WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 11, 829-860. Research Article ISSN 2277– 7105

IN VITRO MICROPROPAGATION OF *LAWSCNIA INERMIS* (HENNA) A NATURAL DYE PLANT.

Gajendra Singh[1] Pushkar Choudhary[1] Sajad Ahmad Mir[2] Rajveer Singh Rawat[3], Dr. Bhanwar Lal Jat[4]*

[1]Department of Agriculture, Bhagwant University Ajmer, Rajasthan, India.

[2]Department of Botany, Bhagwant University Ajmer, Rajasthan, India.

[3]RV Book Company, Ajmer, Rajasthan, India.

[4]*Department of Agriculture Biotechnology, Bhagwant University Ajmer, Rajasthan, India.

Article Received on
13 Sept. 2016,

Revised on 03 Oct. 2016,
Accepted on 23 Oct. 2016

DOI: 10.20959/wjpr201611-7213

***Corresponding Author**
Dr. Bhanwar Lal Jat
Department of Agriculture Biotechnology, Bhagwant University Ajmer, Rajasthan, India.

ABSTRACT

Plant based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in the recent years due to fewer side effects in comparison to synthetic drugs and antibiotics has high lightened the need for the conservation and propagation of medicinal plants and there *in vitro* conservation of germplasm. The present study showed that it was possible to explore the morphogenetic potential of *Lawscnia inermis* by application of growth regulators and light condition. With the use of different combinations of cytokinins, the induction and multiplication of shoots derived from especial and Axillary meristems. The regulatory action of cytokinins and the apical dominance. The maximum shoot induction and multiplication was observed MS medium supplemented with 0.5mg/lit BA, 0.5 mg/l KN within 4 weeks of culture under a 14 hr photoperiod. At higher concentrations of BA or KN, the rate of shoot proliferation declined. The Axillary meristems produced more number of shoots than the apical meristems. Similar results were reported in Psoralea corylifolia. The results are consistent with earlier reports indicating that cytokinins and auxins affect shoot multiplication in other plants using shoot tip or Axillary bud explants. The results showed that the number of shoots per culture was increased in continuous light both in apical and Axillary meristems than in the 14 hr photoperiod. The interaction of photoperiod and plant growth regulators had a significant effect on shoot morphogenesis as reported earlier. With the increase in the concentration of either BA or KN, the percentage of shoot multiplication

declined. The results also implied that there were differences among the treatments for both the percentage of culture developing multiple shoots and the mean numbers of shoots per culture. The variation of response was due to the varying concentration of growth regulators used in the medium and light condition as reported earlier in *Lavandula latifolia* and *Zingiber officinal*. The rate of multiplication was high and stable up to the 5th subculture and declined in subsequent subcultures. This might be due to the balancing of the endogenous and exogenous growth regulators and the ionic concentration of nutrient salts as reported earlier in other plants. The elongated shoots were rooted the maximum in MS basal salts supplemented with 0.25mg/Lit IBA with 2% sucrose. The rooting ability was reduced with the increase in the concentration of IAA or IBA in the medium. The percentage of shoots forming roots and days to rooting significantly varied with different concertinos of IAA or IBA.

KEYWORDS: IAA, IBA**,** *Lawscnia inermis* (Henna), PGR, MS, *In vitro.*

INTRODUCTION

If cloning is the norm among animals and there is no chance for matting, then the earth would be full of the organisms as ‘Dollies’. Similarly in a situation where all plants multiply by simple detachment of parts and growth of each part into a faithful photocopy of the mother plant, then there would be very little room for variation and evolution without which living organisms cannot adapt to unique and changing environments, pests and diseases. Fortunately it is not the case to be. Variety is the spice of life and basic characteristic of life is its unlimited diversity. Nature has myriads of life forms on this planet among which variations are of ubiquitous occurrence. It is particularly so in the mega centers of diversity in the tropics which harbor approximately two/thirds of the biota and where many species of economic importance presumably had their origin. As a matter of fact, variations between individuals of the species were observed over the millennia and were considered as real ‘hot 5 spots’ of evolution.

The notion that no two individuals of a sexually reproduction population are 100% identical prevailed even when methods of scientific scrutiny were not available. In 17th century when the concept of a species was poorly understood, the English naturalist john Ray (1628-1705) showed that within a species there might occur individuals different from the normal in one or more characters. Later Charles Darwin (1809-1882) developed this concept into a supreme and effectively linked the variations with natural selection, survival of the fittest and origin of

new species. Down the years, variations among plants and animals had always fascinated an inquisitive mind and helped an evolutionary biologist or a breeder to select a desirable variant or breed a new form of greater agronomic value. Traditional physicians and village doctors of yester years were no different. They collected herbs of certain morphological attributes (of flowers, fruits, leaves etc) and preferred root drugs of specific colour, smell, size, fibrous content, itching quality etc. obviously from locations known only to them. Even within a medicinal plant species, sometimes one variety was preferred over others. It is also not surprising that curative properties of a plant species change according to seasons or Development stages and hence vaidyas prefer to collect required plants or their parts during certain periods only (Bharat, 1997). In the wilderness of the tropics, plants grow in extreme situations along longitudinal, latitudinal and temperature gradients and therefore variations within and between populations of a species are not uncommon, although plants in general show habitat and distribution preferences, there are many a species which are neutral and adapted to other environmental regimes. Much of the variations in phenotype observed in natural populations of a species were earlier attributed to environmental influences (Briggs and Walters, 1984). Thus individuals of a species adapted to a particular soil type and climatic zone were designated as edaphic ecotypes and climatic ecotypes respectively. Many botanists reasoned that distinct intraspecific variations of plants were me due to habitat modifications and adaptation to environment was by phenotypic plastic response. Phenotype was accepted as a resultant product of interaction between genotype and environment and different phenotypes of a given genotype could occur in different environments. Since a plant remains static and possesses persistent meristems and succession of organs of limited growth. It was thought to show greater phenotypic variability than a higher animal. In certain plants developmental variations were observed as evident from differential morphological characteristics of the juvenile and adult forms. In early 19205, the European botanist Turesson added yet another dimension to the problem of variations by demonstrating the persistence of morphological variations in the same species under standard conditions of cultivation (Turesson, 1922 a, b). The results of his ingenious experiments proved beyond doubt the wide spread occurrence of intraspecific, habitat correlated genetic variations. it was soon realized that adaptation to environment was sometimes by plastic responses but more frequently had a genetic basis. Since selection operated in natural populations, well-adapted genotypes were thought to be selected preferentially over others in each habitat. The problem of variations is further compounded in medicinal plants which apart from displaying visible variations, synthesize and accumulate an array of plant-specific chemicals. These compounds

together called secondary metabolites are mostly high value, low volume compounds biosynthetically derived from primary metabolism and accumulated by certain plants or groups of plants in trace quantities. In plants 'defense chemicals' form a therapeutic arsenal to fight a variety of biotic and a biotic stresses. A study of variation in the active principles is often an important element in the investigation of variation in such plants. Although only limited number individuals in a plant population were usually subjected to chemical scrutiny to represent the population of a taxon in earlier studies, subsequent investigations brought out significant variations within as well as between populations. Thus it became reasonable to assume that chemotypes or chemical variants occurred in wild populations of medicinal plant species.

A wide spectrum of simple and overlapping variations is now documented in plants (Sen and Sharma, 1990; Connoly et al, 1994; Stewart and porter, 1995; Demeke et al, 1996; Sonnante et al, 1997; James and Ashburner, 1997). In general, all observed variations are broadly grouped into two categories; epigenetic and genetic. Genetic variations in plants are strictly heritable i.e. truthfully passed bon from one generation to another through seeds and do not change under conditions of cultivation. They occur invariably due to alterations in the genetic material and may affect both phenotypic and chemical characteristics of medicinal plant. Epigenetic variations on the other hand, are mostly induced by the environment in which the plants grow and are also partially affected by developmental events. Epigenetic changes in medicinal plants in general include morphological and chemical as well as physiological variations. Therefore a great deal of information-morphological, biochemical, physiological and genetic is necessary before the observed pattern of variation may be interpreted. It is also true that beneath these intraspecific variations, there exists a fixed unchangeable genetic spectrum of characteristics that makes up the species. India is a known mega-diversity centre harboring a multitude of medicinal plant species each presumably studded with as yet unknown genetic and chemical variations of economic importance. Out of an estimated l7, 000 higher plant species occurring in India, more than 1000 species are used our several centuries in the traditional systems of medicine viz. Ayurveda, Siddha, Unani and Amchi. The villagers and tribal folks spread across the length and breadth of the country make use of more than 7000 plant species through oral traditions (pushpangadan et a1. 1997). Nearly 3/4 of the herbal drugs and perfumery products used in the world are available in natural state in India. Therefore, the rich and varied plant diversity, especially the genetic diversity of medicinal and aromatic plants, is one of India's important strengths and is the bedrock for all

future bioindustrial developments, Unfortunately, the renowned medicinal plant wealth of India has seldom been subjected to genetic scrutiny keeping In mind the latent and patentable properties and economic utility of the selected plant types. As severe habitat losses and consequent endangerment and extinction of known and hitherto lesser known species of economic value are not uncommon in the Indian subcontinent, it is imperative that heritable variations within the otherwise unimproved natural populations of prospective taxa are studied for selection, improvement and development of suitable cultivars. Otherwise called bioprospecting, this line of research is essential to fish out useful genes and gene products for commercialization in the now unfolded patent regime. Knowledge of the genetic diversity is also a prerequisite for any in situ and ex situ conservation schemes (Hamrick et al., 1991) as it is not practical to conserve all genotypes of a given species against the mass extinction spasm projected for the 21st century (Raven, 1999).

For Cultivation of Medicinal Plants

If efforts are made for systematic cultivation of medicinal plants instead of collecting them from the wild, many of the problems mentioned above will be minimized. Properly identified and certified planting material can be supplied to the growers. Cultivation of the plants can be planned to meet the need of the industry in required Quantities and at the required time. Unintentional adulteration can be avoided and it will be easier to check delivered adulteration. Developing countries can ill afford the products of the western pharmaceutical Industry due to their high and arising prices. The import of such medicines or payment of royalties for local nature of the medicines causes it heavy financial burden for the developing countries. Increased awareness about potential of this group of interesting and useful plants has encouraged many innovative and progressive growers and entrepreneurs to take up their cultivation as a commercially enterprise. A part from health care this enterprise provides means of livelihood to scores of people.

Propagation of Medicinal Plants

During the past two decades, there has been a great interest and progress *in vitro* propagation of medicinal plants using techniques of plant tissue culture. The earlier work is tissue culture of medicinal plants was done I view of getting secondary metabolites in vitro. The results have not been very encouraging. The process is also costly in terms of equipments, inputs and specialized manpower. Transgenic plants have been obtained from a number of plant species, but only a few medicinal plants. Plant tissue culture relies on the fact that many plant cells

have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, or (less commonly) roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones. Tissue cultured plants are clones, if the original mother plant used to produce the first explants is susceptible to a pathogen or environmental condition, the entire would be susceptible to the same problem, and conversely any positive traits would remain within the line also. Plant tissue culture is used widely in plant science; it also has a number of commercial applications.

Applications include

(i) Micropropagation is widely used in forestry and in floriculture, Micropropagation can also be used to conserve rare or endangered plant species. (ii) A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance. (iii) Large-scale growth of plant cells in liquid culture inside bioreactors as a source of secondary products, like phyto-chemicals [secondary metabolites] and recombinant proteins used as bio-pharmaceuticals. (iv) To cross distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue) (v) For production of doubled monoploid (dihaploid) plants from haploid cultures to achieve homozygous lines more rapidly in breeding programmes, usually by treatment with colchicines which causes doubling of the chromosome number. (vi) As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants. (vii) Certain techniques such as meristems tip culture can be used to produce clean plant material from viruses stock. Such as potatoes and many species of son fruit. The application of modern biotechnology techniques like plant tissue culture has proven to be a powerful tool in medicinal plant improvement programs. Scientists hit upon a technique where by not only can these plants be preserved from being lost but are also able to develop a complete plant from a small plant part. Most method of plant transformation applied to Gm crops requires that a whole plant is regenerated from isolated plant cells or tissues which have been genetically transformed. This regeneration is conducted in vitro so that the environment and growth medium can be manipulated to ensure a high frequency of regeneration. In addition to a high frequency of regeneration. The regenerable cells must be accessible to gene transfer by whatever technique is chosen. The primary aim is therefore to produce as easily and as quickly as possible, a large number of regenerable cells that are accessible to gene transfer. Recent progress in the field or plant cell and tissue culture has made this area of research as one of the most dynamic and promising in experimental

biology. The introduction and development of these techniques has allowed studying the problems previously inaccessible and has and turned the dreams of Haberlandt, Whites and Gautheret into realities. The pre-requisites of successful plant cell and tissue culture are tissue specificity (genotype, source), media composition (minerals, phytohormones, other organics, and supporting agents) culture conditions (light, temperature, gases, size of vessels), and culture requirements of all these factors are species specific. The different pathways for plant production by tissue culture are:- (a) Axillary's shoot elongation. (b) Organogenesis. (c) Somatic embryo genesis. During the past two decades these has been a great interest and progress in in-vitro propagation of medicinal plants using techniques of tissue, meristems, protoplast and organ culture. For the commercial propagation of plant, axillary shoot induction and elongation is preferred. In most of the herbaceous as well as woody species, micro propagation has been achieved by inducing multiple shoots from axillary and apical buds. It is the easiest method and maintains genetic stability better than propagation by organogenesis. The merit of using this technique as a means of regeneration is that incipient shoots has already been differentiated in vivo. Thus, to establish a complete plant, only multiplication, elongation and root differentiation are required. This system now days described as conservative because of its ability to produce true-to-type plants without any genetic change. In organogenesis, adventitious shoots are induced from sites other than bud meristems. Rarely are roots induced before shoots and often there is a passage through a callus stage. Organogenesis has great potential for multiplication. However, it faces several problems, first of all the plantlets regenerated are not genetically true to type. Secondly, there is variation in growth behavior of the planlets which leads to heterogeneous growth under field condition. This is because *in vitro* conditions, some clones show plagiotropic growth and poor vigour, while other clones show orthographic growth and vigour typical of young seedlings. This is an important consideration of in case of medicinal plant where such changes can modify the quality and effectiveness of the active principles. In the present era Herbal Science has emerged as a major focus for trade as there has been a worldwide acceptance of herbal products. However new innovations and an increased willingness to adopt new techniques for wider acceptance of such herbal products globally. Synthetic dyes are easily available and have created a comfortable niche in today's ever-growing market. However, a huge market for herbal products exists which needs attention. India has a rich heritage of using medicinal plants in tradional medicines such as Ayurveda, Siddha and Unani besides folklore practices. Lawsonia inermis syn. Lawsonia Alba (Henna) is a sole species in the genus in the family Lythraceae. Henna has been found to exhibit Antibacterial

and Dermatological properties. It is useful in coloring of skin, scalp and nails etc. Henna has also shown anti-diarrhoel, diuretic, emmanagogue and aboritifacient prophetically and is found to be practically nontoxic.

Plant Introduction

Lawsonia inermis lirm. (Lythraceae) is an important medicinal and agricultural cultivated plant, distributed in northern Africa and south-west Asia (Anonymous 1962). *Lawsonia inermis* is a much-branched glabrous shrub or small tree 2-6m in height, which may be spiny. Henna plant is native to tropical and subtropical regions of Africa, South Asia, and Northern Australasia in Semi-arid zoned. The small shrub of *Lawsonia inermis* is widely cultivated and used in many oriental, Middle Eastern and northern African countries. This dwarf shrub grows outdoors unsheltered at temperatures higher than 1l^{o}C (60^{o}F). It needs around 5 years to mature and produce leaves with useful levels with useful levels of tannins. It grows better in arid regions than the moist or wet regions and achieves a height of 8 to 10 feet. Bark grayish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular green but turn red with age. Leaves opposite, entire sub sessile, elliptic to broadly lanceolate, 1.5-5 x .5-2cm, glabrous, acuminate; veins on the upper surface depressed. Flowers small, white, numerous; in large pyramidal terminal cymes, fragrant, 1 cm across, 4 petals crumpled in the bud, calyx with 2-mm tube and 3-mm spread lobes; petals orbicular to obovate, white or red; stamens 8, inserted in pairs on the rim of the calyx tube; ovary 4 celled, style up to 5mm long, erect. Fruits small, brown, globose capsules 4-8mm in diameter, many-seeded, opening irregularly, split into 4 sections, with a persistent style. Seeds 3 mm across angular, with thick seed coat. It grows mainly along watercourses and in semiarid region and is adapted to a wide range of conditions. It can withstand low air humidity and drought. Henna requires high temperatures for germination, growth and development. Botanical Name:-*Lawsonia inerrnis Linn*. Family: Lythraceae. Sanskrit Synonyms**:**-Madayantika, Mendhi, Mendhika, Nakharanjani Ayurvedic Properties**:**-Rasa-Tikta, Kashaya, Guna- Lakhu, Rooksha, Virya- Seeta, Vipaka-Katu Plant Names in different Languages**:**-English-Henna, Ehyptian Priven Cypress Shrub, Hindi- Mehanti, Hena, Malayalam- Mailanji , Mayilanji. Distribution Cultivated through India; and in dry deciduous forests.

Cultivation

The henna plant is native to tropical and subtropical regions of Africa, Southern Asia and northern Australasia in semi-arid zones is the tropical Savannah and tropical arid zone, in latitudes 15°N and 25°S from Africa to the western Pacific Rim, and produced highest dye content in temperatures between 35°C and 45°C. During the onset of precipitation intervals, the plant grows rapidly; putting out new shoots, then growth sloes. The leaves gradually yellow and fall during prolonged dry or cool intervals. It does not thrive where minimum temperatures are below 11°C. Temperatures below 5°C will kill the henna plant. Henna is commercially cultivated in UAE Morocco Yemen, Tunisia Saudi Arabia Egypt, western India, Iran, Pakistan, Bangladesh, Afghanistan Turkey, Somalia and Sudan. Presently the Pali district of Rajasthan is the most heavily cultivated henna production area in India. With over 100 henna processors operating in Sojat city. Henna, *Lawsonia inermis* is a small desert tree, but you can grow it as a houseplant. You can grow it outdoors if temperatures never drop below 50°F or 11°C. If it is indoors, keep it in the sunniest place possible, and plan on it getting about 8 ‘tall in five years. When you get a new henna plant, put it in a large pot of potting soil, and give it one drenching watering, then put it in your sunniest window. It may drop its leaves from the shock. If it drops its leaves, prune at back and let it shoot out new growth.

Chemistry of Lawsonia

The main constituents reported are Naphthoquinone derivatives alphatic coponents, triterpenes and sterols phenolic derivatives, coumarins, xanthones flavononids and other chemical constituents. The coloring component in the leves of henna is lawsonean identified as 2-hydroxy-1,4-napthoquinone which is present in dried leaves of hens contain about 0.5-1.5%, traditionally used to produce colour fast orange, red and brown dyes. The leaves also contain glucose but starch, Gallic acid is present to report. The presence of mannitol in Heena stem, flowers and roots has been investigated. Lawsone is the chief constituent responsible for the dying properties of the plant. Henna extract contains lawsome, the active ingredient and a naturally occurring naphthoquinone (2-hydroxdy-1,4-naphthoquinone2). When applied to wool and nylon it behaves as an acid leveling non metallised acid dye. Dye uptake increases with increased pH (Badri 1993) and it stains tissue preparations in histological colouring leather and skins. It was widely used in Europe from 1890 for tinting hair with many shades prepared by mixing the leaves with other plants such as, indigo catechu or Lucerne. Heena brown colourings constituent are of a retinoid fracture having chemical

properties similar to tannins, hence the name hennotannic acid. Naphthoquinones are widely distributed in plants, fungi and some animals. Their biological activities have long been reported to include antibacterial effects on several species of both aerobic and anaerobic organisms (Dirdy 1968) and parasite (Wendel 1946). Early discovery of heena's medicinal properties seeking healing by using plants is an ancient pactice. Various cultures applied poulitices and imbedded infusions of hundreds, if not thousands of indigenous plants dating back to prehistory. Reports of Heanderthals living 60,000 years ago in present day Iraq used plants as hollyhock (Alcea spp] stockwell 1988, Thomson 1978). *Lawsonia inermis* a well known enthomedicinal plant used cosmetically and medicinally for over 9,000 years. Its use in the Indian traditional folk medicines is well documented. Table-1 indicates the use of different parts of *Lawsonia inermis* in traditional system of medicines.

Phyto-chemistry of *Lawsonia inermis lirm*

Much work is done in the field of phytochemical investigation of the plant. The chemical constituents isolated from L. inermis are napthoquinone derivatives, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, amino acids and other constituents.

Phytochemicals found in various parts of *Lawsonia inermis lirm*:-(Uses of *Lawsonia inermis*)

In the present era Herbal Science has emerged as a major focus for trade as there has been a worldwide acceptance of herbal products. However new innovations and an increased willingness to adopt new techniques are required for wider acceptance of such herbal products globally. Synthetic dyes are easily available and have created a comfortable niche in today's ever-growing market. However, a huge market for herbal products exist which needs attention. The plant *Lawsonia inermis* (Lythraceae) is known for its cosmetic properties. Fresh leaves of L. (Crushed) of dry henna Leaf powder is widely accept [ted as a hair of herbal origin. *Lawsonia inermis* is also used traditionally for the decorating hands and feet. In west and the Middle East henna is used in hair dyes, shampoo and conditioners. The leaves are used as a prophylactic against skin diseases. They are used externally in the form of a paste or decoction against boils, burns, bruises and skin inflammations. A decoction is used as gurgle against sore throat. Lawsonia leaves show mild anti bacterial activity against Micrococcus pyogenes var. aureus and Esherichia coli (Kritikar and Basu 1981). It is also known as good medicinal plant which is said to have properties of astringent, anti-

hemorrhagic, intestinal anti-neoplastic, hypotensive and sedative effects. The flower of Lawsonia has a strong aroma with high commercial value. It is extensively used as a dye in silk and wool industry. Traditionally henna is used to create beautiful designs on hands and other body parts on various occasions like weddings and festivals. Applying henna on wedding and festivals is considered auspicious and important.

Medicinal Properties

Lawsonia inermis is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots flowers and seeds have also been used in traditional medicine. The plant is reported to contain carbohydrates, proteins, Havinoids, tannins and phenolic. Compounds, alkaloids, terpenoids, quinines, coumarins, xanthones and fatty acids. The hair care plant is reported to possess immune-modulatory, antiviral, antibacterial, antifungal, neotropic, antifertility, hepatoprotective, tuberculoostatic activity, anti-mitotic, analgesic and anti-inflammatory, anti-carcinogenic and antioxidant properties.

Ethno-medicinal uses of different parts of L. inermis

Plant parts	Traditional uses (as/in)
Root	Bitter, depurative, diuretic, emmenagogue, arbori-facient, burning sensation, leprosy, skin diseases, amenorrhoea, dysmenorrhoea and premature graying of hair (15).
Leaves	Bitter, astringent, acrid, diuretic, emetic, edema, expectorant, anodyne, anti inflammatory, constipating, depurative, liver tonic, haematinic, styptic, febrifuge, trichogenous, wound, ulcers, strangury, cough, bronchitis, burning sensation, cephalalgia, hemicranias, lumbago, rheumatalgia, inflammations, diarrhea, dysentery, leprosy, leucoderma, scabies, bolls, hepatopathy, splenopathy, anemia, hemorrhages, hemoptysis, fever, ophthalmia, amenorrhoea, falling of hair, greyness of hair, jaundice (15-18)
Flowers	Cardiotonic, refrigerant, soporific, febrifuge, tonic, cephalalgia, burning sensation, cardiopathy,amentia, onsomnia, fevers (15)
Seeds	Antipyretic, intellect promoting, constipating, intermittent fevers, insanity, amentia, diarrhea, dysentsary and, gesteropathy (15).

Antibacterial Activity

The antibacterial activity of the natural napthaquione products alkannin and shilonin and their derivatives has been investigated (Riffel 2002). In general they are active against gram positive bacteria such as Staphylococcus aureus, Enterococcus faecium and Bacillus subtilis, but are inactive against gram negative bacteria (papageorgiou 1999). In nosocomil infection, staphylococcus aureus is one of the most prevalent microorganisms worldwide. Methicillin resistant strains represent 15-45% of all staphylococcus auyreus isolates (Emori 1993). This

may explain the arduos search antimicrobial agents as an important line of research. Hence newer analogues of the aminoacetate derivatives (of diosoyrin dimethyl ether) have to be designed to resolve the structure activity relationship in this series leading to the development of more effective anti micorobacterial agents. However lawsome was shown to elicit in vivo lower toxic effects in mussel tissues than tissues in higher organisms. This may be due to the lower detectable levels of xanthine oxidase in the invertebrate muscles (Osman 2004). For the napthoquinones to have such antimicrobial activity, active compounds must possess at least a substitution at position 2 or 3, which is either an electron releasing or weaker electron withdrawing group (Greshon 1975). This structure activity relationship is reinforced further with studies that indicated the antimicrobial activity of a family of isoxazolylmaphthoquinones requires a free keto group at position I, and the substituent at position 2 must be a hydroxyl group (Bogdanov 1993). Such compounds were found to protect mice infected with staphylococcus aureus, inhibiting septicaemia *in vivo* (Albesa 1995).

Antimycotic Activity

Lawsome has been shown to be effective against oral Candida albicans isolated from patients with HIV/AIDS (Prasirst 2004). We must reported activity against Candida albicans using Omani henna (Habbal 2005). During antifungal screening of higher plants, the leaves of lawsonia inermis were found to exhibit strong fungitoxicity where napthoquinones were found to be the active factor (Tripathi 1978). Fungotoxic studies against ringworm were demonstrated. Barks of 30 plant species were screened against Microsporum gypsum and Trichophyton mentagrophytes; only Lawsonia inermis exhibited absolute toxicity (Singh 1989). The lawsonia bark extract was found to possess a fungistatic nature at its maximum inhibitory dilution of 1:30 (W/V) against both test pathogens, but became fungicidal at 1:10 (W/V) (Bogdanov 1993). The extract showed broad fungi toxic spectrum when tested against 13 ringworm fungi (Singh 1989). This role of a cosmetic and antimycotic has been reported by others (Itani 1973). Molluscicidal activity of leaf, bark and seed of lawsonia inermis against Lymnaea acuminate and indoplanorbis exustus was studied with the highest toxicity observed in the seed of the plant (Singh 2001).

Virucidal Activity

Bhandarkar and khan (2003) suggest hepatoprotective and antioxidant activities activity of extract of lawsonia Alba lam, against hepatic damage in albino rats. This may indirectly

indicate an important role of henna as antiviral agent. Hepatitis related viruses such as hecatoititis c virus. HCV and hepapatis E virus HEV and the new infections diseases such as the Ebola virus, Legionella pneunoophila and human immune deficiency virus HIV, have been discovered in the past 20 years. The emergence of drug resistant strains in a big challenge that faces antibacterial medicine.

Anticarcinogenic Activity of Henna

Henna's anticarcinogenic property was reported (Endrini 2002) using a chloroform extract of lawsonia inermis by the culture tetrazolliumsalt (MTT) assay on the human breast, colon and liver carcinogeni9c cell lines and normal human liver cell lines (Enedrini 2002). The preliminary results showed that henna extract displayed cytotoxic effects against HepG2 (liver cells) and M,cf-7 (hormone dependent breast cells), but no significant activity was recorded against colonic, hormone nondependent breast cell lines and normal liver cells at the concentrations tested. These results indicate the selectivity of such cytotoxic activity.

Antioxidative Activity

The Antioxidative activity of this henna extract was found to be highest compared to vitamin E or {alpha} tochopherol, attributing to the strong cytotoxic activity of the extract (Endrini 2002). Additional inhibition of malignant cell growth in culture by quinines using HGT-15 cells derived from human colon carcinoma was shown to be due to lawsome as a member group of the quinones group (Kamei 1998). Dried powered leaves of henna contain about 0.5-1% lawsone, traditionally used to produce colour fast orange, red and brown dyes. Due to having demand as herbal dye and medicinal importance its large cultivation and conservation is essential. For this biological diversity is most important and this has raised the urgency for preserving natural strains for future. The application of modern biotechnology techniques like plant tissue culture has proven to be a powerful tool in medicinal plant improvement programs. Scientists hit upon a technique where by not only can these plants be preserved from being lost but are also able to develop a complete plant from a small plant part. During the last few years Micropropagation, technique has emerged as a promising technique for rapid and large scale propagation. Technique has emerged as a promising technique for rapid and large scale propagation of vascular plants. Compare to conventional propagation, micro-propagation has the advantage of allowing propagation in limited time and space. A more promising propagation technique for the economical production of large number of plantlets is somatic embryogenesis. Somatic embryos morphologically resemble zygotic embryos.

They are bipolar and bear typical embryonic organs, the radical, hypocotyls and cotyledons. Somatic embryogenesis is the *in vitro* production of embryo the structure from somatic tissue which is potential miniature plants. Rapid exploitation of medicinal plants for their uses caused depletion of particular plants species. So many ways and methods are being developed to save and preserve the valuable plant wealth, especially from the tropical forests. The exploitation of some of the plant species have led them to get listed among rare and endangered are medicinal plants already threatened by over exploitation and habitat loss. In addition to solving problems related with mass multiplication, plant cell and tissue culture has tremendous potential for the production of active principles from plants which are important in pharmaceuticals. The role of plant cell and tissue culture in the production of important compounds directly in culture has proven over the years. More over higher level of secondary compounds than the plant can be produced. The culture portion of metabolites promise the high value low volume products to the great demands for the constituents. It will be a viable alternative source for the production of Phytochemicals without losing the whole plants. In general, the need for reliable supplies makes the prospects of Synthesizing commercial compounds in the lab more appealing than that of cutting them from the wild of the 119 plant derived drugs on the market today fewer than a dozen are synthesized commercially or produced by simple chemical modification of the active chemical modification of the active chemicals modification of the active chemicals, the rest are extracted and purified directly from plants. It is well known that plants are in important source for a variety of chemicals used for a variety of purposes including pharmacy, medicine and industry. In recent years, plant cell suspension cultures and immobilized cells are being utilized for the production of these following advantages over extraction from whole plants. (i) They yield and quality of the product is more consistent in cell cultures because it is not influenced by the environment. (ii) The production schedule can be predicated and controlled in the laboratory or industry. The most important chemicals produced using cell cultures are secondary metabolites, which are defined as those cell constituents which are not essential for survival. Some of the important secondary metabolites obtained from plants are listed and plant cell culture technique can be profitably utilized to produce them at a commercial level (also consult on bioprocess engineering). In the indigenous system of medicine, Henna leaves are used as a prophylactic against skin diseases. It has been used for the treatment of boils, bums, bruises and skin inflammations. Although a lot of pharmacological investigations have been carried out based on the in present but a lot more can still be explored and utilized. The challenge of synthesizing derivatives of natural antimicrobial napthoquinones to improve their

pharmaceutical properties has been accepted and practiced by many laboratories. Indeed the synthesis and evaluation of antimicrobial activity of bioactive analogues of certain substances has been reported (Nagata 1998, Olivera 2001). American Indian has used plants containing napthoquinones in treating a number of diseases including cancer (Pinto 1977, Kapadia 1997). Such activity was confirmed by several research reports (Sieber 1976, Dinnen 1997, Pink 2000). Furthermore several other biological activities for napthoquinones have been described such as being anti-inflammatory (De Almeida 1990). Henna the potential medicinal plant is the unique source of various pharmacologically important compounds. Extensive investigation is needed to make the most of their pharmaceutical and therapeutic utility to fight various diseases. As the global scenario is now changing towards the use of safer non toxic plant products having traditional medicinal use, development of modern drugs from henna should be emphasized for the control of various diseases. In fact, time has come to exploit the centuries-old knowledge on henna through modern approaches of drug development. For the last few years, there has been an increasing trend and awareness in herbal research. Significant numbers of research works have already been carried out during the past few decades in exploring the chemistry of different parts of henna. Several therapeutically and industrially useful preparations and compounds have also been marketed. This generates much interest among the scientists in exploring more information about this versatile herb. An extensive research and development work should be undertaken on henna and its products for their better economic and therapeutic utilization. Conventional methods of propagation of L. inermis, sexual as well as vegetative, are besets with many problems that restrict their multiplication on large scale. Propagation through seed is unreliable because of disease and peat problems, short viability and heavy rains during the seeding season in the natural habitat. An unplanned exploitation by the ever growing human population has resulted in the rapid depletion of plant resources, particularly the economically important plants. Cosmetic and pharmaceutical companies largely depend upon materials procured from naturally occurring stands, raising concern about possible extinction and providing justification for development of in vitro techniques for mass propagation of *L. inermis*. Preservation of genetic stability in germplasm collections and Micro-propagation of elite plants is of utmost importance and propagation of plants through apical of axillary meristem culture allows recovery of genetically stable and true to type progeny (Hu and Wang 1983, George and Sherrington 1984). There is very few works has been reported on Micropropagation of L. inermis. The present work describes standardization of the protocol for in vitro clonal propagation photochemical production and analysis of *Lawsonia inermis*

which helps to requires planting material for plantation and conserving a highly medicinally potent endangered plant. Therefore a biotechnological approach was made as an attempt to capitalize on the potential of henna as an herbal dye to fulfill the market demand. Plant tissue culture forms the backbone of plant biotechnology i.e. Micro-propagation, induction of somaclones, somatic hybridization, cryopreservation and regeneration of transgenic plants. Plant tissue culture is an essential component of plant Biotechnology. Plant cell and tissue culture has already contributed significantly to crop improvement and has great potential for the future (Kumar and Kumar, 1996). Research efforts in plant cell and tissue culture have increased dramatically worldwide in recent years including efforts in developing nations. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cells, tissues and organs under sterile and controlled environmental conditions (Murashige & Skoog, 1974). In India, Tissue culture research began nearly four decades ago with the first report on production of test tube fertilization (Kanta et al. 1962). Tissue culture techniques are now being widely applied for improvement of field crop, forest, and horticulture and plantation crop for increased agricultural and forestry production. Today tissue culture technology is for large-scale production or Micropropagation of elite planting material with desirable characteristics. This technology has now been commercialized globally and has contributed significantly towards the enhanced production of high quality planting material. Recently emphasis has been on genetic transformation, especially for increased production of secondary metabolites, production of alkaloids, pharmaceutics, nematocidal compounds, and also some novel; compounds not found in the whole plant, regeneration of plant resistant to herbicides, diseases, and pests, scale up cultures in bioreactors, plants with different morphological traits, and transgenic plants for the production of vaccines etc. these developments have far reaching implications of the improvement of medicinal plants as well. (Bajaj, 1998). Micropropagation has been useful for the rapid release of new varieties prior to multiplication by conventional methods, e.g. pineapple (Drew, 1980) and strawberry (Smith and Drew, 1990). Micropropagation is also used to promote gerrnplasm storage for maintenance of disease-free stock is controlled environmental conditions (Withers, 1980). According to pinto et al., (1977) Lawsonia plant possessing naphthoquinones have been used in treating a disease like cancer by American and Indians. Pink et al., (2000) also declared that several research reports have also confirmed cancer treatment activity of Lawsonia. De Almeida et al., (1990) described napthoquinones being anti inflammatory, fungicidal, (Gafner et al., 1996) virucidal (Heinrich et al., 2004), bacterial (Binutu et al., 1996), trypanocidal, and anti- malaria (De Moura et al.,

2001). Plant regeneration and induction of coloured callus from henna (*Lawsonia inermis* syn. *Lawsonia alba*) has been reported by Fatimah Abdul Rahim and Rosna Mat Taha (2011). Tissue culture studies of *Lawsonia inermis*, syn. *Lawsonia Alba* (henna) were carried out to induce multiple shoots, plant regeneration and colored callus on MS media supplemented with various hormones. Various explants, such as sections of leaves, stems and roots, from 4month-old aseptic seedlings were used. Different concentrations of 2, 4dichlorophenoxyacetic acid (2, 4-D), naphthalene acetic acid (NAA), benzyl aminopurine (BAP), kinetin and indole acetic acid (1AA) hormones were used. The results showed that efficient regeneration system for henna plant *in vitro* could be achieved. Different colored callus were obtained from henna tissue culture on MS supplemented with different combinations and concentrations of hormones 0.5mg/L 2,4-D and 0.5mg/L IAA, 2.0mg/L NAA and 2.0mg/lit BAP, 0.5mg/lit NAA and 0.5mg/lit BAP, 1.5mg/L NAA and 1.5mg/L BAP and also 0.5mg/lit IAA and 0.5mg/L BAP and thus. Production of important secondary metabolites in vitro in possible for this important species. Formation of embryogenic callus was observed during the plant morphogenesis in MS medium supplemented with combination of 0.5mg/L IAA and 0.5mg/L 2,4-D hormones which will enhance more production of in vitro plantlets and ultimately promote new propagules of this species. Conventional methods of propagation of L. inermis, sexual as well as vegetative, are besets with many problems that restrict their multiplication on a large scale. Propagation through seed is unreliable because of disease and pest problems, short viability and heavy rains during the seeding season in the natural habitat. An unplanned exploitation by the ever growing human population has resulted in the rapid depletion of plant resources, particularly the economically important plants. Cosmetic and pharmaceutical companies largely depend upon materials procured from naturally occurring stands, raising concern about possible extinction and providing justification for development of in vitro techniques for mass propagation of L. inermis. Preservation of genetic stability in germplasm collections and Micropropagation of elite plants is of utmost importance and propagation of plants through apical or axillary meristem culture allows recovery of genetically stable and true to type progeny (Hu and Wang l983, George and Sherrington 1984). There is a very less report on Micropropagation of *Lawsonia inermis* has reported. The present communication describes a successful protocol for mass propagation of L .inermis Linn.

MATERIALS AND METHODS

Materials required for equipment's required for plant. Culture the necessary of required for setting up tissues culture laboratory are rested blow (Table-1) for a small scale simpler require can be used table 1.0 equipment require for plant tissues culture. (i) Gas, water, Electricity supply. (ii) Hot plate & magnetic stirrer. (iii) Sensitive balance. (iv) PH meter. (v) Distillation apparatus. (vi) D-ionizer for sterilization process. (vii) Autoclave & pressure cooker. (viii) Flaming instruments. (ix) Culture rake. (x) Tempt. Control unit. (xi) Laminar air flow cabinet. Techniques involve sterilization inoculation and regeneration of plant cell tissue organ under aseptic condition and culture vials constraining Synthetic nutrient medium. Both chemical composition of the medium and e controlled environment condition (temperature, light, humidity, aeration) effectively control the expression of any genotype or phenotypic potential in the explants. A tissue culture laboratory should be equipped to facilitate the following procedures. (i) Sufficient space for washing, sterilization of glass ware and other equipment's. (ii) Aseptic transfer condition. (iii) Preparation sterilization & storage of nutrient medium. (iv) Bio chemical analysis of the material studies of cells & tissue etc.

Methods Involved

The technique of tissue culture is broadly carried out in following three main phase. (1). Pre-experiment phase. (2). Experiment phase. (3). Post experimental phase.

1. Pre-experiment phase-it is the preparatory and include following steps. (A). Sterilization of rooms. (B). Washing of glassware. (C). Preparation of stock solution.

Sterilization of rooms

The maintenance of highly aseptic condition is a vital factor for successful tissue culture laboratory thus the room such laboratory should be first washed with disinfecentant followed by wiping with 20% sodium hypochlorite solution or 95% ethyl alcohol commercially available disinfectants (Razadan, I993). The final sterilization of the rooms should be done either by Organo mercury lamps of low pressure or by more recently by ozone generating system, which gives 90-100% ted area, can be used 8 minutes after sterilization is over (Kumar, 1998).

Washing of Glassware

All the glass ware used in tissue culture technique is washed with hot or warm water containing soap detergent (lanoline) the soap detergent is removed under running tap water

followed by a final washing with double distilled water after washing the glassware should be subjected to over drying at 70 degree centigrade.

Choice of media

The selection of medium for achieving successful result is essential Criteria in tissue culture technique, the composition of tissue culture medium may range from simple salt mixture to highly complex of organic and inorganic Salts.

Media Ingredients

The composition of nutrient medium is the governing factor successful growth and morphogenesis of plant tissue in culture is as under. (i) Inorganic macronutrients, inorganic micronutrients. (ii) Carbon source, Vitamins, phytoharmones, Amino acid. (iii) Solidifying agents or supporting materials, Anti oxidants. (iv) Undefined organic, Miscellaneous.

Preparation of Stock Solution

(a) Stock solution of nutrient medium; stock solution are concentrated individually or in groups prepared well in advance and used sub sequentially to prepare before experimentation because dissolution or different components during media preparation is time consuming. It's necessary to dissolve each constituent in double, distilled water completely before adding another otherwise precipitation may occur. Stock solution after preparation should be placed in a refrigerator. Storage life to different stock solution varies and is representative in Table-4 before using the stock solution; they should be examining for any contamination precipitation. The procedure for the preparation of stock solution of must commonly used medium i.e. Murashige & Skoog (MS) medium (1962) is given in Table-5. (b) Stock Solution of phytoharmones; The concentration of phytohormones in tissue culture media is usually representative in milligram (mg), parts per millions (PPM) or micro moles. The procedure for the preparation of stock solution of phytoharmones is given in Table-6.

Table – 1- Solution of MS (1962) Macro Salts (X-10)				
Constituents	**Amount (mg/Lit) Present in original medium**	**Amount (mg/Lit) to be Taken for stock solution (X100)**	**Final Volume of Stock Solution (ML)**	**Amount to be used l/ml**
NH_4NO_3	1650	16.5		
KNO_3	1900	19.0		
Cad22H_2O	440	4.4	1000	100
MgSo47H_2O	370	3.7		
Kn2PO_4	170	1.7		
Table– 2- Solution of MS (1962) micro salts (X100)				

Constituents	Amount (mg/Lit) present in original medium	Amount (gm/Lit) to be taken for stock solution (X100)	Final Volume of Stock Solution (ML)	Amount to be used l/ml
KL	0.83	83		
H_3Bo_3	6.2	620		
$MnSo_4$, $4H_2O$	22.3	2230		
$ZnSO_4$, $7H_2O$	8.6	860	500	5
Na_2MoO_4,$2J_2O$	0.25	25		
$CuSo_4$,$5H_2O$	0.025	2.5		
CaC_{l2},$6H_2O$	0.025	2.5		
Table– 3- Stock Solution of MS (1962) Iron Source (X100)				
Constituents	**Amount (mg/Lit) present in original medium**	**Amount (gm/Lit) to be taken for stock solution (X100)**	**Final Volume of Stock Solution (ML)**	**Amount to be used l/ml**
$FeSO_4$, $7H_2O$	27.6	2.78		
Na_2EDTA, $2H_2O$	37.3	3.73	500	5
Table – 4- Stock Solution of MS (1962) Myo-inositol (X50)				
Constituents	**Amount (mg/Lit) present in original medium**	**Amount (gm/Lit) to be taken for stock solution (X100)**	**Final Volume of Stock Solution (ML)**	**Amount to be used l/ml**
Myo-inositol	100	5	250	5
Table– 5- Stock Solution of MS (1962).Vitamins (X100)				
Constituents	**Amount (mg/Lit) present in original medium**	**Amount (gm/Lit) to be taken for stock solution (X100)**	**Final Volume of Stock Solution (ML)**	**Amount to be used l/ml**
Thiamine Hcl	0.1	10		
Nictinic Acid	0.5	50	500	5
Pyrodoxine	0.5	50		
Glycine	2.2	200		

Preparation of Media

The preparation of one liter MS (1962) medium involves following steps. (i) Take 500ml of distilled water in a sterile flask (2000ml). (ii) Add 30 grams (3% w/v) sucrose and shake to dissolve it. (iii) Add 100ml of macro salts, 5ml of vitamin myo-inositol and 5ml of iron source from their stock solution. (iv) All required quantity of growth hormones. (v) Add more double distilled water making the volume of medium to 900m. (vi) Adjust the pH between 5.2-8 by adding O.N. NaOH or OJN HCl drop wise. (vii) Make final volume of the medium (i.e. l000ml) by the addition of double distilled water. (viii) Add 8g (0.85) of agar. Heat the medium and boil to dissolve thec agar and then dispense in culture vials. (ix) The culture vials are final plugged. Labeled and autoclaved at 15psi and 121ºC for 15-20 minutes. The nutrient media sterilized by autoclaving at 121ºC (15-20 lb) for 20 minutes. The minimum time required for sterilization depends upon the volume of the medium in the vessel prolonged autoclaving May results in braking and denaturation of various media ingredients.

Therefore, it is better to dispense media in small aliquots, whenever possible to prevent denaturation of media ingredients.

Collection of Plant Material

Juvenile shoots were obtained from mature plants of *Lawsonia inermis* (L.) growing in the Botanical Garden of the Grow tips Biotech lab of Bhopal. Axillary nodes, young leaves and internodes were used as explants. The experiments were performed in replicates of ten for each type of explants and all experiments were repeated three times. The growth responses of the explants were studied at weekly intervals in terms of the initiation band distribution sites of shoots and root regeneration.

Explant Selection and Sterilization

The disease free, young and healthy nodal explants were selected for carrying out study as young cells are supposed to have retained their ton-potency.

Explant sterilization

The leaves were removed from the explants and then washed under running Tap water for 30 minutes bin order to wash off the external dust Contaminants. (i) In the next step explants were soaked in an aqueous solution containing 0.2% Bavistin (BASF, India limited and 0.03% Streptomycin (Ambistryn --S, Sarabhai Piramal) for 10 minutes in Laminar flow hood. (ii) This was followed by gentle wash in sterile double distilled water for 5 minutes for two cycles. (iii) Then the explants were immersed in aqueous solutions of Savlon (Johnson &0 Johnson) (1.5% v/v chlorohexidine gluconate solution and 3.0% w/v cetrimide) For 10 minutes and were shaked regularly. (iv) Then the explants were washed thoroughly with sterile double distilled water for 5 minutes (two cycles). (v) After this treatment, the explants were sterilized with 0.01% Mercuric chloride (Ranbaxy) aqueous solution for 4-8 minutes. Then the explants were removed from the sterilized solution and rinsed thoroughly for two times with sterile Double distilled water.

Initiation of Cultures

Explants were inoculated on the medium MS with 3% sucrose (WW) and 4.5 gm of Agar. The pH of all medium was adjusted to 5.8 before autoclaving. The cultures were incubated in a culture room at 25±2°C under sixteen hours photo period provided by cool white fluorescent tubes (Philips India). For the present investigation the Ms medium supplemented with different concentration of growth hormones like cytokinins (BAP and KN), Auxin

(IAA-NAA) were used for establishing the culture, For initiation experiment the Axillary apical explants were inoculated on MS medium supplemented with different concentration of BAP (0.1 -1 mg/Lit), alone and with IAA (0.1-0.5mg/Lit) and NAA (0.01-0.05mg/Lit).

Followed protocol

(i) Sterilized explants were transferred aseptically to sterilized glass under plate the laminar flow hood. (ii) Then a cut was given on both basal as well as the top portion of the explants to remove undesirable/ dead portions after surface sterilization. (iii) The forceps were earlier rinsed in the 70% ethanol (Bengal chemicals and pharmaceuticals ltd.) and were flamed and then kept for some time to get cool. Then the lid from one test tube was removed and test tube's mouth was flamed to avoid any chance of contamination. (iv) The lid was finally closed carefully, flamed lightly and sealed with alkaline film. The forceps were then again rinsed with 70% alcohol to avoid any chance of cross contamination. The same procedure was undertaken for all the explants. (v) These jars were finally kept in the growth room with temperature conditions 25±2°C, with a period of 16 hours daylight and 8 hrs night break under the cool white fluorescent light of average 2500 lux (cool white fluorescent Tube light 40WGE).

Different medium used in Initiation Experiment

(1). MS+0.01mg/Lit BAP (2). MS+0.02mg/Lit BAP (3). MS+0.05mg/Lit BAP (4). MS+1.0mg/Lit BAP (5). MS+0.5mg/Lit BAP+0.1mg/Lit NAA (6). MS+1.0mg/Lit BAP+0.1 mg/Lit NAA.

Multiplication of shoots

The shoot starts to initiate alter 3rd day of inoculation. The initiated shoot were sub culture in the same medium; Experiments were also carried out to check the effect of higher concentration of cytokinins alone in combination with IAA. The experiments were performed in replicates of ten for each type of medium. The growth response of explants was studied at weekly interval. The parameters were taken as the number of shoots initiated, the height of regenerated shoots and the callus developed.

Different Media Used for the Multiplication of shoot

(1) MS+0.05mg/Lit BAP (2). MS+0.05mg/Lit BAP+0.05 NAA (3). MS+0.0mg/Lit BAP + 0.05 NAA (4). MS + 0.05mg/Lit BAP+0.05 NAA (5). MS+1.0mg/Lit BAP+0.05 IAA (6). MS+10.mg/ Lit BAP+1.0 NAA (7). MS+1.0mg/ Lit BAP+1.0IAA

Root Initiation

Axillary shoots developed in cultures in the presence of cytokinnin generally black roots. To obtain full plants the shoots must be transferred to a rooting medium. This is different from the shoot multiplication medium. Especially in its hormonal composition. A low salt medium is found satisfactory for rooting of shoots in large Number of plants species. After multiplication phase the regenerated multiplied shoots were separated and inoculated for root induction in the medium containing Auxin like NAA, 18A and activated charcoal (AC) the growth parameter was observed as percentage of root initiation. No of roots, and the callus formed.

Rooting Protocols

(i) In the laminar flow, under sterile conditions the cap was removed from the culture bottles in sterile condition (laminar flow hood) and with the help of sterile forceps the multiplied shoots were removed from the medium and placed on the sterile glass plate. (ii) With the help of sterile scalpel elongated up to 1-2 cm in length were cut and placed into the rooting medium. The culture bottles were then capped and placed in the growth room under condition. The time required for in vitro rooting of shoots the same may vary from 10 -15 days.

Different Media used for root production

(i) MS+0.05mg/lit NAA (ii) MS+0.05mg/Lit IBA (iii) MS+200 mg/Lit AC. (iv) MS+V_2+200mg/Lit AC.

Ex -Vitro Rooting

The technique can be used for many plants. The technique is particularly suitable for species which root easily and it has been said to have special advantages for woody plants in which secondary thickening is important for proper root function. In many of these plants, the absence of a vascular cambium may that roots formed in-vitro are incapable of proper water movement.

Protocol Followed

(i) Plantlets were taken out of the culture bottles (multiplication sub forceps and washed thoroughly with water to remove any Remaining of the medium. (ii) 0.015 Bavistin treatments were given to the plants in order to protect them from the fungal attack in the near future. (iii) Plantlets were separated into single shoots by cutting their bases gently either the

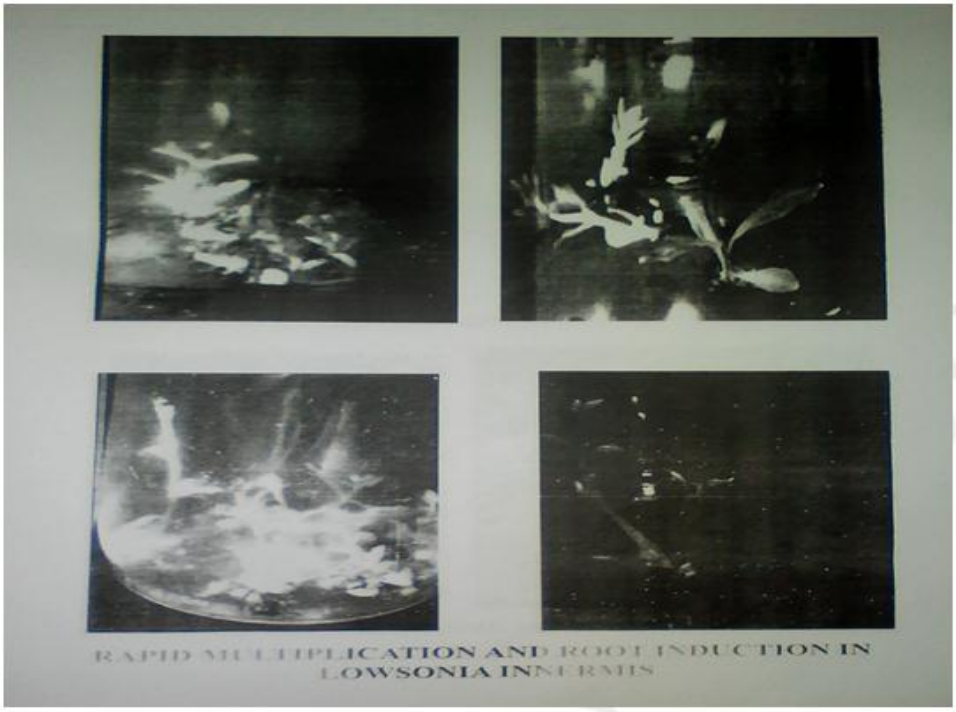
help of blade. (iv) Single shoots were dipped in IBA solution (200 ppm) before planting into a hardening mixture. (v) After this the single shoots are carefully planted in the trays containing soil and agro peat vs. (M/s Varsha Enterprises, Bangalore, India) mixture in 1:1 ratio.

Transplantation and acclimatization of the plantlets

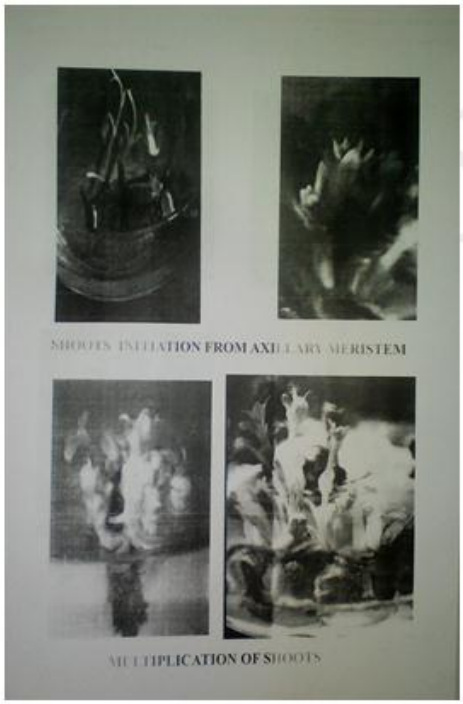
The transfer of plants from the culture vessel to the soil requires a careful, stepwise procedure. The roots of the plants are gently washed to remove the agar sticking to them. The most essential requirement for the successful transplantation is to maintain the plants under a very high humidity (90-100%). For the first 10 -15 days by keeping tem under mist or covering them clear plastic. Some small holes may be poked in the plastic for air circulation. Inside the culture vessel the humidity is high and thus, the natural protective covering of cuticle is not fully developed. During this time plant attain ability to synthesize more food and develop cuticular. Plants are maintained under shade and are then ready to be used in open nursery.

Protocol followed

(i) After 10-15 days of culture on rooting media, the rooted plantlets were transplanted ton pots on trays of hardening prior to their final transfer to soil. (ii) Rooted plantlets were taken out of the culture bottles with the help of forceps and washed thoroughly with water to remove any remaining of the medium. 0.01% Bavistin treatment was given to the plaints in order to protect them from the fun gal attack in the near future. (iii) After this the plants are carefully planted in the polybags containing different soil mixtures in different ratios as shown in the table. (iv) VAM (l.A.R.I, New Delhi), Fannaryad Manure and Vermicopodst from local source, flyash (Thermal Power Station, PSEB. Ropar, Kindly provided by Dr. Dinesh Goyal, DBTED). (v) After planting, plantlets are thoroughly watered and kept in polyhouse under humidity range of approximately 80%. These plantlets should be sprinkled with water time to time as per the requirement and after two weeks should be transferred to shade house having humidity range of approximately 60%. (vi) The plantlets are then transferred to open area after 9-10 days and kept there for ten days prior transferring them to the field.



RAPID MULTIPLICATION AND ROOT INDUCTION IN LOWSONIA INNERMIS



SHOOTS INITIATION FROM AXILLARY MERISTEM

MULTIPLICATION OF SHOOTS

HENNA PLANT IN FIELD FRUITS FLOWERS AND SEEDS

RESULTS AND DISCUSSION

Present study the earlier research on *Lawsonia inermis* reported plant regeneration through axillaries nodes, inner nodes and young leaves on media with higher concentration of cytokinins (Rout GR, Das G, Samantaray S, Das P 2003). However, we worked in the present in the present experiments on one step medium with low concentration of cytokinins and Auxin that were found suitable for Axillary and apical explants for rapid and large scale

multiplication at a cost effective level. The standardized time duration for surface sterilization of explants with 0.1% $HgCl_2$ solution was optimized at 4-5 minutes. The explants show 70-80% sterile conditions. For initiation of shoots the explants shows response in every medium tried for experiment the bud break and the initiation of shoots was reported maximum in the medium containing 0.1% $HgCl_2$ solution was optimized at 4-5 minutes. The explants show 70-80% sterile conditions. For initiation of shoots the explants response in every medium tried for experiment the bud break and the initiation of shoots was reported maximum ion the medium containing 0.01-0.5 BAP alone. 1-4 shoots initiated which is 1-2 cm long within one week. The addition of NAA promotes the percentage of shoots initiation about 4-5 shoots were initiated within one week at low concentration of BAP and Auxin. High concentration of cytokinins and Auxin produces callus at the lower end of shoots. The initiated shoots were transferred in the fresh medium containing BAP alone and with combination of Auxin. The higher multiplication of shoots was reported in the medium containing 0.05mg/lit BAP alone and with combination of 0.05 NAA. Both concentration and combination of growth hormones show multiplication within two weeks of incubation. Addition of NAA (0.05mg/I) enhanced the shoot production from the nodes and emergence of shoot buds at the base of internodes which later differentiated into shoots. Proliferation of shoot bud and elongation growth of shoots was comparatively higher in the medium containing BAP and NAA. Increase in shoot length was observed faster in the medium about 40 -50 shoots of 5.6 to six cm length has been achieved within 15 -20 days of culture which increase with the age of culture. The multiplication pattern which was observing in *Lawsonia inermis* was that the shoots multiplied at the lower end of the explants with the formation of the callus. Research is said to be more successful if it cost effective. The number of shoots as per subculture and media quantity per subculture was standardized from the commercial point of View. Within a period of three subcultures, the number of shoots at each subculture generated from Axillary nodes and internodes were: 12100, 49, 784 shoots, respectively the system demonstrated a continuous supply of shoots up to ten cycles without any decline in their number in subsequent subcultures. Subcultures were performed frequently (3 weeks), as delayed subcultures (more than Four weeks) in the liquid medium were found to cause vitrification of shoots explants were found to be optimal. The separated multiple shoots show 809-0% root induction in the medium containing activated charcoal and NAA (mg/lit) within 10 days. 2-3 roots in lower and the shoot which becomes thick and hard within 20 days. We reported high shoot bud regeneration from Axillary and apical explants with continues proliferation and elongation of shoot buds. Shoot buds the root induction on MS medium supplemented with

0.2-1.0mg/lit BAP with 0.05 NAA. Thus a commercially viable protocol has been established for mass propagation of *Lawsonia inermis*. The procedure described here will go a long way to meeting a one demand of the pharmaceutical industries and on the other hand save the species from the extinction.

Table – 6- Effect of Growth Regulators on shoot initiation in *Lawsonia inermis*

S. No	Medium + growth Hormones Mg/l	% age of response	Average No of Shoots	Average Shoot Length in cm	Callusing
1	MS + 0.1BAP	70%	1-3	2-3	-
2	MS + 0.2 BAP	705	1-2	2-3	-
3	MS + 0.5 BAP	85%	3-4	2-5	-
4	MS + 1.0 BAP	79%	3-4	2-3	-
5	MS + 0.5 BAP + 0.5 NAA	70%	2-3	1-2	++
6	MS + 1.0BAP + 0.1 NAA	60%	2-3	1-2	++

Table -7- Effect of Growth Regulators on shoot multiplication in *Lawsonia inermis*

S. No	Medium + growth Hormones Mg/l	% age of Shoot induction	No. of shoots per culture	Average Shoot Length in cm	Callusing
1	MS + 0.5BAP	70%	50	3-4	-
2	MS + 0.5 BAP +0.5 IAA	85%	60-65	3-4cm	-
3	MS + 1.0 BAP + 0.5 NAA	85%	60	4.5 cm	-
4	MS + 0.5 BAP + 0.5 NAA	70%	55	2-3	-
5	MS + 1.0 BAP + 0.1 IAA	42%	20-5	1-3	++
6	MS+1.0 BAP + 1.0 NAA	45%	40	12	++

Table -8- Effect of different Media on root induction in *Lawsonia inermis*

S. No	Medium + growth Hormones Mg/l	% age of Shoots rooting	Root Length	Root Morphology
1	MS + 0.5 NAA	50-60	2-3	Thin, Short
2	MS + 0.05 IBA	70-75	2-5	Thin, Short
3	MS + 200 AC	85-90	3-5	Thin, Short
4	MS ½ 200 AC	82-90	3-5	Thin, Short

Rooting

After two cycles of multiplication subculture, elongated shoots of 2-3cm in length were excised and cultured on MS basal medium having different combinations of Sugar and agar with MS basal (MS + Sugar 3O gm/I Agar~ 89 gm/) as control. The experiments were conducted twice, with 3 replications (with 3 shoots per bottle). Rooted shoots were taken after 2 weeks, shoot length, root length band no of roots per explants (total 9 explants per treatment each time), fresh wt dry wt (keeping them in an oven with 500ºC for 24 hrs) were measured. Initiation of rooting took place alter 56 days of inoculation. Single and multiple roots were formed from the base and the nodal portions and the length of the roots were 1-2cm within 8 10 days. It was observed during multiplication that rooting in lawsonia inermis in vitro culture is fairly spontaneous and no addition of growth regulators is further

necessary. Hence the experiment is designed to study the rooting response with different treatment combination with two variations of agar (7 and 8gm/Lit) and sugar (0, 10,20,30gm/lit). It was postulated that highest shoot root ratio and biomass accumulation may indicate positive responses. It was observed that RTS (MS + agar 7gm/ lit+ Sugar 20gm/lit) Recorded highest S/R (2.97) and 2.6 for RT6 (MS+agar 8 gm/lit+sugar 20gm/lit) with considerable higher no of roots (7.320 and 7.72 respectively). Similar response was also no observed in case of RT-7 and MS in terms of S/R of 2.50 band 2.15 and root Number of 5.58 and 2.20 respectively. Minimal media (RTI and RT 2) i.e. Having 0% Sugar and lower concentration of agar also show positive results so this combination can also be used for rooting of explants. Our results indicate 100°C root formation in all the mediums? (MS basal without any additional growth regulator). In case of Withania, shoot were rooted best (87%) on ms medium containing 2mg/I (9.9uM) indole -3-butyric acid (Rani er al, 2003) and root induction in B. Monnieri was observed in Mushage and Skoog medium supplemented with BAP 0.5 mg / liter within 6 days of culture (Singh et al, 1999). Tiwari et al, 2000, observe3d highest rate of rooting was also carried out using single of 1-2cm in height derived from MS medium and then transplanted in Soil; agro peat mixture in the ratio of 4: 1 which gave 100% survival rate.

CONCLUSION

Plant based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in the recent years due to fewer side effects in comparison to synthetic drugs and antibiotics has high lightened the need for the conservation and propagation of medicinal plants and their *in vitro* conservation of germplasm. The present study showed that it was possible to explore the morphogenetic potential of *Lawsonia inermis* by application of growth regulators and light condition. With the use of different combinations of cytokinins, the induction and multiplication of shoots derived from especial and Axillary meristems. The regulatory action of cytokinins and the apical dominance (Wickson and Thiemann 1958). The maximum shoot induction and multiplication was observed MS medium supplemented with 0.5mg /me BA, 0.5 mg/l Kn within 4 weeks of culture under a 14 hr photoperiod. At higher concentrations of BA or Kn, the rate of shoot proliferation declined. The Axillary meristems produced more number of shoots than the apical meristems. Similar results were reported in Psoralea corylifolia (saxena et al, 1998), Picrohiza kurroa (Lal et al, 1998, Upadhyay el al, 1989) and plumbago zeylanica (Rout et al, 1999). The results are consistent with earlier reports indicating that cytokinins

and auxins affect shoot multiplication in other plants using shoot tip or Axillary bud explants (Mathew and Hariharan 1990, Rout et al, 1992, Rout and Das 1993, Rout et al, 1999). The results showed that the number of shoots per culture was increased in continuous light both in apical and Axillary meristems than in the 14 hr photoperiod. The interaction of photoperiod and plant growth regulators had a significant effect on shoot morphogenesis as reported earlier (Baraldi et al, 1988, Samantaray et al, 1995). With the increase in the concentration of either BA or KN, the percentage of shoot multiplication declined. The results also implied that there were differences among the treatments for both the percentage of culture developing multiple shoots and the mean numbers of shoots per culture. The variation of response was due to the varying concentration of growth regulators used in the medium and light condition as reported earlier in *Lavandula latifolia* (Calvo and Segura 1989) and *Zingiber officinal* (Rout and Das 1997). The rate of multiplication was high and stable up to the 5th subculture and declined in subsequent subcultures. This might be due to the balancing of the endogenous and exogenous growth regulators and the ionic concentration of nutrient salts as reported earlier in other plants (Zimmerman 1985, Rout and Das 1997). The elongated shoots were rooted the maximum in MS basal salts supplemented with 0.25 mg/I IBA with 2% sucrose. The rooting ability was reduced with the increase in the concentration of IAA or IBA in the medium. The percentage of shoots forming roots and days to rooting significantly varied with different concentrations of IAA or IBA. Similar observations were made in plumbago rosea (satheesh Kumar and Bhavandan 1988) and psoralea corylifolia (Saxena et al, 1998). The rooted plantlets were established in the field and grew normally. Henna has been used since earliest times as a medicine, preservative and cosmetic. It has long been recommended in tradional Eastern medicine as an astringent, purgative and aboritifacient. Results come to reinforce the work completed on this plant and extend the fact that the *Lawsonia inermis* plants have an antibacterial capacity that can be exploited. Lawsone appears to be responsible of the antimicrobial activity showed. In conclusion, an attempt was made to develop an in vitro protocol for mass multiplication of L. inermis by manipulating the nutrient salts, growth regulators and culture conditions. The pattern of morphogenesis on various phytoharmones regimes largely confirm those reported in other plant species (Koblitz et al, 1983, Ideda et al, 1988, La] et al, 1988). This investigation may be useful for conservation of economic plant species. In the present study we have attempted to work on the Micropropagation protocol. Observation and conclusion in the present study:- (i) In identifying the sterilization protocol for *Lawsonia inermis* explants the following protocol was found satisfactory. Washing of explants under running tap water for 20 minutes

(two cycles). These were then treated with streptomycin for 15 minutes. These were taken to laminar flow for surface sterilization where the explants were treated with Savlon and Tween -20 for 10-20 minutes. (ii) It was also it was also it was also observed that the media combination which gave best results for explants length, number of nodes per explants, no of shoots/node and number for leaves for initiation and establishments i.e. MS+BAP 0.05mg/lit NAA 0.05mg/lit + 30gm/1 for sugar, shoot length of 2.6cm; number of nodes per explants 1.7 cm and number have newly sprouted shoots 2.9. (iii) Further in multiplication stage the trend that we obtained during the initiation and establishment phase continued and it was observed that the media (MS+BAP 0.5-1.0mg/lit & NAA 0.05 mg/lit) continued to give best results for explants length i.e. 5.5 cm and shoot bud per node was 15-20 and number of nodes /explants 5.1cm and leaves per shoot 6.1.(iv) For rooting general observation shows that media combination of (MS+0.5mg/lit NAA+30gm/lit sugar) gave results for number of roots and length of roots.

REFERENCES

1. AI-Arnaoutt S, AIArnaoutt AK. 1987. in AlJozieh IK Prophetic Medicine. Beirut: Al-Risala publishing. ASE. 1997. Status report on the development and application of in vitro techniques for the conservation and use of plant genetic resources. International plant Genetic Resources Institute, Rome, Italy. 67 p.
2. AL-Abri A, Al-Abri H, AlHashmi N, AlMukhaini N, EI-Haq A, Habbal 0, Omani henna (Lawsonia inermis) as a natural histological stain, unpublished.
3. Ali BH, Bashir AK, Tanira MO. 1995. Antiinflammatory, antipyretic and analgesic effects of lawsonia inermis L (henna) in rats. Pharmacol, 51: 356 -63.
4. Anand KK, Singh B, Chand D, Chandan BK, 1992. An evaluation of *Lawsonia inermis* alba extract as hepatoprotectiveagent. Plants Med, 58(1): 22-5.
5. Anissa Boubaya Nidhal Marzougui, Laila Ben Yahia Ali Ferchichi Chemical diversity analysis of Tunisian Lawsonia L. Populations African journal of Biotechnology, 8 June, 2011; 10(26): 49804987.
6. Ashok Chaudhury, poonam Duhan, Anil Poonia and Subhash Kajla Efficient method for Micropropagation of lawsonia inermis India BIOTECHNOLOGY» 2011.
7. Badri BM, Burkinshaw SAM. 1993. Dyeing of Wool and nylon 6.6 with henna and lawsone. Dyes & pigments, 22(1): 15-25.
8. Bakkali, A.T., Jaziri M, Foriers, a, Heyden, V, Y., Vanhaelen, M. and Homes, j. (19907). Lawsone accumulation in normal and transformed cultures of henna, 51: 83-87.

9. Balachandran SM, Bhat SR, Chandel KPS. In vitro clonal Ahmad RU. 1993. Medicinal plants used in ISM their procurement, cultivation, regeneration and import/ export aspects: a report. In: JN Govil, VK Sing and S Hashmi (editors). Medicinal plants: New Vistas of Research, part I. Today and tomorrow publishers, New Delhi, India, 221-258.
10. Bhandarkar M, Khan A. 2003. Protective effect of *Lawsonia Alba* lam, against CC14 induced hepatic damage in albino rats. Indian J Exp Biol, 41(I): 85 -7.
11. Bhuvaneswari K, Gnana poongathai S, kuruvilla A, Appala Raju A. 2002. Inhibitory concentrations of lawsonai innermis dry poeder for urinary pathogens. Indian J pharmacol, 34: 260-3.
12. Endrini S, Rahmat A, Patimah I, Yap Yun Hin T. 2002, Carcinogenic properties and antioxidant activity of henna (lawsonia inemiis). J med Sci., 2(4): 194-7.
13. Girard M, Either J-C Kindack D, Dawson BAA, Wang DVC. 1987. Mass spectral characterization of napthoquinones related lapachol. J Nat prod, 50(6): 1149-51.
14. Fatimah Abdul Rahiman and Rosna Mat Taha has reported on plant regeneration and induction of colored callus from henna (*Lawsonia inermis* syn. *Lawsonia alba*) Received 12 January 201 l,m accepted 126 April 2011. Pak. J. Bot, 2011; 43(l): 383-389.
15. Fatimah Abdul Rahiman and Rosna Matv Taha (2011) plant regeneration and induction of coloured callus from henna (*Lawsonia inermis* syn. *Lawsonia Alba*) journal of Food, Agriculture & Environment, 9(2): 397-399.
16. Habbal 0A, Al-Jabri AA, El-Haq AH, ALMahrooqi ZIL ALa Hashmi NA. 2005. *In vitro* antimicrobial activity of lawsmia inermis Linn (henna). A pilot study on the Omani Henna. Saudi Med J, 26(1): 447-50.
17. Lal, N., P.S. Ahuja, A.K. Kukreja & B. Pandey. 1988. Clonal propagation of *Picrorhiza kurroa* Royk ex Benth by shoot tip culture. Plant cell Rep. 7: 202-205.
18. Malekzadeh F. 1968. Antimicrobial activity of *Lawsonia inermis* L, App Microbiol, 16: 4; 663-4.
19. Mathew, MK & M. Hariharan. 1990. *In vitro* multiple shoot regeneration in *Syzgium aromaticum.* Ann. Bot. 65: 277-279.
20. Muhammad HS and Muhammad S. 2005. The use of *Lawsonia inermis Linn.* (Henna) in the management of burn wound infections. Africa J Biotech, 4: 9; 934-7.
21. Anonymous. 1962. The wealth of India: A dictionary of Indian raw materials and industrial products. Vol. 6. Council of Scientific Industrial Research, New Delhi, India, 47-50 p.
22. RAUT, G.R & P. Das. 1997. *In vitro* organogenesis in ginger (*Zingiber officinal Rose.*) 1.

Herb. Spice. Med. Plant. 4: 41 -5 l.

23. Rout G.R & P. Das. 1993. Micropropagation of *Madhuca longfolia* (Koenig) Mac bride var, latifolia Roxby, plant cell Rep. 12: 513-516.
24. Rout G.r., u.c. Mallick & P. Das. 1992. *In vitro* plant regeneration liom leaf callus of *Cephaelis pecacuanha* A. Richard Adv. Plant Sci. 5: 608-613.
25. Rout GR, Das G, Samantaray S, Das P. In vitro Micropropagation of *Lawsonia inermis* (Lythraceae). Rev biol trop. 2001 sepdec, 49(34): 757-63.
26. Rout, G.R, C. Saxena, S, Samantaray & P. Das. 1999. Rapid clonal propagation of *Plumbago zeylanica Linn.* Plant Growth Regul. 28: 1-4.
27. Samantaray, S., g.r. Rout & P. Das. 1995. An in vitro study on organogenesis in *Trema orientalis (Blume) Linn*. Plant Sci. 105: 87-94.
28. Satheesh Kumar, K. K.V. Bhavanandan. 1998. Micropropagation of *Plumbago rosea* Linn. Plant cell Tiss. Org. Cult. 15: 275-278.
29. Saxena, C., g.r. Rout & P. Das 1998. Micropropagation of *Psoralea corylifolia*. J. Med. Arom Plant cell. Sci., 20: 15-18.