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Endangered plants used as Medicine: A review

Sohan Singh Chouhan *.
Associate Professor, Head of department of Pharmacology.
Charak Institute of Pharmacy, Mandleshwar.

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ABSTRACT: India has a very rich plant biodiversity, many of which are medicinally useful. The rich resource is disappearing at an alarming rate as a result of over-exploitation. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Plant in vitro regeneration is a biotechnological tool that offers a tremendous potential solution for the propagation of endangered and superior genotypes of medicinal plants which could be released to their natural habitat or cultivated on a large scale for the pharmaceutical product of interest. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. Some of these endangered medicinal plants are Saussaurea lappa, Picorrhiza kurroa, Ginkgo biloba, Swertia chirata, Gymnema sylvestre, Tinospora cordifolia, Salaca oblonga, Holostemma, Celastrus paniculata, Oroxylum indicum, Glycyrrhiza glabra, Tylophora indica, Bacopa monnieri, Rauwolfia serpentina. The conventional means of propagation takes a long time for multiplication and also clonal non uniform. Conventionally, there are two methods of conservation: in situ and ex situ conservation, both are complementary to each other. In situ methods allow conservation to occur with ongoing natural evolutionary processes ex situ conservation via in vitro propagation also acts as a viable alternative for increase and conservation of populations of existing bioresources in the wild and to meet the commercial requirements. A review highlighting various in vitro protocols developed for selected rare and threatened plant species of India has been done to highlight the significance of ex situ conservation in cases where regeneration through conventional methods is difficult to undertake and species are left with low population in the wild.

KEYWORDS: Endangered, in vitro, medicinal plants.

I. INTRODUCTION

Medicinal plants have been the subjects of man's curiosity since time immemorial (Constable, 1990). Almost every civilization has a history of medicinal plant use (Ensminger et al., 1983). Approximately 80% of the people in the world's developing countries rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993). India has 2.4% of world's area with 8% of global bioth diversity and it is one of the 12 mega diversity hotspot countries of the world with a rich diversity of biotic resources. Out of 34 hotspots recognized, India has two major hotspots - the Eastern Himalayas and the Western Ghats. The bio-geographic position of India is so unique that all known types of ecosystems range from coldest place like the Nubra Valley with "57°C, dry cold deserts of Ladakh, temperate and Alpine and subtropical regions of the North-West and trans-Himalayas, rain forests with the world's highest rainfall in Cherrapunji in Meghalaya, wet evergreen humid tropics of Western Ghats, arid and semiarid conditions of Peninsular India, dry desert conditions of Rajasthan and Gujarat to the tidal mangroves of the Sunderban which harbours about 47000 species of plants of which 17 000 are angiosperms (Bapat et al., 2008). India is also rich in medicinal plant diversity with all the three levels of biodiversity such as species diversity, genetic diversity, and habitat diversity (Mukherjee and Wahile, 2006). Across the country, the forests are estimated to harbour 90% of India's total medicinal plants diversity. Only about 10% of the known medicinal plants of India are restricted to nonforest habitats (Wakdikar, 2004). Concerning the total number of flowering plant species, although only 18,665, the intraspecific variability found in them makes it one of the highest in the world. Out of 18,665 plants, the classic systems of medicines like Ayurveda, Siddha, and Unani make use of only about 3000 plants in various formulations (Schippmann et al., 2006). Although, there is no

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reliable figure for the total number of medicinal plants on Earth, and numbers and percentages for countries and regions vary greatly but estimates for the numbers of species used medicinally include: 35,000-70,000 or 53,000 worldwide (Schippmann et al., 2002); 10,000- 11,250 in China (He and Gu, 1997; Pei, 2002; Xiao and Yong, 1998); 7500 in India (Shiva, 1996); 2237 in Mexico (Toledo, 1995); and 2572 traditionally by North American Indians (Moerman, 1998).

The World Health Organization (WHO) has estimated that the present demand for medicinal plants is approximately US $14 billion per year. The demand for medicinal plantbased raw materials is growing at the rate of 15 to 25% annually, and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion in 2050. In India, the medicinal plant-related trade is estimated to be approximately US $1 billion per year (Kala et al., 2006). According to Schippmann et al. (1990), one fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value and which are in use. But according to Hamilton (2003), India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the total number of medicinal plants present worldwide, the fact remains true that India with rich biodiversity ranks first in per cent flora, which contain active medicinal ingredient (Mandal, 1999).

A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. On a global basis, the IUCN has estimated that about 12.5% of the world's vascular plants, totalling about 34 000 species are under varying degrees of threat (Phartyal et al., 2002). IUCN recognises the following categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. Species with small populations that are not at present endangered or vulnerable but are at risk are called rare. (Singh et al., 2006). Many of them are facing extinction. In the past few decades, there has been an ever-increasing global inclination towards herbal medicine, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants (Bodeker, 2002). The plants used in the phytopharmaceutical preparations are obtained mainly from the naturally growing areas. The genetic diversity of medicinal plants in the world is getting endangered at alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization etc. is other factors, thus challenging their existence (Gupta et al., 1998). In view of the tremendously growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem, etc the natural habitat for a great number of herbs and trees are dwindling and of per capita consumption has resulted in unsustainable exploitation of Earth's biological diversity, exacerbated by climate change, ocean acidification, and other anthropogenic environmental impacts (Rands et al., 2010). Alarge sum of money is pumped every year to replenish the lost biodiversity and large numbers of protocols are available at present. Unfortunately, we are not witnessing any improvement in the status of these plant species in nature and the number of threatened plant species is increasing gradually (Tripathi, 2008). In order to safeguard this knowledge, it should be documented, preserved and patented (Mukherjee, 2009). Even the United Nations Conference on Environment and Development (UNCED), held recently at Rio de Janeiro, Brazil helped to place the loss of biodiversity and its conservation on the global agenda. Therefore, the management of traditional medicinal plant resources has become the matter of urgency. Hence, conservation of such a buffer is considered fundamental and provided priority in all sectors of global development (Tandon et al., 2009). Although species conservation is achieved most effectively through the management of wild populations and natural habitats (in situ conservation) but most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Even plants raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items (Murch et al., 2000). Thus mass multiplication of disease free planting material becomes a general problem. In order to

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overcome these barriers, ex situ techniques can be used to complement in situ methods and, in some instances, may be the only option for some species (Sarasan et al., 2006; Negash et al., 2001). Therefore, conservation of medicinal plants can be accomplished by the ex situ, that is, outside natural habitat by cultivating and maintaining plants through long-term preservation of plant propagules in plant tissue culture repositories (Rands et al., 2010).

In vitro techniques have been increasingly applied for mass propagation and conservation of germplasm as it has superiority over conventional method ofpropagation and offer some distinct advantage over alternative strategies. Some of these are as follows: (1) collection may occur at anytime independent of flowering period for each species (this assumes that seed material is not required), (2) there is the potential of virus elimination from contaminated tissue through meristem culture, (3) clonal material can be produced where this is useful for the maintenance of elite genotypes, (4) rapid multiplication may occur at any time where stocks are required using micropropagation procedures, (5) germination of difficult or immature seed or embryo may be facilitated for breeding programmes, and (6) distribution across the border may be safer, in terms of germplasm health status using in vitro cultures. Some more general positive advantages of in vitro techniques include the fact that storage space requirements are vastly reduced compared with field storage. Storage facilities may be established at any geographical location and cultures are not subject to environmental disturbances such as temperature fluctuation, cyclones, insect, pests, and pathogen (Bhojwani and Dennis, 1999; Shibli et al., 2006). In this regard the micro-propagation holds significant promise for true to type, rapid and mass multiplication under disease free conditions. Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetatively propagated plant species. Tissue culture has emerged as a promising technique for multiplying and conserving the medicinally important species within short period and limited space, which are difficult to regenerate by conventional methods and save them from extinction. In recent years, in-vitro cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale re-vegetation and for genetic manipulation studies under precisely controlled physical and chemical conditions. Combinations of in vitro propagation techniques (Fay, 1992) and cryopreservation may help in conservation of biodiversity of locally used medicinal plants (Singh et al., 2006).

II. IMPORTANT MEDICINAL PLANTS OF INDIA

Celastrus paniculatus

Celastrus paniculatus Willd. (Family Celastraceae) commonly known as Malkangni, Jyotishmati and Bitter sweet is a rare and endangered important medicinal plant believed to sharpen the memory and also used to cure a number of diseases. It is a large, woody, unarmed climbing shrub occurring naturally in hilly parts of India up to an altitude of 1200 m. This plant is widely used to cure depression, paralysis, leprosy, fever, abdominal disorders and cancerous tumors. Chemical constituents of seeds as revealed by phytochemical analysis were sesquiterpene alkaloids like celapagine, celapanigine and celapanine (Sharma et al., 2001).

Aegle marmelos

Aegle marmelos (L.) Corr., (Family Rutaceae) commonly known as “Bael Tree” is a popular vulnerable medicinal plant mostly found in tropical and subtropical regions. Almost all parts of the tree are used in preparing herbal medicine for treating diarrhea, dysentery, dyspepsia, malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema.The plant is rich in alkaloids, among which aegline, marmesin, marmin, and marmelosin are the major ones (Kala, 2006).

Acorus calamus

Acorus calamus Linn. (Family Araceae) commonly known as “sweet flag” or “Bach” is an important endangered medicinal plant. It is a semi aquatic herb with creeping rhizomes and sword shaped long leaves. The rhizomes possess anti-spasmodic, carminative and anthelmintic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and tumors (Anon, 2000).

Commiphora mukul

Commiphora mukul (Hook. ex Stocks) Engl. (Family Burseraceae) popularly known as “Guggul”, is an important endangered medicinal plant species. It is widely distributed in tropical regions of Africa and Asia. It grows wild in the

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arid, rocky tracts of northwestern regions of India. The plant exudes a medicinal oleo-gum resin ('Guggul') from incisions made on the bark in cold season. Gum is bitter, acrid, aromatic, pungent, carminative and stomachic stimulating the appetite and improving digestion. It is astringent, expectorant, anthelmintic, antispasmodic, antiinflammatory, diuretic, depurative, anodyne, vulnerary, themogenic, antiseptic, nervine tonic, aphrodisiac, stimulant, emmenagogue and diaphoretic (Sosa et al., 1993). It also possesses strong purifying and rejuvenating properties and is said to be a uterine stimulant. The main constituents of guggul include phytosterols, gugulipids and the ketonic steroidcompound (guggulsterones) mainly E and Z gugguisterones. These are responsible for the lipid lowering effects of guggul (Singh et al., 1997).

Peganum harmala

Peganum harmala L. (Syrian Rue), a medicinally important perennial herb of family Nitrariaceae, distributed over semi arid areas of North-West India, North-Africa and central Asia. Medicinally the fruits and seeds of this plant are digestive, diuretic, hallucinogenic, hypnotic, antipyretic, antispasmodic, nauseant, emetic, narcotic and uterine stimulant (Chatterjee, 1997). A red dye obtained from seeds is widely used in Turkey and Iran for colouring carpets. Leaves are useful in asthma, colic, dysmenorrhea, hiccup, hysteria, neuralgia and rheumatism. The plant has also been used as antimicrobial, antitumoral, in curing malaria and has insecticidal potential (Kiritikar, 1995).

Prosopis cineraria

Prosopis cineraria (Family Fabaceae) is a versatile species commonly known as Jhand or Khezri. Prosopis species are the dominant species in Indian desert. P. cinerarium has a very good economic importance in arid regions and is assumed to treat snake bite and scorpion stings. Green pods of this plant are used as food. This species is highly drought tolerant and can withstand in the area having 50mm rainfall annually.

Simmondsia chinensis(Jojoba)

It belongs to family Simmondsiaceae. It is an evergreen, dioecious desert shrub which grows wildly in Sonora desert of Arizona, northern Mexico, southern and Baja California. Jojoba is now cultivated commercially in Argentina, Australia, Chile, Egypt, India, Israel, Mexico, Peru, South Africa and the USA. Jojoba seed oil is being utilized in industrial lubricants, and in pharmaceutical and cosmetic industries. The liquid wax that makes 40–60 % of seed dry weight has properties similar to sperm whale oil. Due to the ban on the import of sperm whale products into USA and other countries, jojoba is gaining commercial importance at international level (Reddy and Chikara, 2010).

Spilanthes acmellaMurr

It belongs to the Asteraceae family and is commonly known as Akarkara or Toothache plant. This plant is widely distributed in the tropical and subtropical regions. The flowers and leaves of this plant have been used as traditional medicine for stammering, toothache, stomatitis and throat complaints. It has potent diuretic activity and the ability to dissolve urinary calculi. The plants have shown anti-inflammatory, antibacterial and antifungal properties. Spilanthol, the most active antiseptic alkaloid extracted from this plant, is found effective at extremely low concentrations against blood parasites, and indeed is a poison to most invertebrates while remaining harmless to warm-blooded creatures (Anon., 1989).

Stevia rebaudianaBertoni

It belongs to the Asteraceae family, a natural sweetener perennial herb commonly known as "Sweet Weed", "Sweet Leaf", "Sweet Herbs" and "Honey Leaf". The leaves of this plant are estimated to be 300 times sweeter than sucrose and the sweetness is due to glycosides of which the most abundant is stevioside.(Dushyant et al., 2014) The increasing consumption of sugar (sucrose) has resulted in several nutritional and medical problems, such as obesity. Therefore, low caloric sweeteners have been investigated to substitute sugar. The refined extracts of leaves of this plant are officially used as high potency natural-source, low calorie (non sucrose) sweetener in processed foods, artificial diets and pharmaceuticals. The sweet compounds pass through the digestive process without chemically breaking down, making it safe for diabetic and obese people (Mizutani and Tanaka, 2002).

Sapindus mukorossi

Sapindus mukorossi (Family: Sapindaceae) popularly known as 'Ritha' and 'Soapnut', is a most important deciduous tree of tropical and sub- tropical regions of Asia. The fruit of this tree contains saponins, the most active secondary metabolites extracted from this plant. It is a good substitute for washing soap and is as such

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used in preparation of quality shampoos, detergents etc. The fruit is of considerable importance for its medicinal value for treating a number of diseases like common cold, pimples, epilepsy, constipation, nausea etc. It is also used as expectorant and anthelmintic in small doses (Anon., 1992).

Bacopa monnieri

Bacopa monnieri belonging to the family Scrophulariaceae is a very popular herb in India for longevity and mental function. It is used to decrease fatigue and depression, and to stimulate the sex drive. It energizes the central nervous system, and aids the circulatory system, soothes and minimizes varicoseveins and helps to minimize scarring. It is also useful in repairing skin and connective tissues and smoothing out cellulite. It is generally considered an Ayurvedic "age tonic" restoring youth and vitality. Brahmi has been used by Ayurveda in India for almost 3000 years. The Ayurvedic treatise, the Charaka Samhita (100A.D.), recommends Brahmi in formulations for a range of mental conditions including anxiety, poor cognition and lack of concentration. In India, Brahmi is currently recognized as being effective in the treatment of mental illness and epilepsy.

Glycyrrhiza glabra

It was one of the most widely known medicines in ancient history, and records of its use include Assyrian tablets of around 2000 BC and Chinese herbals of the same period. Theophrastos of Lesbos, writing in the fourth century BC wrote that 'it has the property of quenching thirst if one holds it in the mouth'. Dioscorides gave the plant its botanical name (Greek glukos = sweet, riza = root). Its 13th century English name was Lycorys, a corruption of glycyrrhiza.. Liquorice (Glycyrrhiza glabra) belonging to the family Fabaceae has long been used for both culinary and medical purposes. Used for flavoring and sweetening candies and medical remedies, licorice also has potent effects of its own, particularly for ulcers and adrenal insufficiencies. It is also used for asthmatic coughs, as an antispasmodic and ulcer remedy, and to cool 'hot'conditions.

Holostemma ada-kaodien

Holostemma ada-kodien (Synonym – Leptadenia reticulate, W & A) commonly known as jivanti belongs to family Asclepiadaceae. From the plant, stigma sterol and tocopherols are isolated, hentriacontanol amyrin, amyrin, stigmasterol and sitosterol are also isolated from the stem and leaves. From the roots – lupeol, amyrin, sitosterol, alanine, aspartic acid, glycine, serine, threonine and valine can be isolated. Jivanti is sweet in taste, sweet in the post digestive effect and has cold potency. It alleviates all the three doshas, namely, vata, pitta and kapha. It possesses light and oily attributes. It is a rejuvenative, heart (caksusya and hrdya). It is used in diseases like fever, tuberculosis, burning sensation of the body and raktapittal. Mainly the roots and the whole plant are used for medicinal purposes. Externally the paste of its leaves and roots alleviate oedema due to vitiation of pitta dosa. The herb is beneficial for external use in various skin diseases, wounds and inflammation of the skin.

Oroxylum indicum

It belongs to family Bignoniaceae. Syonaka is astringent and bitter in taste, pungent in the post digestive effect and has cold potency. It possesses light and dry attributes. It is used in rheumatic disorders, diarrhea, cough, diabetes and cystitis. The skin of roots of syonaka is used for medicinal purpose, both, externally as well as internally. Used externally as a paste of its skin of roots, it dries up the discharges and promotes the wound healing. The tub bath with its decoction relieves the swelling and pain in rheumatic disorders. The medicated oil of syonaka in sesame oil base instilled into ears mitigates the pain in otitis. The decoction of its sroot-skin is an effective gargle in stomatitis. The root skin is also useful in dressing the wounds in soft chancre (upadamsa). Internally, syonaka is a panacea for arthritis and rheumatism. The decoction of the roots is commonly used for arthritis. In diarrhea and dysentery the decoction combined with mocarasa (gum of samali Bombax malabaricum). It is given along with honey. Syonaka also stimulates appetite, improves digestion and is vermicidal.

Picrorhiza kurroa

Kutki or Picrorhiza Kurroa is a herbal medicinal plant from Scrophulariaceae family. It is also known as hellbore, katuka, kurri, Katuko, Kuru, Katukarogani. It is found in Himalayan region in India. This herbal medicine has shown effective therapeutic action in liver disorders. The crude extract of plant shows good results in liver damage caused by carbon tetrachloride, paracetamol, galactosamine and alcohol. According to Ayurveda the plant has utility as laxative, liver-stimulant, appetite and stimulant, febrifuge. The plant is also beneficial in bronchial asthma and epidemic jaundice. It is also used to ease stomachache, and is believed to promote appetite.

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The herb is also effective in 'Kapha'disorders, billow fever, urinary discharge, hiccup, blood troubles, burning sensations and leucoderma.

Saussurea lappa

Saussurea lappa (Family Asteraceae) is a tall, robust perennial Herb; leaves simple, large pubescent, heart shaped radical leaves with long petiole. The genus Saussurea has many endemic species in Hindukush, Himalayan region. The species is mainly confined to Kashmir. In the northern areas, it is confined to Astore and Minimerg forest ranges. The most important locations where these species grow wild include Kalapani, Kamari and Thanknala, Mapno and Kilshai where this species is found growing inbetula forests on hill slopes at a height of 2438-3657 meters in Himalayas. In Pakistan this species is found in Kaghan and Azad Kashmir. Roots is tonic, stomachic, stimulant, carminative, used for asthama, diuretic, antiseptic, cough, cholera, aphrodisiac, anthelmintic and also used to insecticide, pesticide. The roots are highly aromatic used in perfumeries, also used for skin diseases. Locally it is used against the heart diseases of cattle and for toothache. The powdered roots are sprinkled over crops as insecticides. Externally the roots are used as an ointment or powder for the treatment of maggotinfested wound.

Swertia chirata

Swertia chirata (Family Gentianaceae) is found is in the temperate Himalayas at an altitude of 1,200- 3,000m from Kashmir to Bhutan and in the Khasi hills in Meghalaya at a height of 1,200-1,500 m. According to Ayurveda, this herb is a bitter tonic, stomachic. It is useful in liver disorders, eyes, heart. It is an excellent remedy for a weak stomach, especially when this gives rise to nausea, indigestion and bloating and it has also been shown to protect the liver. It is best known as the main ingredient in Mahasudarshana churna, a remedy containing more than 50 herbs. It also contains xanthones which are reputedly effective against malaria and tuberculosis, and also amarogentin, a glycoside that may protect the liver against carbon tetrachloride poisoning. The whole plant is an excellent drug for intermittent fevers, skin diseases intestinal worms, bronchial asthma, burning of the body, regulating the bowels. The root of the plant is useful in checking hiccups and vomiting. It is used in the liquor industry as a bitter ingredient.

Tinospora cordifolia

Tinospora cordifolia commonly known as Guduchi belonging to the family Menispermaceae is a famous plant of traditional use and also a powerfulrasayana mentioned in Indian ayurvedic literature. It is considered as a bitter tonic and powerful immuno modulator. Guduchi is a perennial plant of weak and fleshy stem found throughout the India. The aerial roots that arise from the stem are thread like. The leaf is heart shaped and smooth. The flowers are yellowish in colour emerges in bunch in rainy season. The fruits of guduchi are pea like which are seen in winter in India. Guduchi acts as a diuretic and found to be effective against renal obstruction like calculi and other urinary disorders. Guduchi acts as a memory booster, develops intelligence, and promotes mental clarity. It is described as one of the Medhya Rasayana (mental rejuvenative) in the Charak Samhita (The oldest and most potent book of ayurvedic medicine). Guduchi is regarded as a liver protector.

Table: List of the some endangered and economically important medicinal plants of India

S. No	Plant Species	Family	Explants	References
1	Aegle marmelos	Rutaceae and	Nodal segments	Yadav and shoot tip Singh (2011a)
2	Acorus calamus	Araceae	Rhizome tip and Rhizome segments	Yadav et al.(2011)
3	Celastrus paniculatus	Celastraceae ,	Seeds, nodal segments & shoot tip	Lal and Singh (2010) Lal et al. (2010)
4	Commiphora mukul	Burseraceae	Leaf segments, apical and nodal segments	Singh et al. (2010b)
5	Peganum harmala	Nitrariaceae	Seeds	Goel et al., (2009)
6	Prosopis cineraria	Fabaceae	Seeds	Kumar and Singh (2009)

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7	Simmondsia chinensis	Simmondsia ceae	Nodal segments	Kumar et al. (2010)
8	Spilanthes acmella	Asteraceae	Nodal and intermodal segments	Yadav and Singh (2010) Yadav and Singh (2011)
9	Stevia rebaudiana	Asteraceae	Apical and nodal segments	Kumar and Singh (2009) Singh et al. (2011)
10	Sapindus mukorossi	Sapindaceae	Leaf segments, apical and nodal segments	Singh et al. (2010a)
11	Bacopa monnieri	Scrophularia ceae	Leaf explants and and nodal segments	Mohapatra Rath (2005)
12	Ginkgo biloba	Ginkgoaceae	Apical and nodal segments	Tommasi & Scaramuzzi (2004)
13	Glycyrrhiza glabra	Papilionacea e	Nodal segments	Vadodaria et al., (2007)
14	Gymnema sylvestre	Asclepiadac eae	Seeds	Komalavalli & Rao (2000)
15	Holostemma ada-kodien	Asclepiadad eae	Nodal segments	Martin (2002)
16	Oroxylum indicum	Bignoniacea e	Nodal segments	Dalal & Rai (2004)
17	Picrorhiza kurroa	Scrophularia cae	Nodal segments	Martin et al. (2006)
18	Saussurea lappa	Compositae	Shoot tip	Johnson et al. (2007)
19	Swertia chirata	Gentianacea e	Shoot tip	Balaraju et al. (2009)
20	Tinospora cordifolia	Meninsperm aceae	Nodal segments	Gururaj et al. (2007)

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