1. INTRODUCTION

Plants remain a major source of pharmaceuticals and over 80% of the approximately 30,000 known natural products are of plant origin. As many as 121 clinically useful prescription drugs have been derived from plants and 75% of the world’s population relies on plants for traditional medicines even today (Gómez-Galera et al., 2007; Ramachandra Rao and Ravishankar, 2002). The isolation of morphine (‘principium somniferum’) by Friedrich Wilhelm Sertürner about 200 years ago is generally accepted as the beginning of scientific phytochemistry (plant secondary products research). For about 150 years, this research almost exclusively addressed the isolation and structure elucidation of new plant products. It had great impact on the development of modern organic chemistry and pharmaceutical industry and provided the chemical basis for biological research on plant secondary metabolism, which began about 50 years ago (Hartmann, 2007). Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment, besides controlling essential functions of growth and reproduction, and adding to their therapeutic potential (Molyneux et al., 2007). The medicinal properties of plants are generally conferred by the secondary metabolites they produce and the extraction of these compounds from the plants is very taxing as it leads to depletion of their natural populations. Innovative conservation solutions will be required if the impending wave of extinction is to be averted. Major threatening processes are varied, and include land clearance, salinity, burning, weed encroachment, disease and pests (Swarts and Dixon, 2009). Plant in vitro cultures are able to produce and accumulate many medicinally valuable secondary metabolites (Matkowski, 2008). The genetic manipulation of plants together with the establishment of in vitro plant regeneration systems facilitates efforts to engineer secondary product metabolic pathways (Gómez-Galera et al., 2007). In recent years there has been a dramatic increase in the application of plant biotechnology for the production of a variety of commercially valuable simple and complex biological molecules for use in human and animal healthcare (Teli and Timko, 2004). In this connection, plant cell and tissue culture, an alternative method to extract the secondary metabolites in vitro, has proved beneficial as it enables their increased production in lesser time, in addition to saving the precious natural populations of the medicinal plants.
A large number of medicinally and economically important plants have been propagated by using either shoot-tip or other explants for the production of secondary metabolites. Shoot and root cultures have been successfully tapped for extracting artemisin, atropine, vindoline, quinine, withanolides, betalaines, indigo and indirubin, hyoscyamine, volatile oils, solasodine, and tropane alkaloids (Heble, 1985; Kang et al., 2004). The technique, however, has limited utility for the production of compounds at a commercial scale, being successful in only a few cases like, the production of anti-inflammatory drug, shikonin, from cell cultures of Lithospermum erythrorhizon, berberine from Coptis japonica and sanguinarine from Papaver somniferum (Ramachandra Rao and Ravishankar, 2002). In most of the cases, the rate of production of the desired compound is very poor. To maintain elevated productivity, it is necessary to screen repeatedly for the desired product, or else the culture reverts to lower productive capacity; this inherent instability is associated with changes at the genomic levels leading to variable patterns of gene expression (Rao and Ravishankar, 2002). Efforts to commercially exploit native plant secondary metabolite production patterns in cell culture systems have been largely thwarted by the repression of secondary metabolism under growth-oriented culture conditions (Ellis, 1986). A development which revolutionized the in vitro production of secondary metabolites was the discovery of rapidly growing, productive and stable hairy root cultures obtained by genetic transformation of the plant tissue by Agrobacterium rhizogenes (cf. Rao and Ravishankar, 2002). Certain disadvantages of the tissue culture technique like instability of cell lines, low yields, slow growth, and scale-up problems have led to the exploitation of hairy root culture (Saito et al., 1992). Rol genes, belonging to T-DNA and transferred by A. rhizogenes into plant cells, affect plant development which is regulated by the host (Altamura, 2004).

The hairy roots are unique in being genetically and biosynthetically stable. They grow fast, show low doubling time, have easy maintenance and synthesize a variety of chemical compounds (Giri and Lakshmi, 2000). Hairy root cultures produce secondary metabolites over successive generations without losing genetic or biosynthetic stability. These cultures produce root-derived compounds as well as novel compounds by biotransformation and by tailormade Ri plasmid. Earlier, Tepfer (1990) listed 116 plants belonging to 30 dicotyledonous families wherein hairy roots have been induced. There
have been some additional reports of hairy root cultures and pharmaceutical production from dicotyledonous plants such as Arnica montana for thymol derivatives; Bidens species for polyacetylenes; Catharanthus roseus for ajmalicine and serpentine; Centaurium erythraea for secoiridoids; Cinchona ledgeriana for quinoline alkaloids; Coleus forskohlii for rosmarinic acid; Echinacea purpurea for caffeic acid derivatives; Gentiana macrophylla for secoiridoid glucoside gentiopicroside; Glycyrrhiza uralensis for glycyrrhizin; Hyoscyamus for hyoscyamine; Lithospermum canescens for shikonin derivatives and pyrrolizidine; Saussurea meduca for jaceosidin; Tropaeolum majus for glucotropaeolin; Tylophora indica for tylophorine; Withania somniferum for withanolide (Abbasi et al., 2007; Bandyopadhyay et al., 2007; Li et al., 2005; Piatezak et al., 2006; Pietrosiuk et al., 2006; Tiwari et al., 2007; Wielanek and Urbanek, 2006). Particle bombardment technique has been used for the transformation of dicotyledons since long e. g. Cicer arietinum (Indurker et al., 2007). However, reports of genetic transformation on monocotyledonous plants have been quite a few. Agrobacterium-mediated stable transformation of Alstroemeria, Hordeum vulgare, indica rice, japonica rice, Lilium, and Musa acuminata has been reported. Genetic transformation of floriculturally important orchids like Calanthe through in vivo electrophoresis (Griesbach, 1993) and Cymbidium by particle bombardment (Yang et al., 1999) has been attempted. Transformation of Cymbidium niveo-marginatum, Dendrobium, Oncidium, Phalaenopsis, and Zygopetalum mackaii has been carried out using A. tumefaciens (Yoshiyuki et al., 2001, 2002). However, no reports of transformation of orchid species using A. rhizogenes are available as yet.

The members of Solanaceae, one of the dicotyledonous families, are economically very important. Species of Atropa, Datura, Duboisia, Hyoscyamus, Nicotiana, and Solanum are familiar as the sources of some important alkaloids like atropine, hyoscyamine, nicotine, and solasodine. Solanum is noted for the production of C27 steroidal alkaloids in many species. Solasodine, a steroidal alkaloid of spirostanol type (Δ5,22β-25X-spirosolan-3β-ol; C27H45O2N), is obtained in varying degrees from various species of Solanum. Solasodine is the aglycone of the glycoalkaloid solasonine. Solasodine is a nitrogen analogue of diosgenin (obtained from Dioscorea). Diosgenin is used for synthesizing oral contraceptives, sex hormones, and corticosteroids.
Introduction

But continuous exploitation and collection pressures have led to the severe depletion of *Dioscorea*. With a view to releasing pressures due to its over exploitation, the option of using solasodine yielding plants like *Solanum aviculare*, *S. khasianum*, and *S. laciniatum* was exploited. Solasodine has been reported to reduce body temperature; induce congenital craniofacial malformations; increase liver weight in mice; affect morphology, motility, and glycolytic enzymes of human and bull spermatozoa; affect central nervous system; and affect adrenal glands. It is also known to have immunomodulating effect; cardiotonic effect; *in vitro* preferential cytotoxicity for human cancer cells; cholinergic activities; anti-coagulative effect, decrease antithrombin activity and increase fibrinolysis level; antifungal activity; effective in malignant and benign human skin tumors and carcinomas while having no adverse effect on liver, kidneys and haemopoietic system; and has activity toward DNA repair deficient yeast mutants.

*Solanum xanthocarpum* Schrad. and Wendle (= *Solanum surattense* Burm. f.; *S. mccanni* Sant./ *S. virginianum* L.), The Yellow-Berried Nightshade, commonly known as Kantakari, Katheli, is an annual, perennial, prickly diffuse herb found in South East Asia, Malaysia, and tropical Australia, very commonly found throughout plains from seashore to hills. In India, it occurs in wastelands, along the roadside from plains to an altitude of 7000 ft (Kaushik and Dhiman, 2000). The important formulations using the drug are ‘Kantakari ghritam’, ‘Putikaranjasavam’ and ‘Surandi leham’ (Swamy et al., 2005). Its root is an expectorant, forming an ingredient of the well-known Ayurvedic medicine, *Dasamula* and is used in ayurvedic preparation, ‘Chavyanprash’. The whole dried plant is sold as ‘Panchang’. It is employed in cough, asthma and chest pain, has diuretic properties and is used to cure dropsy. A decoction of the plant is also given in gonorrhoea and to promote conception. An ayurvedic compound ‘Arkadhi’, with this herb is useful in dengue, acute bronchitis and fever accompanied by chest infections. It is used to relieve pain, rheumatism, check vomiting; and is considered expectorant, diuretic, anti-asthmatic and anti-emetic. The pulp of the fruits is made into a paste and applied on skin infections (Kaushik and Dhiman, 2000). Bitter fruits are used in Indian curries. Extracts of shoot and fruit show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in phosphate buffer. Like roots, seeds are also administered as an expectorant in asthma
Introduction

(Tamakswasa) and cough (Kasa roga); the vapours of burning seeds are used as an expectorant in asthma, coughs and in toothache (The Wealth of India, 1981).

Clinical efficacy of the species in bronchial asthma has been well established (Govindan et al., 1999). The beneficial effect of the drug may be attributed to the depletion of histamine from bronchial and lung tissue (Gupta et al., 1967). The use of Solanum xanthocarpum for curing diabetes (Sharma and Sharma, 2000), cardiac problems (Sajid et al., 1996), control of Culex larvae (Mohan et al., 2005, 2006; Muthukrishnan and Pushpalatha, 1995) and malaria vectors i.e., Anopheles culicifacies, A. stephensi, A. aegypti (Singh and Bansal, 2003), antiseptic properties (Pandey and Chauhan, 1999), skin pathological conditions (Zanchi, 1998) has also been very well documented. The use of its extract as a contraceptive for male albino rats (Bhagat and Purohit, 2000), and antispermatogenic in rats (Mali et al., 1996) and as biopesticide (Kumbhai et al., 1998) has also been reported. Solasodine is teratogenic in rats and guinea pig. The species has a high concentration of solasodine, a starting material for the manufacture of cortisone and sex hormones.

Malaxis Solander ex Swartz, a group of Adder’s Mouth orchids, belongs to tribe Epidendreae, subtribe Liparidinae of family Orchidaceae and comprises nearly 300 species distributed in Europe, Asia and North and South America and absent from South Africa and Australia (Coblet, 1980). In India, it is epitomized by 18 species, many of which including M. acuminata, M. muscifera, M. cylindrostachya, M. aphioglossoides, M. versicolor, and M. rheedi, are therapeutically important and rich in alkaloids.

Malaxis acuminata D. Don (= Microstylis wallichii Lindl.), The Crimson Shield Orchid, is a widely distributed species found in Thailand, China, Burma and Indo-China. In India, it is available in temperate and subtropical Himalaya (from Himachal Pradesh to Sikkim; 6000-7000 ft), Western Ghats, Nilgiri Hills and Andaman Islands. It is an erect, terrestrial herb, 30 cm high having stem with few sheaths at the base (Handa, 1986). The species forms an important ingredient of ‘Ashtavarga’ drugs used in the preparation of an ayurvedic tonic ‘Chavyanprash’ and is marketed under the trade name ‘Rshbhak’ (Handa, 1986). ‘Rshbhak’ provides strength, enhances sperm formation and makes a person free from diseases borne by vat, pit, kaf and tuberculosis (Khasim and Mohana Rao, 1999; Hegde and Ingalhalli, 1988).
Malaxin, first isolated by Leander and Luning (1967) from *Malaxis congesta* belongs to pyrrolizidine group and is an ester of laburnine and malaxinic acid \((C_{26}H_{37}O_8N)\) (Leander and Luning, 1967; Luning, 1974). Malaxin has also been found in *Liparis bicallosa* and its variant *L. hachijoensis* (Lawler and Slaytor, 1962; Slaytor, 1977; Nishikawa et al., 1969). *M. biloba*, *M. fastigota*, *M. paludosa*, *M. siamensis*, *M. soulei*, *M. khasiana*, *M. acuminata*, and *M. grandifolia* have been tested positive for alkaloids (Luning, 1967; Lindstrom et al., 1971). *M. densiflora* and *M. versicolor* were tested positive for flavonoids from Western Ghats (Jorapur, 1986). Brandänge et al. (1973) reported the synthesis of some pyrrolizidine derivatives. Tetraacetates of malaxin and kuramerine were synthesized by Tanino et al. (1969).

Conservation through reserves alone is now considered unlikely to achieve protection of plant species necessary to mitigate direct losses of habitat and the pervasive impact of global climate change. Assisted translocation/migration represents new challenges in the face of climate change; species, particularly orchids, will need artificial assistance to migrate from hostile environments, across ecological barriers (alienated lands such as farmlands and built infrastructure) to new climatically buffered sites. The technology and science to underpin assisted migration concepts are in their infancy for plants in general, and orchids, with their high degree of rarity, represent a particularly challenging group for which these principles need to be developed. It is likely that orchids, more than any other plant family, will be in the front-line of species to suffer large-scale extinction events as a result of climate change (Swarts and Dixon, 2009). The problem is compounded by highly specialized pollinators (locally endemic native invertebrates) and, in the most threatened groups such as hammer orchids (*Drakaea*) and spider orchids (*Caladenia*), high levels of mycorrhizal specialization. Management and development of effective conservation strategies for orchids require a wide range of integrated scientific approaches to mitigate impacts that directly influence ecological traits critical for survival. In response to threats to orchid species, integrated conservation approaches have to be adopted (including *ex situ* and translocation principles) in the Indian sub-continent with the result that a significant, multidisciplinary approach is under development to facilitate conservation of some of the most threatened taxa and build expertise to carry out assisted migration to new sites (Swarts and Dixon, 2009).
Introduction

Multiplication through tissue culture thus emerged as an important strategy to release their natural populations from profit-making collection pressures. Possibilities of replenishing their natural stocks, by using tissue culture and raised plantlets are also being increasingly realized.

A heavy urbanization and the recent population explosion have led to the felling of a large proportion of Indian forests, thereby turning the diversity of ecosphere into monocultural agrosphere and technosphere. The natural populations of both the above selected species have been detrimentally affected under the duress of unregulated commercial collections, progressive habitat loss and poor natural regeneration potential. There have been, nevertheless, only some reports on the *in vitro* propagation of the two species (Malpathak and David, 1992; Prasad and Chaturvedi, 1982; Rao and Narayanaswami, 1968; Deb and Temjensangba, 2006, Pathak *et al.*, 2001), but they have been few and far-between. The present studies were, therefore, undertaken with a view to

- develop protocols for propagating these two medicinally important taxa, *Malaxis acuminata* (Orchidaceae) and *Solanum xanthocarpum* (Solanaceae) *in vitro* by using various explants and nutrient recipes;
- assess the alkaloid contents of both *in vivo* sourced plant parts and *in vitro* raised cultures; and
- raise hairy root cultures of the two species.