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Orchid micropropagation: Regeneration competence of anther culture

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Androgenesis in flowering plants is a unique biological phenomenon. The principle of androgenesis is to arrest the development of the pollen grains and to force them towards a somatic pathway. Antherculture is the main technique for haploid induction in crop improvement.

Since Guha and Maheshwari [1964, 1966] reported the induction of haploid plants from Datura innoxia, this technique became important for plant breeding and crop improvement [Clapham, 1973]. Anther culture has become a powerful tool for the rapid production of haploid and inbred lines used for obtaining hybrid cultivars and it has reduced the time required for breeding new cultivars by at least 3 to 5 years [Tai, 2003]. Hence, this system provides an unparalleled opportunity to shorten the breeding cycle and fix agronomic traits in the homozygous state and provides excellent material for research, plant breeding and plant transforma [Datta, 2005]. Production of haploids plants has been useful in providing access to recessive genes and for biotechnological manipulations, while using as a tool for cultivar development [Tai, 2003]. Therefore, in vitro techniques are considered to be alternative tools of conventional method of plants improvement[Sayem,2010].It is particularly useful in out breeders like orchids which generate a great deal of heterozygosity in the progenies.

Till now there is one report on anther culture in Orchids[Suryowinoto, and Somaryo,1985] , but attempts to assess a similar competence of anther culture in monocots including Orchidaceae, have remained almost negligible because of the following reasons(a)Little success in inducing callus and maintaining proper growth,(b)difficult to obtain suitable size of homogenous tissue from monocotyledonous plants.

R. retusa Bl. (Orchidaceae), a genus of fox tail orchid, is an important stem herb. The stem extract of R.retusa commonly known as 'Rasna'is used as expectorant for curing rheumatic diseases [Lawler, 1984]. Besides being victim of its own beauty & utility R. retusa is progressively losing its natural habitat and heading towards extinction particularly, in Sri Lanka [Wicramasingh, 1992] and conforming to these, in this paper, we report the possibility of using anthers for initiating in vitro cultures of R. retusa.

The study was designed to study the effects of physiological status,stage of microspore development and pre-cold treatment on androgenic response in Orchids. The anthers from open flowers (2 days of anthesis) in tetrad stage failed to respond despite variations in the chemical regime; whereas those from unopened buds (1.25-1.35cm long) with an intact operculum & pre-cold treatment with microspores in early & late-uninucleate decussate regenerated provided their nutritional complexities were satisfied through an exogeneous supply of Peptone (2mg/l) in the BAP (10mg/l) and NAA enriched Vij and Sharma, 2011 medium. The best response was obtained when a 24hrs cold treatment was employed at 4°c in darkness. The callus induction was promoted under darkness, but was inhibited by light. The complete plantlet (2-3 leaves &1-2 roots) was formed in 24wks.The plantlet was accilimatized & the survival rate is 70%.

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