In Vitro Micropropagation of Dendrobium Crepidatum Lindl Using Fruit Capsule

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Abstract **- Dendrobium.Crepidatum is one of the largest genesus in the orchidaceae,of which about 1200 species are distributed widely on the Asia pacific region dendrobium.Crepidatum Lindl. Endemic epiphyte, Pseudobulbous orchid, found in Kodagu and Shimago. Flowering and fruiting is from April – June. dendrobium.Crepidatum Lindl. fruits was used with the various medium. VW, B5, MS and KC medium was used for micropropagation. KC medium with 2mg BAP+0.5mg NAA gave good plantlet formation and KC medium with 2mg BAP+1mg IAA was used for invitro rooting comparing to other medium this gave good results.**

Keywords **- Dendrobium crepidatum Lindl., VW, B5, MS,KC, NAA, IAA, BAP, AC & CM.**

Abbreviations **- VW - Vacin and Went medium, B5 - Gamborg B5 medium, MS- Murashige and Skoog medium, KC – Knudson C, NAA – Naphthalene Acetic Acid, IAA – Indole Acetic Acid, BAP – Benzyl Amino Purine, AC – Activated Charcoal & CM–Coconut Milk**

I. INTRODUCTION

Dendrobium Crepidatum Lindl. The family Orchidaceae is one of the largest groups among the angiosperms and distributed throughout the world. The genus dendrobium is the second largest group among the orchids plant in India and exhibit diverse shapes, colour and morphological characters. The stems of several Dendrobium species are used as 'Shi-Hu' in traditional Chinese medicine for a long time for the purpose of Cleaning the stomach and promoting the production of the body fluid, nourishing and cleaning heat. *dendrobium*. Crepidatum species is used as a biological source of 'Shi-Hu' and its flowers are very beautiful and get the name "Rosa Shi-Hu" in Chinese *dendrobium*. Crepidatum was selected as a plant material the fruits and the stem are very useful in the world of medicine.

Scientific Classification

Binomial Name: *Dendrobium crepidatum* Lindl. & Paxton

Kingdom	Plantae	**Tribe**	Dendrobieae
Order	Asparagales	**Subtribe**	Dendrobiinae
Family	Orchidaceae	**Genus**	Dendrobium
Subfamily	Epidendroideae	**Species**	*D. crepidatum*

Materials and Methods: Dendrobium macrostachyum specimens were colleceted from sagar between 7th May 2012 and 9th May 2012.sagar is surrounded by water bodies and forest regions. Plant specimens were collected from the natural environment in perforated, clean, polythene bags. Care was taken to ensure to retain the mother plant intact in its natural epiphytic territory.They were planted in green house of St.joseph's Post-Graduate and Research Centre. standard protocol and voucher specimens were deposited in the herbaria of St.joseph's Post-Graduate and Research Centre, Bangalore.

Surface sterilization

Green capsules of wild were collected and then rinsed thoroughly three times with sterile distilled water, followed by dipping them in 70% ethanol for 30 seconds. Sterilized capsules were dried and then split longitudinally with sterile surgical blade. Seeds were inoculated on different nutrient media like MS medium, B_5 medium, KC medium and VW medium which were prepared with various concentrations and combinations of phytohormones and other additives. KC medium gave the best results in comparison to all other media. So KC medium was standardized for *Dendrobium Crepidatum.*

Seed cultures were placed in growth chamber at 25 ± 20 °C and 70 –80% relative humidity under 24h-light and under 16h-light/8h-dark with light provided by cool white fluorescent lamps for 70 days. Sub-culturing was regularly done every 15 days and observations were made

***In vitro* rooting**

In vitro rooting was successful with KC media supplemented with 2 mg BAP, 1.5 mg NAA, 50 ml CM and 500 mg AC.

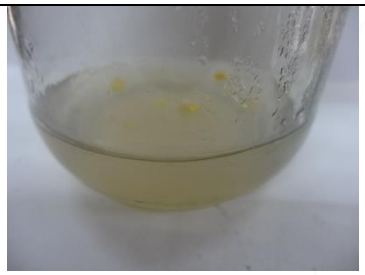
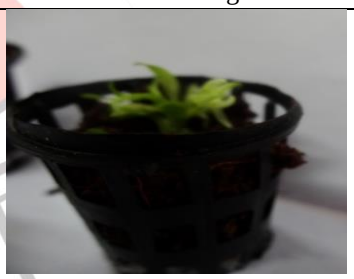
***Ex vitro* rooting**

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA (made in distilled water) then planted in small pots containing solrite (potting mix) sprayed with bavistin to avoid fungal infection. *In vitro* rooted plants in the pot trays containing potting mixture maintained under mist chamber and covered with perforated plastic cups.

Hardening

Well grown shoots were directly transferred to small pots containing soil, sand and solrite (mixture of pearlite and peat moss) and were kept in the green house. Successfully established plantlets were subsequently transferred to field condition.

II. OBSERVATIONS

<table>
<tr><td> 
1.Dendrobium crepidatum in green house</td><td> 
2.dendrobium crepidatum with fruits</td><td> 
3.Dendrobium crepidatum fruit to be inoculated</td></tr>
<tr><td> 
4 – Inoculation</td><td> 
5– Slight swelling of seeds</td><td> 
6 – Initial stages</td></tr>
<tr><td> 
7– Plantlet formation</td><td> 
8 - Sub-culturing</td><td> 
9 – Ex-vitro Rooting</td></tr>
<tr><td> 
10– Hardening</td><td> 
11 - Hardening in Green House</td><td> 
12 – Transfer to Green House</td></tr>
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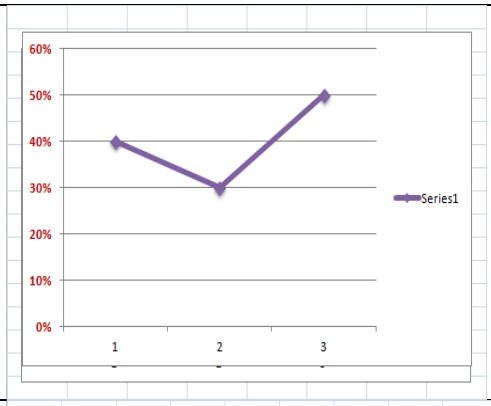
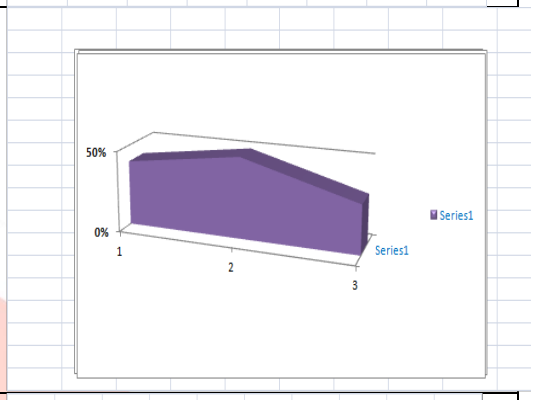
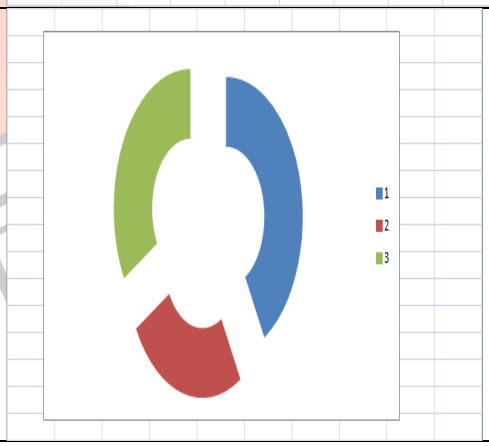
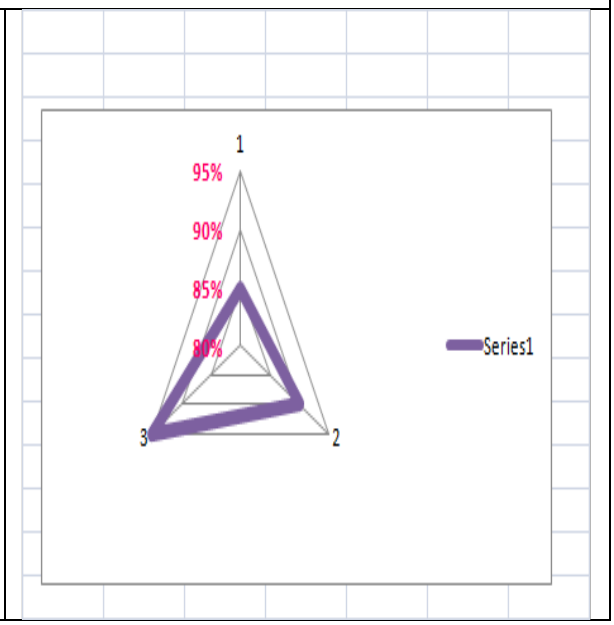
III. RESULTS AND DISCUSSION

MS, B5 and KC media was used (Table 1)

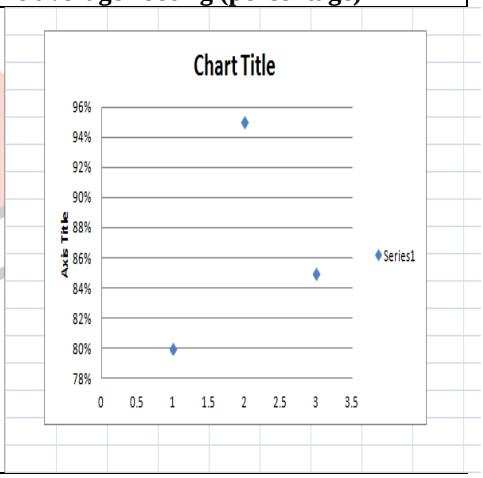
Media used	Media composition	Average plantlet formation (percentage)	
VW	2 mg BAP +1.5 mg NAA + 50 ml CM 1 mg BAP + 0.5 mg NAA + 50 ml CM 2.5mg BAP + 2 mg NAA + 50 ml CM	40% 30% 50%	
B_5	2 mg BAP + 1mg NAA + 50ml CM 1.5mg BAP + 0.5mg NAA+50ml CM 2 mg BAP + 1.5 mg NAA + 50 ml CM	40% 50% 30%	
MS	2 mg BAP + 1 mg NAA + 50 ml CM 1.5 mg BAP + 0.5 mg NAA + 50 ml CM 1 mg BAP +1.5 mg NAA + 50 ml CM	50% 30% 40%	

Media used	Media composition	Average plantlet formation (percentage)
KC	3 mg BAP + 1 mg NAA+ 50 ml CM 2.5 mg BAP + 1.5 mg NAA + 50 ml CM 2 mg BAP + 0.5 mg NAA + 50 ml CM	85% 90% 95%



KC - For the *In vitro* Rooting (Table 3)

Media Used	Media Composition	Results The average rooting (percentage)
KC	Basal KC Medium + 1 mg BAP+ 3mg NAA+ 50 ml CM +250 mg AC	80%
	Basal KC Medium + 2 mg BAP+ 5mg NAA + 50 ml CM+750 mg AC	95%
	2mg BAP+ 1mg IAA + 50 ml CM + 500 mg AC	85%



IV. CONCLUSION

From these studies it can be concluded that KC medium suitable for *Dendrobium Crepidatum fruit germination. This study also revealed that a low concentration of 2* mg BAP + 0.5 mg NAA + 50 ml CM was found to be more suitable for plantlets and multiple plantlets. KC medium supplemented with Basal KC Medium + 1 mg BAP+ 3mg NAA+ 50 ml CM +250 mg AC giving highest percentage was found to be suitable for In vitro Rooting.

V. REFERENCE

1. Jarret, RI and DF Austin (1994). Genetic diversity and systematic relationship in sweet potato (Ipomoea batatas (L.) Lam) and related species as revealed by RAPD analysis. Genetic Resources and Crop Evolution 41(3) 165-173.
2. Villordon, AQ and DR LaBonte (1995). Variation in randomly amplified DNA markers and storage root yield in 'Jewel' sweet potato clones. Journal of the American Society for Horticultural Science 120(5)734-740.
3. Zhang K, Leung HW, Yeung HW and Wong RN (2001). Molecular markers in herbal drug technology. Planta Medica 67 781-783. 5)
4. ZhaoY., SonY.O., Kim S.S.,Jang Y.S., Lee J.C., J. Biochem. Mol. Biol.,40,670—677 (2007). Arditi J (1992). Classfication and naming of orchids. In: The Fundamentals of Orchid Biology, John Wiley and Sons Press, pp. 55-101.
5. Besses SDD, Bory, G, Bellec D (2004). RAPD genetic diversity in cultivated vanilla: Vanilla planifolia, and relationships with V. tahitensis and V. pompo., Plant Sci. 167: 379-385. Blakeslee AF, Avery AG (1930). Method of inducing doubling of chromosomes in plants by treatment with colchicine.

6. J. Hered. 28: 393-411. Chen WH, Fu YM, Hsieh RM, Wu CC, Chyou MS, Tsai WT (1994). Modern breeding in Phalaenopsis orchid. In: 7th International Congr. SABRAO WSAA, Taichung, Taiwan, pp. 291-296. Dirk VA, Rosaa JG, Harpstead DD (1956). Colchicine induce true breeding chimera sectors in flax.
7. J. Hered. 47:229-233. Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus. 12: 13-15.
8. Duren V, Morpurgo R, Dolezel J, Afza R (1996). Induction and verification of autoteteraploids in diploid banana (Musa acuminate) by in vitro techniques. Euphytica 88: 25-34. Ferreira WDM, Kerbauy GB, Costa APP (2006). Micropropagation and genetic stability of a Dendrobium hybrid (Orcidaceae). In Vitro Cell Dev. Biol. Plant. 42: 568-571.
9.Goh MWK, Kumar PP, Lim SH, Tan HTW (2005). Random amplified polymorphic DNA analysis of the moth orchids, Phalaenopsis (Epidendroideae: Orchidaceae). Euphytica. 141: 11-22. Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Sci. 163: 223-270. Jerardo A (2005). Floriculture and nursery crops situation and outlook yearbook. Market and Trade Economics Division, Economic Research Service, United States Department of Agriculture, p. 131.
10. Jong LH, Mohd- Saad RM, Hamir NA (1997). Cut flower production in Malaysia. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand.
11.Koh TL, Khoo G, Fan LQ, Phang VPE (1999). Genetic diversity among wild forms and cultivated varieties of Discuc Symphysodon spp. as revealed by random amplified polymorphic DNA RAPD fingerprinting. Aquaculture. 173: 485-497.
12.Kuehnle AR (2007). Chapter 20: Orchids, Dendrobium. In: N.O Anderson (ed.), Flower Breeding and Genetics, Springer, pp. 539560.
13.Murashige T, Skoog F (1962). A revised medium for rapid growth of and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.
14.Rohlf FJ (1993). NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Version 1.80. Applied Biostatistics Inc., New York. Sneath PHA, Sokal RR (1973). A statistical method for evaluation systematic relationships. University Kansas Science Bulletin. 38: 1409-1438.
15. Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant science. Plant Cell Rep 27: 617–631
16.Ahuja S, Mandal BB, Dixit S, Srivastava PS (2002) Molecular, phenotypic and biosynthetic stability in Dioscorea floribunda plants derived from cryopreserved shoot tips. Plant Sci 163: 971–977 Antony J, Keng CL, Rathinam X, Subramaniam S (2010) Preliminary study on cryopreservation of Dendrobium Bobby Messina protocorm-like bodies by Vitrification Technique.