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In vitro Micro propagation of Medicinal plant *Abroma Augusta L.* (Ulatkambal)

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Abstract: *Tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries in all over the world. Callus cultures were established from shoot tit explants of diploid Abromaaugusta (Ulatkambal) on a modified basal medium of Murashige and Skoog (1962) supplemented with either 2 mg/l^{-1} 2,4-D+15% (v/v) CM or 4 mg/l^{-1} 2,4-D+2 mg/l^{-1} NAA+2 mg/l^{-1} KN+1 g/l^{-1} YE. Shoot primordia developed after 2–3 subcultures in that medium. Increased growth of shoot primordia was obtained in media containing less auxins and vitamins. Rooted bulbous plantlets obtained were maintained in MS medium with 0.5% sucrose. Adventitious shoots were induced from ad axial epidermal cells of outer scales of regenerated bulbs used as secondary explants in presence of 1 mg/l^{-1} of 2, 4-D with slightly higher concentration of the three vitamins of MS medium. From each scale leaf, approximately 400 bulblets were produced in 18 weeks in liquid culture. 90% of the plants transferred to potted soil have survived. The micro propagation protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development.*

Keywords: *Abromaaugusta, Root tip, shoot tip, Regeneration, Transplantable Plantlets, Medicinal, Explants*

I. Introduction

Among the south Asian countries, Bangladesh has a rich and prestigious heritage of herbal medicines. Out of 500 species of medicinal plants about 250 species are used for the preparation of traditional medicines in Bangladesh. Several Abromaaugusta species have been investigated for in vitro regeneration [6-9].

Since ancient times, mankind has been dependent on plants for food, flavours, medicinal and many other uses. Ancient written records of many civilizations (i.e. Egyptian, Roman, Chinese) give strong evidence regarding use of medicinal plant [1],for example ayurveda documents record the use of medicinal plants to cure many ailments [2-3]

Abromaaugusta (Ulatkambal), a member of malvaceae, which is medicinally important for bufadienolide content, is not amongst those examined. Chemical analysis of different cytolyses has revealed strains with elevated cardiac bufadienolide contents [10]. The objective of the present study was to establish a method for in vitro multiplication of the high yielding diploid cytolyses[11-13].

The use of herbal medicines is growing in developed countries, presently 25% of the UK population use herbal medicine [4-5]. Similar trends in medicinal plant research were also seen in the other parts of world [15]. Due to successful research carried out during this period plant tissue culture is an established field in plant cell biotechnology.

II. Materials

- 1 Vial of Murashige Skoog (MS) media. (If you wish to make up your own growing medium you could use the recipe for the Murashige medium given at the end of this section.), 1 L sterile distilled water
- 1.5 L or 2 L container in which to prepare the growth medium, 10 g of agar/L, 30 g sucrose/L
- small amounts of 1M NaOH and 1M HCl to adjust the pH of the media
- 60 flat bottom culture tubes with closures, Glass aquarium or box lined with plastic
- Plastic sheet to cover the top of the aquarium Adhesive tape, 2 or 3 beakers or jars of sterile water
- 10% Bleach in a spray bottle, 70% alcohol in a spray bottle
- Forceps or tweezers, Gloves, Pressure cooker, Beaker or jar in which to wash the plant material
- Cutting equipment such as a scalpel blade or razor blade, Detergent mixture 1ml detergent per liter of water

- ► 2 bottles of sterile distilled water (purchase at the grocery store)
- ► Your chosen plant (cauliflower, rose, African violet or carnation)
- ► Paper towel for cutting on or sterile petri dishes if available
- ► Bleach sterilizing solution dilutes commercial bleach (56% sodium hypochlorite) to a final concentration of 1-2% sodium hypochlorite in distilled water in a large beaker or jar.
- ► A well-lit area away from direct sunlight or use full-spectrum gro-lights
- ► Hormones such as BAP (benzyl amino purine) and NAA (naphthalene acetic acid) to stimulate growth and root development, respectively

Murashige Minimal Organics Medium recipe (MMOM)

Inorganic salts	**mg/L**
NH4NO3	1,650.00
KNO3	1,900.00
CaCl2 (anhydrous)	332.20
MgSO4 (anhydrous)	180.70
KH2PO4	170.00
Na2EDTA	37.25
FeSO4.7H2O	27.80
H3BO3	6.20
MnSO4.H2O	16.90
ZnSO4.H2O	5.37
KI	0.83
Na2MoO4.2H2O	0.25
CuSO4 (anhydrous)	0.016
CoCl2 (anhydrous)	0.014
Sucrose	30,000.00
I-Inositol	100.00
Thiamine.HCl	0.40

The pH is adjusted to 5.7 using 0.1 M HCl or NaOH.

III. Methods

One high yielding diploid cytotype of Abroma*augusta* (Ulatkambal) collection from Botanical garden (Bangladesh) was used in this study. Only undamaged root tip or shoot tip were taken and the outer dry and dirty scales were peeled off and rejected.

Lower halves of root tip or shoot tip containing the basal disc were then cut into 4 sectors and washed thoroughly in 5% teepol for 15 min. The quarters were surface sterilized with 0.1% mercuric chloride for 25 min followed by five washed with sterile distilled water. Finally, the material was immersed in 70% ethanol for 1-2 min. The quarters were dried with sterile filter paper.

From each sector (quarter),2-5 explants were dissected, the scalpel blade being dipped in 90% ethanol and flamed between cuts. Each explant (5-8 min high and 2-3 min wide) consisted of 3-5 scales joined at the base by a small piece also disc. Individual scales of the same size or 2-4 mm^2 axialdiscs were also used as explants.

Secondary explants

Individual outer scales and longitudinal quadrant (3-4 mm wide) of shoot tip regenerated in vitro were used as secondary explants

Nutrient medium

Thebasal nutrient medium (BM) was Murashinge and Shoogs (1962) medium supplemented with 10 mg/l^{-1} thiamine HCl,5 mg/l^{-1} nicotinic acid, 1 mg/l^{-1}pyridoxine HCl, various combinations of auxins and cytokinins viz. 2, 4 dichlorophenoxyacetic acid (2,4 D), α-naphthaleneacetic acid (NAA), indoleacetic acid (IAA),indolebutyric acid (IBA), 6-furfurylaminopurine (kinetin, Kn) and 6-benzylaminopurine (BAP) with or without coconut milk(CM) and yeast extract (YE). The pH of the media was adjusted to 5.6,solidified with 0.6% agar and sterilized for 15 min at 1.05Kg/cm^2pressure.

Explants were placed in culture tubes containing 25 ml medium and sub cultured after 28 days. For liquid culture, secondary explants were culture in 250 ml conical flasks containing 50 ml medium on a rotary shaker (70 rev/min).

Fig. 1: Abroma *augusta species culture in* culture tubes under Aseptic condition

Each experiment was set up with ten replicates and repeated thrice. The cultures were grown at 22-25°C and maximum relative humidity of 55-60% and under Phillips fluorescent day light tubes emitting 3200 Lux for 16/8 h light/dark period.

Histological and cytological preparation

Sample for histological study were fixed in formalin-acetic acid alcohol, dehydrated in tertiary butanol series of johansen and embedded in paraffin wax. Sections were cut at 10 µm and stained following Foster's tannic acid/iron chloride method.For cytological study, shoot tips were pretreated and stained following aceto-orcein technique.

IV. Results and Discussion

Induction of callus from field grown plants. Isolated axial disc and individual scales (outer and inner) did not respond to any cultural condition but explants consisting of 3-5 scales attached to the axial disc did so with eighty percent proliferating in 15-20 days. Callus is actively dividing non-organized tissue of undifferentiated and differentiated cells often developing from injury (wounding) or in tissue culture [16]

After development and sufficient growth of roots, the rooted shoots were planted in small poly bags containing garden soil and compost in the ratio 1:1. The bags were covered with a plastic film to retain moisture and gradually acclimatized to outdoor condition. The ultimate survival rate of the transferred plantlets to soil was about 90% and their growth in such condition was satisfactory. Mortality of about 10% occurred during transplantation of plantlets due to shifting between containers, injuries to the root system and excessive evaporation [14]

Callus induction was confided to the basal part of each scale in proximity with the disc. None of the auxins alone viz., 2,4-D,NAA, IAA, and IBA in the concentration.

V. Conclusion

Plants are rich sources of pharmaceutically important compounds; but there is a need to synthesis these compounds within laboratory conditions. Micropropagation is an important technology since many secondary plant metabolites can't be synthesized chemically. Many plant species are undiscovered and their medicinal properties unknown; and even the medicinal remedies past down from generations are being lost. Further research and conservation of all plant species including medicinal plants is needed to preserve nature's natural drugs. Advances in plant tissue culture will enable rapid multiplication and sustainable use of medicinal plants for future generations. Tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries

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