



Micropropagation of Some Medicinal Plants: An Overview

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KEYWORDS:-*Life science, Plant and animal science, Plant Tissue, Traditional Health*

INTRODUCTION

"There is nothing in this universe, which is non-medicinal, which cannot be made use of for many purposes and by many modes." This definition rightly suggests that "in principle" all plants have a potential medicinal value, although in practice a plant is referred to as medicinal when it is so used by some system of medicine. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. Early humans recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Physical evidence of use of herbal remedies goes back some 60,000 years to a burial site of a Neanderthal man uncovered in 1960 (Solecki, 1975). All cultures have long folk medicine histories that include the use of plants. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The first written records detailing the use of herbs in the treatment of illness are the Mesopotamian clay tablet writings and the Egyptian papyrus. About 2000 B.C., King Assurbanipal of Sumeria ordered the compilation of the first known materia medica—an ancient form of today's United States Pharmacopoeia—containing 250 herbal drugs (including garlic, still a favorite of herbal doctors). The Ebers Papyrus, the most important of the preserved Egyptian manuscripts, was written around 1500 B.C. and includes much earlier information. It contains 876 prescriptions made up of more than 500 different substances, including many herbs (Ackerknecht, 1973). About one-quarter of the prescription drugs dispensed by community pharmacies in the United States contain at least one active ingredient derived from plant material (Famsworth and Morris, 1976). The widespread use of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. The use of traditional medicine and medicinal plants in most developing countries has been widely observed. According to the World Health Organisation (WHO) more than 1 billion people rely on herbal medicines to some extent. The WHO has listed 21,000 plants that have reported medicinal uses around the world. India has a rich medicinal plant flora of some 2500 species. Of these, 2000 to 2300 species are used in traditional medicines while at least 150 species are used commercially on a fairly large scale. India and Brazil are the largest exporters of medicinal plants (Hanfee, 1998). WHO estimates that about 80% of the rural population living in developing countries relies almost exclusively on traditional medicine for their primary health care needs (Bannerman et al., 1993). In almost all traditional medicine systems, medicinal plants play a major role and constitute the backbone of traditional medicine. Indian people had an incredible knowledge of phyto-medicine driven apparently by a tremendous passion for the study of medicinal plants. This is evident both in the living folk traditions in the rural communities as well as the scholarly traditions of the codified knowledge systems - i.e., Ayurveda, Siddha Unani etc. India has a rich medicinal plant flora of some 2500 species. Of these, 2000 to 2300 species are used in traditional medicines while at least 150 species are used commercially on a fairly large scale (Hanfee, 1998). At the folk level, in every ecosystem from the trans-Himalayas to the coast, local communities have keenly studied the medicinal plants found in their locality. The maximum numbers of medicinal plants are utilized by the folk traditions, followed by Ayurveda, Siddha, Unani, Homeopathy, Tibetan and Modern respectively (Shankar et al, 2000). The Indian materia medica includes about 2000 drugs of natural origin, almost all of which are derived from India's different traditional systems and folklore practices (Narayana et al., 1998). Out of these drugs derived from traditional systems, 400 are of mineral and animal origin while the rest are of vegetable origin. India has a rich heritage of traditional medicine and traditional health care systems have been flourishing for many centuries. According to Hindu mythology there are four Veda written by the Aryans: Rig Veda, Sham Veda, Yayur Veda, and Atharva Veda. Among these the Rig Veda, the oldest, was written after 1500 BC. Ayurveda is said to be an Upaveda of Atharva Veda whereas the Charaka Samhita (1900 BC) devoted to the concept of the practice of Ayurveda. Sushruta Samhita (600 BC), which emphasizes surgery. Ayurveda was divided into eight sections or "Astanga" (Mukherjee, 2001)

OBJECTIVES AND INTRODUCTION TO THE TAXA

The present study was undertaken with three taxa of high medicinal value viz.

1. Bacopamonnieri Linn. Pennell

2. *Boerhaviadiffusa* Linn.
3. *Plumbagozeylanica* Linn.

The study was conducted with the following objectives:

1. To standardize protocol for micro propagation of all the three taxa for large scale propagation and conservation of the elite clones. The various methods of micro propagation investigated in the present study are:
 - Plant regeneration through callus mediated indirect organogenesis.
 - Plant regeneration through somatic embryogenesis in cell suspension cultures in *Bacopamonnieri*.
 - Plant regeneration through direct organogenesis.
2. Comparison of the phytochemical profile of the cultured roots of *Plumbagozeylanica* with roots of the field grown plants by the process of High performance thin layer chromatography (HPTLC). Comparison of the phytochemical profile of the in vitro raised plants with the field plants of both *Bacopamonnieri* Linn, and *Boerhaviadiffusa* Linn Pennell by TLC and HPTLC

***Boerhaviadiffusa* Linn:**

In the present work with *Boerhaviadiffusa*, for the first time an efficient protocol of large scale shoot multiplication by exploiting the regeneration potentialities of the nodal explants is established. Since direct shoot induction through axillary proliferation did not produce large number of shoots, callus mediated indirect organogenesis was adopted to increase the number of shoots. Callus was produced from leaf, internodal and nodal explants on media supplemented with different concentrations and combinations of PGRs. The callus was subcultured on media supporting shoot induction and proliferation. It was found that only the nodal segment- derived callus responded to shoot induction. Large scale shoot proliferation was achieved only on media supplemented with BA/Kn and Ads. Inclusion of Ads in the shooting media promoted rapid shoot induction and multiplication. Ads worked well at a higher concentration. Its positive effect might be due to its metabolic change into some favorable cytokinin as being an adenine derivative it can be the precursor for a number of cytokinins. In the present investigation the callus subcultured on Ads supplemented media consistently showed excellent response for shoot differentiation and multiplication. Roots were either spontaneously induced on the shoot induction media (SIM) or were produced on rooting media supplemented with IAA/ IBA. The cultured plantlets were acclimatized and were transferred to field where they are showing healthy growth without any morphological variations. The phytochemical profile of the cultured plants was compared with the field grown plants by thin layer chromatographic techniques (TLC) and was found to be matching. In the present protocol, large number of shoots were obtained from a single node within a short period of time which can be a useful method for large- scale propagation and ex situ conservation of the elite plants.

***Bacopamonnieri* (Luin)Pennell:**

Large numbers of shoots were directly induced on the leaf, internodal and nodal segments in *Bacopamonnieri*. Although the leaf explants were found to exhibit maximum potential for direct shoot regeneration on media supplemented with BA and IAA, but in the present investigation, nodal segments were also found to show good shoot regeneration on media supplemented with same media combinations. In the present work it has been shown that the number of shoots produced by the nodal segments was higher than that produced with TDZ in previous work with this plant. Since better result was obtained with much cheaper PGRs the protocol established in the present investigation is cost effective. In the present work it was found that although the number of shoots produced in solid media in callus mediated organogenesis was not as good as that produced in direct organogenesis, but the nodal segment-derived callus showed very good shoot multiplication on liquid MS media producing large number of shoots. The protocol for 150 large scale shoot multiplication of the nodal segment-derived callus in liquid (stationary) MS media is reported for the first time. In the present work for the first time somatic embryogenesis was achieved on suspension culture where the leaf segment derived callus produced maximum number of embryoids. Previous reports of somatic embryogenesis of *Bacopamonnieri* was achieved on solid media. The total number of embryoids produced in the process is not reported in the previous protocol however suspension culture is known to be superior in producing higher number of embryoids. In the present work, the somatic embryos reached the germinating stage in the induction media and afterwards these were transferred to the shoot induction media. The somatic embryos produced in this process can also be used for preparing synthetic seeds. Phytochemical profile of the cultured plant was analysed and compared with the field grown plants through the process of HPTLC. The profile was found to be identical. In the TLC scan three more additional peaks were obtained from the cultured plants which might be some new compounds or some intermediates. The cultured plants were easily acclimatized and showed 100 % survival.

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