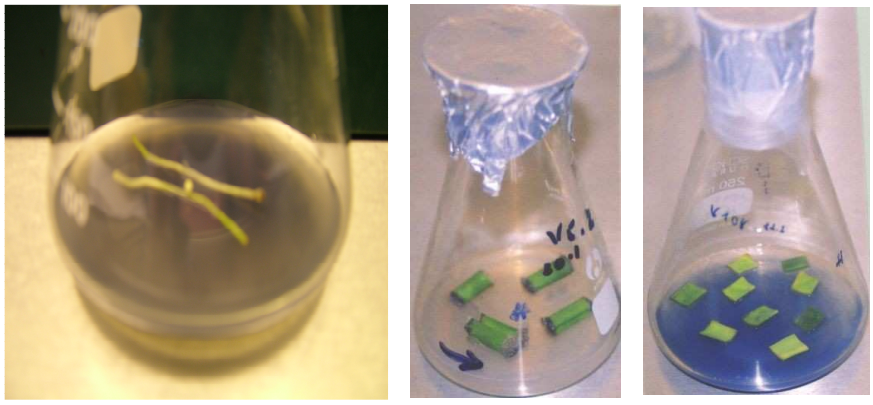
Initiation and subculture of embryogenesis callus

Preliminary experiments with shoot segments showed that only root tips formed callus in all treatments with combination of thiadiazol and 2,4 D on ½ MS basal medium. Thus, only the segments containing root tips with meristems were used root explants for callus induction. Between 4 and 5 weeks after planting, root tips of the cultured root segments (figure 1). Cut surfaces of stem segments of Figure 1b exhibited embryogenesis calli. Explants from leaves (5-7cm in length) did not form any visible callus. Table 1 shows frequencies of callus induction in relation to the types of explants and growth regulator treatments.

The highest percentage of callus induction and the best growth occurred in root explants. The combinations of 3mg/l TDZ with either 3 or 10mg/l 2,4-D favored callusing from root tips, while lesser dosages of TDZ and 2,4 D were required for callusing from stem explants. In the presence of TDZ, ranging from 0,3 to 3mg/ l , a higher dosage (10 mg/l) 2,4 –D was required for callusing from explants of young leaves of 5 cm in length.

Table 1: Effects of 2,4 D and TDZ on callus induction and proliferation from leaf , stem, and root explants of vanda

2,4D	TDZ	Leaf		Stem		Root	
		% Callusing	Proliferation rate	% Callusing	Proliferation rate	% Callusing	Proliferation rate
1	0	0		0		0	
1	0,3	0		0		0	
1	1	0		12	45	10	2
1	3	0		0		0	
3	0	0		0		0	
3	0,3	0		10	37	0	
3	1	0		10	23	0	
3	3	0		5	34	25	2.4
10	0	0		0		0	
10	0,3	10	30	0		0	
10	1	10	25	0		0	
10	3	2	36	0		5	2.8



a b c

Fig 1. Callus initiation. (a): Callusing from tip of a root explant, (b) Callusing from cutting faces of stem explant. (c) Callusing from cutting surface of a young leaf explant

Embryo formation

In light, the callus turned green and somatic embryos form the surfaces of callus for 3-4 weeks after transfer into a hormone free basal medium. Embryo formation was strongly affected by origins of callus. In general, the frequency of embryoformation of root derived callus was higher than stem and leaf derived callus (Jen et al. 2000). However, this experiment the frequency of embryoformation of stem derived callus was higher then root and leaf derived callus (table 2). Different degrees of somatic embryogenesis in callus lines of the same origin were found. The

highest frequency (90%) of somatic embryogenesis and average 5.9 embryos per callus were found in stem derived callus on the basal medium supplemented with 0.1 mg/l NAA and 3 mg/l TDZ (table 2). While the highest frequency of somatic embryogenesis and average number of embryos per callus are 23.8%, average 10.6 embryos per callus were found in stem derived callus on the basal medium supplemented with 0.1 mg/l NAA and 0,3 mg/l TDZ.

Table 2: Effects of NAA and TDZ on Somatic embryogenesis from stem, and root explants of vanda

NAA	TDZ	Line S1 (% Forming embry0)	Embryo/callus	Line R2 (% Forming embryo)	Embryo/callus
0	0	23.6	3.4	0	0
0	0,3	78.3	6.7	0	0
0	1	45.9	23.7	0	0
0	3	46.3	10.8	7.9	1.7
0,1	0	45.5	11.3	0	0
0,1	0,3	98.7	21.8	**23.8**	**10.6**
0,1	1	88.0	8.9	2.8	11.9
0,1	3	**90.0**	**5.9**	7.0	3.2
1	0	56.9	7.4	0	0
1	0,3	56.7	5.9	0	0
1	1	78.3	6.8	0	0
1	3	15.8	3.9	0	0
F		**	**		
LSD		2.18	2.25		

Scaning electron microscopy observation on embryo formation

Somatic embryos emerged from the outer cell layer of callus, and apparently started to form a single cell.

The capacity for embryo formation was strongly affected by the origin of callus. In general, the embryo formation capacity of stem tip derived callus was higher then leaf and root derived callus. This high regeneration capacity may be due to the callus origin from meristematic cells of stem root tips. It was also found that very different ability of somatic embryogenesis between cell lines in the same callus origin.

Plant regeneration

After 6 weeks, culture on the same medium for embryos induction, most embryos expanded sequentially and germination into PLBs with a protrusion in the anterior site and numerous absorbing hairs in the posterior region (figure 2). All regenerants about 5-8cm in height with six leaves and three roots were then potted in sphagnum moss in the greenhouse. These plants all grew well with an almost 90% survival rate .

a b c

Fig 2: Plant regeneration (a) Cluster of embryos/ PLB s with a (b) Young embryoderived PLBs with sheath leaves . (c) A rooted planter recovered from embryos.(d) P

d

Fig 3: Plant regeneration (a) Cluster of embryos/ PLB s with a (b) Young embryo derived PLBs with sheath leaves. (c) A rooted planter recovered from embryos (d) Plantlets

CONCLUSION

There are the first successful attempts to establish consistent callus and embryo formation callus culture protocols from stem, root and lease of vanda. This report showed that the embryogenic calli derived from the segments of roots, stems and leaves of vandal are able to form somatic embryos in 1 hormone free MS medium. The procedure required about one year from the initiation of callus to the plantlet formation. This method for embryo formation from callus is essential to establish *in vitro* culture of Orchidaceae. This method also opens up the prospects of using biotechnological approaches for other Orchidaceae

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