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In Vitro Propagation and Phytochemical Screening of *Papilionanthe teres* (Roxb.) Schltr.

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Abstracts

Papilionanthe teres (Roxb.) Schltr. is a medicinal orchid found in all reserve forests of Southern Assam, India. Mature pods of Papilionanthe teres were collected from natural plants and seeds were inoculated in Murashige and Skoog (MS), Knudson C (KnC), Commercial Orchid Maintenance(OMM) and Commercial Orchid Maintenance Replate (OMR) medium(HiMedia). Best germination and growth were observed in commercial orchid maintenance replate medium supplemented with 2mg\L IAA and 5mg\L KN. Growth of root, shoot were measured after three months of germination. Shoot tip and nodal explant of the plant were tried in different medium viz. MS, White, B5, KnC, Commercial Orchid Maintenance medium etc. containing different growth regulators modification for micro propagation. For phytochemical screening qualitative tests were performed from the dried powder of the plants. From the qualitative test it was observed that the plant extracts contain alkaloid, flavonoid and tannin in petroleum ether extract and alkaloid, tannin and saponin were found in ethyl acetate extract. Steroid was found present in methanol and benzene extract. Flavonoid was found only in acetone extract

Key words: *Papilionanthe teres, in vitro,* phytochemical screening.

Introduction

Orchid constitutes an order of royalty in the world of ornamental plants. They are of immense horticultural importance and also play a very useful role to balance the forest ecosystems (Kaushik, 1983). In India, orchids form nine percent of flora and about 1300 species are found in Himalayas with others scattered in Eastern and Western Ghats (Jain, 1980). The *Orchidaceae*, by far the largest family of the plant kingdom, comprises more than 30,000 species in approximately 750 genera, and is one of the most widespread of all plant families; there are terrestrial, saprophytic and epiphytic species. The use of orchids in herbal medicine has a very long history. A total of 365 plants, including several orchids are listed in the earliest known Chinese Materia Medica (Kong,*et al.*,2003).Orchids have great medicinal value. *Rig Veda* and *Atharva Veda* which are known to be the oldest books provide inquisitive information about medicinal value of orchids (Kaushik,1985). Several species of orchids, e.g. *Dendrobium macrei, Orchis latifolia, Vanda roxburghii*, and *Pholidata palida* are widely used in manufacturing different types of Ayurvedic medicines (Withner *et al.*, 1974 ; Maheswari *et al.*, 1978; Hedge, 1984; Kaushik and Kishore, 1995). *Microstylis walliachi* is used in treatment of tuberculosis .Juice of *Dendrobium ovatum* is helpful in all kinds of stomachache, bile secretion and is used as a laxatives (Kirtikar & Basu,

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1975).*Dendrobium nobile* is used in freshly cut wounds for quick healing .Leaves of *Chleisostoma williamsoni* are used for bone fracture by Monpa tribal of Arunachal Pradesh. *Eulophia noda* tuber extracts are used for blood purification. The native inhabitants of some areas use seeds of Cymbidium *madidum* and pseudo bulbs of *Dendrobium tukai* as oral contraceptive (Bose *et al.*, 1989). In China orchids are used to improve appetite, to stimulate gastric secretion and to promote general health.

As early as 1892, E de Wildeman had already begun investigation of orchid alkaloids in domesticated European orchid species as well as *Dendrobium nobile* and *Phalaenopsis lueddemanniana*. From then until 1896, E de Droog analyzed 104 species in 78 genera. In late 1890s, W Boorsma studied orchid alkaloids at the Bogor Botanical Gardens and detected some in *Paphiopedilum javanicum* and *Liparis parviflora*, among other species .All together about 60 alkaloids from Orchidaceae have been isolated .The interesting ones are nobiline from *Dendrobium nobile*, laburine from *Liparis bicllosa*, malaxine from *Malaxis congesta* and phalaenopsin from *Phalaenopsis manii.*

In vitro seed germination has become an important technique for propagation of a large number of Orchids (Arditti *et al.*, 1982) as well as other crops (Murashige & Skoog, 1962).The orchid seed germination in nature is less than 3%. A single orchid capsule contains millions of seeds, which lack any metabolic machinery and do not have any endosperm. In spite of a very large number of seeds produced, only few seeds germinate in nature (Arditti, 1981). Moreover natural germination orchid seeds require mycorrhizal association and in most cases it does not receive this association.

Papilionanthe teres is an epiphytic orchid having beautiful pink flowers occurs in reserve forests of Assam. Like other orchids this plant is also threatened due to pollution and felling of big trees. *Papilionanthe teres* has also medicinal importance. Paste obtained by crushing aerial roots applied locally as poultice over fractured bones for joining by the Reang Tribe (Dutta Choudhury, 2000). The species is distributed in West Bengal, Assam, Meghalya, Tripura, Andaman & Nicober Islands, tropical valley of Sikkim, India.

In the present study, an attempt was made to have a mass propagation of an orchid, *Papilionanthe teres* within a short span of time as it is medicinally and commercially important. Seeds of *Papilionanthe teres* were cultured in MS medium supplemented with different growth regulators and shoot regeneration was attempted using different explants like shoot tips, nodes, and root tips. The qualitative phytochemical screening of this medicinally important orchid has also been undertaken.

Materials and methods

A) In Vitro Propagation

1. Sterilisation of the spores \ Explants:

(a) Mature pods of *P. teres* were collected from natural conditions. The spores were surface sterilized with 6% Sodium hypochlorite (4% active chlorine) and absolute alcohol. These were then kept in laminar air flow UV chamber for 30 minutes. The pods after 30 minutes were splitted open and the powdery seeds were transferred to various media like MS, B5, White, KnC, OMR and OMM with different growth substances.

(b) Nodes shoot tips and root tips of *P.teres* were first washed vigorously with distilled water and then surface sterilized with 0.1% mercuric chloride. The explants were again washed with distilled water and then transferred to various media like MS, 35, KnC etc with different growth regulators.

(c) The P^H of the medium was adjusted at 5.6 levels. The seeds were inoculated in the media. The inoculation operation was performed under the laminar air flow chamber to check the contamination by microorganisms. After inoculation, the flask containing orchids seeds were incubated inside the tissue culture racks at 25 ± 1^0C temperature. Observations were made every alternate day.

Transfer of the culture was made every fortnightly. The protocorms of orchids were transferred to

the flask containing fresh medium.

The intensity of light was maintained at 1600 lux. The cultures were maintained at 16 hour photo period and 8 hour dark period.

(d) Transplantation: After 6 month of growth the plantlets were transferred to earthen pots containing following potting mixtures:

i) Brick bats + sand + charcoal (1:1:1).-Mixture 1
ii) Brick bats + sand + charcoal + Roots of Water hyacinth (1:1:1:1) – Mixture 2
iii) Brick bats + sand + charcoal + coconut husk.-Mixture3

The plantlets were thoroughly washed with distilled water to remove all medium stuck to roots of the plantlets otherwise it may invite contamination by microbes and consequently infection by microbes may cause less survival of plants. After through wash the plantlets are dipped in broad spectrum fungicide Bavistin (2gm/ litre) for 2 minutes and then transplanted to respective potting mixture (Mazumder and Bhowmik, 1999). The pots containing plantlets are the covered with polythene bags having some holes to check excess of transpiration. The transplanted sprayed with liquid medium (with out agar and sugar) for first 15 days and then with plane water.

B) Phytochemical Screening:

Soft tissues from the stem of *P. teres* (Roxb.) Schltr. plant were collected first and air dried .The dried stems were then powdered and the powdered materials were than kept in different solvent system likely- petroleum ether, ethyl acetate, acetone benzene and methanol separately for 72 hours and the extracts were collected. Qualitative tests for alkaloid, reducing sugar, steroid, flavonoid, saponin and tannin have been done using standard protocol.

Table: 1; Protocol for Qualitative Tests

TEST SAMPLE	TEST SOLUTION	EXPECTED OBSERVATION	INFERENCES
2 ml plant extracts+ 0.2ml dilute HCL	0.1 ml iodine solution	Reddish Brown ppt	Alkaloid Present
5 ml plant extracts	5ml Fehlings A & B solution	Brick red colored ppt	Reducing sugar Present
5 ml plant extracts hydrolyzed with 10% H_2SO_4 & extracted with ether	a. 1ml dilute ammonia solution b.1ml dilute sodium carbonate c.1ml dilute sodium hydroxide solution	Greenish yellow ppt. Pale yellow colour Yellow colouring	Flavonoid Present
10mg of plant extracts in 5 ml glacial acetic acid	1drop conc. H_2S0_4	Reddish ring at the bottom	Steroid present
5 ml plant extracts added to water & shaked vigorously		Stable froth	Saponin present
5 ml plant extracts	1ml 10% $K_2Cr_2O_7$	Yellowish brown ppt.	Tannin present
5 ml plant extracts	1ml 10% $FeCl_3$	Green or black colouration	Tannin Present

Result and discussion

After 45 days of inoculation of orchid seeds germination was observed in different media like, KnC OMR and OMM. MS and B5 medium did not respond for germination. The result of germination was found to be as in Table- 2

Table-2: Result of Media Tried

Murashique & Skoog	-
B5	-
Kn C	+
OMM	++
OMR	+++

(+)→ Responded, (+ +) → Good respond (+ + +) → Excellent, (-)→ no response

Table-3 Composition of Commercial Orchid Maintenance \Replate Medium

Ingrediants	mg\ml
Potassium nitrate	950
Ammonium nitrate	825
Calcium chloride 2 H_20	220
Magnesium sulphate	90.34
Potassium phosphate monobasic	85
Manganese sulphate H_20	8.45
Boric acid	3.1
Potassium iodide	0.42
Molybdic acid, 2 H_20	0.125
Zinc sulphate,7 H_20	5.3
Copper sulphate,5 H_20	0.0125
Cobalt chloride. 6 H_20	0.0125
Ferrous sulphate,7 H_20	27.8
Na_2-EDTA	37.3
Myo-inositol	100
Thiamine HCL	10
Pyridoxine HCL	1
Nicotinic acid	1
Peptone from meat	2000
Banana powder	30000
Sucrose	20000
MES	1000
Agar	7000

MS medium supplemented with certain concentrations of plant growth regulators influenced on seed germination, production of protocorms like bodies, shoot multiplication and root initiation (Kalimuthu, *et al*., 2007). In the present study ,the protocorms like bodies (PLBs) grown in OMM and OMR media were transferred to commercial OMM media & Commercial OMR media supplemented with and without 2mg \ L IAA + 5 mg \ L KN. It was observed that OMR media supplemented with 2mg \ L IAA + 5 mg \ L KN showed better result after two months of inoculation in terms of growth (Table 4)

For subsequent development (of *in vitro* orchid seeds into seedlings), several modifications were made in the media by changing the ingredient and their quality and quantity. The most important development in culture media was the incorporation of growth regulators like auxins and cytokinins ((Kalimuthu, *et al*., 2007.).Various growth regulators and various concentrations of growth hormones used in an attempt to promote seed germination and seedling growth. In majority of the cases auxins (mostly NAA, IAA and IBA) enhanced the germination and seedling growth Arditti, 1979). Higher degree shoot proliferation was induced in the shoot segments of three epiphytic orchids, *Cymbidium aloifolium*, *Dendrobium aphyllum* and *Dendrobium moschatum* (on Murashige and Skoog medium (MS) containing N^6-benzyladenine (BA) or thidiazuron (Nihar *et a*l., 1998).In the present study,

Table-4; Plant growth observed after 2 months of inoculation of PLBs with and without growth hormones (IAA & Kinetin)

Sl no.	Media	Average Shoot length in cm	Average Root length in cm	Average Fresh Weight in mg
1	OMR + 2mg\L IAA + 5mg KN	3.54	1.52	52
2	OMR	2.45	0.8	35

Table-5: Qualitatative phytochemical screening of *Papilionanthe teres*

Extractives	Alkaloid	Reducing sugar	Flavonoid	Steroid	Saponin	Tannin
PET	+	-	-	-	+	+
EtOAc	+	-	-	+	+	+
MeOH	+	+	+	+	+	+
Benzene	-	-	+	-	-	-
Acetone	-	-	+	-	-	-

kinetin (2mg\L & 5mg\L respectively). The quantitative tests of the dried stems of *P.*

(+)=Presence, (-) = Absence, PET=Petroleum ether, EtOAc –Ethyl Acetate, MeOH - Methanol.

measurement of plantlets in above medium it is observed that media supplemented with growth regulators IAA and kinetin showed better growth. The auxin and cytokinin played an important role in development of plantlet.

The explants i.e nodes and shoot tips from *P. teres* were inoculated in media like MS, B5, OMM etc but they failed to respond in all media except MS medium supplemented with 3mg/L 2,4-D. Here nodes produce many multiple shoots after 30 days of transfer. Generally, medium supplemented with 3mg/L 2, 4-D initiate callus but in our case it produced multiple shoots.

Plantlets transplanted to different potting mixtures showed good response in terms survival. Potting mixture containing Brick bats + sand + charcoal + Roots of Water hyacinth (Mixture - 2) showed 85 % survival followed by potting mixtures Brick bats + sand + charcoal + coconut husk (Mixture – 3) and Brick bats + sand + charcoal (Mixture-1) (73 and 65 % respectively). The potting mixture containing water hyacinth roots showed excellent result may be due to water hyacinth roots contain auxin which is supplied to the growing plants.

Preliminary phytochemical screening showed wide range of chemical constituents in *P. teres*.

Flavone C-glycosides are most common in the tropical and subtropical species of the *Epidendroid* and *Vandoid* subfamily (63% contain them), whereas flavonol glycosides are found in *Neottioid* orchids (78% have them). Other flavonoids are less common (Williams, 1979). In late 1890s, W Boorsma studied orchid alkaloids in *Paphiopedilum javanicum* and *Liparis parviflora*, among other species. In the present study, alkaloid was found present in all extracts except benzene & acetone. Test of reducing sugar showed negative result in all extracts except in methanol. Methanol, benzene & acetone extracts showed positive result for flavonoid. While

A B C D E F

Plate1:A.*Papilioanthe teres* flower.B&C. Plants cultured in OMR media.D.Plants in OMM medium& F.Plants acclimatized in Mixture 1,2 &3 respectively.

performing tests for steroid, saponin and tannin, ethyl acetate and methanol extracts gave positive result. Saponin and tannin was also found present in petroleum ether extracts.

Conclusion

From the *in vitro* propagation of *P. teres* it can duly inferred that Commercial Orchid Maintenance \Replate medium is excellent for the growth of *P. teres* supplemented with IAA and *P. teres* showed the presence of alkaloid, reducing sugar, steroid, flavonoid, saponin, tannin. In latter stage, these phytochemicals can be separated and isolated using column chromatography and PTLC, HPLC techniques. Bioactive compounds can be utilized for preparation of drugs after performing sequence of microbial and biochemical tests.

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