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TISSUE CULTURE OF HIGH ALTITUDE MEDICINAL, RARE & ENDANGERED ORCHIDS-*Cremastra appendiculata* (D. Don) Makino, *Pleione maculata* (Lindl.) Lindl. & *Dienia muscifera* Lindl. OF ARUNACHAL PRADESH, INDIA

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Abstract

Regeneration through tissue culture of three medicinal orchids namely *Cremastra appendiculata* (D. Don) Makino, *Pleione maculata* (Lindl.) Lindl. and *Dienia muscifera* Lindl. was tried in ORC Lab, Tippi and successfully produced the plant materials. Young shoot of 20-30 mm long were used as explants. Murashige & Skoog (1962) media was used in which Ferric tartarate & Tricalcium phosphate are added. Excised and discarded axillary meristem from the base and culture the rest. When protocorm-like bodies, protocorms with occasional root and shoot-primordia, or callus are formed, subculture is initiated. There was a few variations (Scully, 1967) in chemicals, phyto-hormones & its application technique for each species.

INTRODUCTION

Arunachal Pradesh is known for rich diversity of orchids about 568 species (Rao, A .N. 2007) distributed from tropical to alpine forest. They are important aesthetically, medicinally and also regarded as ecological indicators (Joshi *et al*., 2009). Orchids are one of the ingredients in ancient Indian systems of medicine like Ayurveda. In the present studies three high altitude medicinal orchids namely *Cremastra appendiculata* (D. Don) Makino, *Pleione maculata* (Lindl.) Lindl. & *Dienia muscifera* Lindl. have been taken for mass production. They are rare and endangered species (Rao, A .N. , 2003). So it is desirable to establish an ideal protocol for tissue culture of these species. So far, there is no report of tissue culture of these three species. As a result, tissue culture of the species are undertaken with various stages of *in vitro* culture.

MATERIALS AND METHOD

During the month of July, August and September 2015, a number of field trips were conducted to various parts of Darjeeling, Sikkim and Arunachal Pradesh. As a result *Pleione maculata* (Lindl.) Lindl., *Cremastra appendiculata* (D. Don) Makino and *Dienia muscifera* Lindl. were collected from Saruk, Sessa orchid sanctuary, T-Gompa; altitude 986 M, 2418 M, 3650 M and geographical location N 27°04'09.6" & E 088°26'57.1", N 27°06'05.3" & E 092°24'48.4", N 27°43'09.1" & E 091°47'59.6" respectively. Young shoot of 20-30 mm long is used for explant. Removed the scale which covers bud. Rinsed & washed tissue with detol soap under running tap water. Then tissue is put in $Hgcl_2$ solution for half an hour. The concentration of solution is in the ratio of 40 : 1 ml (distilled water & $Hgcl_2$). $Hgcl_2$ is removed in 40 ml distilled water. Water on tissue surface is removed with air flow under spirit flame on sterilized petridish. The tissue was then inoculated in the media gently and cultured in growth chamber at 17°C under 2000 lux for 16-18 hours.

Media for initial culture of explants

Basal medium	Vitamins	Auxin (for 500 ml)	Cytokinin (for 500 ml)	Other organic compounds	Other chemicals	Name of species	Phytagel (in mg)
I (MS)	As in MS	NAA: 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm		*Cremastra appendiculata* (D. Don) Makino	3912
II (MS)	As in MS	NAA : 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm	$AgNo_3$: 1.5 ml	*Pleione maculata* (Lindl.) Lindl	3912
III (MS)	As in MS	NAA : 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm		*Dienia muscifera* Lindl.	3912
IV (MS)	As in MS	NAA : 0.5 ml IAA : 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm	Ferric tartarate (1.5 ml) & Tricalcium phosphate (2.5 ml)	*Dienia muscifera* Lindl., *Pleione maculata* (Lindl.) Lindl., *Cremastra appendiculata* (D. Don) Makino	3912

Media for subculture

Basal medium	Vitamins	Auxin (for 500 ml)	Cytokinin (for 500 ml)	Other organic compounds	Other chemicals	Name of species	Phytagel (in mg)
I (MS)	As in MS	NAA : 0.5 ml IAA : 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm	Ferric tartarate (1.5 ml) & Tricalcium phosphate (2.5 ml)	*Dienia muscifera* Lindl., *Pleione maculata* (Lindl.) Lindl., *Cremastra appendiculata* (D. Don) Makino	3912
II (MS)	As in MS	NAA: 0.5 ml	BAP: 1.5 ml	Sucrose 15 gm, coconut oil 5%		*Pleione maculata* (Lindl.) Lindl	3912

Various stages of tissue under *in vitro* culture regenerated from orchid plants

A: Habit of *Dienia muscifera*-terrestrial B:Habit of *Cremastra appendiculata*-terrestrial A1,B1 & C1: Tissues from explants
C: Habit of *Pleione maculata*-epiphytic A3, B3 & C3 : Seedlings
A2, B2 & C2 : Protocorms

RESULTS AND DISCUSSION

Micropropagation of desirable tissue under aseptic condition is one of the methods for mass production. *In vitro* culture of the species was successful. In the initial culture of *Pleione maculata* (Lindl.) Lindl. 1.5 ml $AgNo_3$ and 1.5 to 2 ml vitamins were added to 500 ml of Murashige & Skoog culture media. Tissue of 10-20 mm long is divided within 62 days after fresh inoculation. For micropropagation, just after autoclaving, protocorms were inoculated in the media containing one each of BAP(15 µl), IBA(10 µl), NAA(10 µl) and TDZ (5 µl). It is found that Cytokinin and TDZ added media shows early response resulting to proliferation of tissue to protocorm-like bodies, protocorms with primordial leaf and root or callus formation. Protocorms of *Pleione maculata* (Lindl.) Lindl. were inoculated in the basal MS media. There was no good response in the media and all the tissues died gradually.

Protocorms were inoculated in an another MS media in which Ferric tartarate (1.5 ml) & Tricalcium phosphate (2.5 ml) was added. Ideally, it is found that response of the tissue to the media within 60 days was very good. Initial culture of *Cremastra appendiculata* (D. Don) Makino and *Dienia muscifera* Lindl. has been carried out in MS media. Tissue of 10-20 mm long of *Cremastra appendiculata* (D. Don) Makino is divided longitudinally into two within 180 days where as tissue of *Dienia muscifera* Lindl. within 150 days for transformation of tissue to protocorm-like bodies, protocorms or callus formation. In case of *Cremastra appendiculata* (D. Don) Makino, it is found that response of tissue to media was good but whole margin of leaf of seedling turn brown. But response of tissue of *Cremastra appendiculata* (D. Don) Makino and *Dienia muscifera* Lindl. in Ferric tartarate and Tricalcium phosphate added MS media has been found equally better and brownish margin of leaf of *Cremastra appendiculata* (D. Don) Makino disappeared.

Later, this Ferric tartarate and Tricalcium phosphate added MS media is used for micropropagation of all three species of *Pleione maculata* (Lindl.) Lindl., *Dienia muscifera* Lindl. and *Cremastra appendiculata* (D. Don) Makino. Hence, this is considered as new efficient culture media.

MEDICINAL USES

In Arunachal Pradesh more than 100 species are now found to have medicinal value and few orchids are used for treatment of cut injury and bone fracture. Asthavarga an important ingredient in many classical formulations viz. Chavyanprasa is reported to contain *Malaxis muscifera* (Lindl.) Kuntze or *Dienia muscifera* Lindl. (Singh and Duggal, 2009). The species is also useful in sterility, seminal weakness, dysentery, fever and general debility as a tonic. Decoction of tuber of *Malaxis muscifera* (Lindl.) Kuntze or *Dienia muscifera* Lindl. is used as tonic to strengthen kidneys (A Nageswara Rao, 2003). *Pleione maculata* (Lindl.) Lindl. is used for liver and stomach ailments (Bijaya Pant, 2013). In China *Cremastra appendiculata* (D. Don) Makino is used externally to heal boils, swelling, to remove freckles and as an antidote to snake bites and in Japan the species is used to assuage toothache.

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