Review Article

Comprehensive review on origin, distribution, micropropagation and agronomical practices of Gymnema sylvestre R. Br

Abstract

Gymnema sylvestre (Retz.) R.Br. ex Sm. is a plant that belongs to the Apocynaceae family and is found throughout many parts of Asia, Africa, and Australia. This herb is used in several traditional medicine practices for a wide range of ailments, including in Ayurevda, for its potential to reduce blood glucose levels. It is used as a pharmacological ingredient, mainly for the presence of bioactive phytochemicals such as gurmarin, gymnemic acid, and gymnemasaponins, which have been shown to lower glucose levels. Gymnema sylvestre is also recognized as an important nutritional supplement for its antioxidant, antibacterial, anti-inflammatory, antiviral, gastro- and hepatoprotective, anti-cancer, and lipid-lowering properties. The main objective of this study is to have a comprehensive review of Gymnema sylvestre on origin and distribution, germplasm availability, a package of practices and post-harvest practices to increase the area under cultivation of this herb. In spite of having a well-developed package of practices, this herb is rarely cultivated and nearly 80% of the required quantities are being collected from wild sources, which will lead to extinction. This study will provide an unabridged repository of references regarding the species for its effective and safe utilization as a "potential medicinal herb" for creating awareness on the use of plant-based medicine.

Keyword: Botany, Cultivation, Organic, Phytohormone and Propagation

1 Introduction

More than 80% of the world's population uses natural medicines and depends on medicinal plants for health care. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded within and across the countries. At present, 90% collection of herbal raw drugs used in the manufacture of Ayurveda, Siddha, Unani, and Homeopathy systems of medicine is largely from the wild out of which 70% collection involves destructive harvesting. Due to this spurt, medicinal plants are being overexploited and many of them are pushed to the brink of extinction (Gowthami et al. 2021) Many medicinal plants are highly sensitive to the level of harvest and fragility of the ecosystem; one of them is Madhunashini (Gymnema sylvestre R. Br.) commonly known as Australian cow plant, small Indian Ipecaunha or periploca of the woods in English and 'Gudmar' in Hindi. It has been mentioned in literature like Ayurveda and Sushruta Sahitya to destroy diabetes (glycosuria) and other urinary disorders. It neutralises the excess sugar in the body that is present in diabetes mellitus. When its fresh leaves are chewed in the mouth, it neutralises the taste of sweetness for some time. For these potential antidiabetic medicinal properties, it is popularly known as "Gudmar" or "Madhunashini" G. sylvestre is one of the

most popular medicinal plant on the global market, requiring a cost-effective and simple method of cultivation to meet its growing demand. The availability of the species in natural forests is decreasing very fast due to overharvesting and unsustainable harvesting. The present demand is mostly met by wild collection. Therefore, the only way to meet the increasing demand and reduce the pressure of harvesting in the wild is through large-scale cultivation.

The World Health Organization (WHO) considers that the quality of raw materials and finished products, depend on many factors including cultivation techniques, collection methods, harvesting methods, post-harvest, processing, transport and storage practices. WHO prescribed general guidelines for good cultivation and collection practices (GACP) for supply of quality medicinal herbs (WHO, 2003)

Table :1. Vernacular names of Gymnema sylvestre (Farooqui and Sreeramu, 2004)

English	Ram's horn, Small Indian Ipecacunaha, Australian cowplant and Periploca of the wood
Sanskrit	Meshshringi, Madhunashini
Hindi	Gudmar, Merasing, Gurmar
Tamil	Adigam, Cherukurinja
Telugu	Podapathri
Kannada	Sannagresehambu, Kadhasige
Malayalam	Chakkarakolli, Madhunashin
Gujarathi	Dhulet, Mardashingi
Marathi	Kavali, Kalikardori, vakundi
Bengali	Mera – singi

1.1 Origin and Distribution

In addition to India, it is widely distributed in many countries such as Malaysia, Sri Lanka, Australia, Indonesia, Japan, Vietnam, tropical Africa, and the southwestern regions of the People's Republic of China. This plant is found in tropical and sub-tropical regions. In India, it is found in the forests of the Western Ghats, Konkan, Madhya Pradesh, Chhattisgarh, Bihar, Tamil Nadu and Karnataka at an altitude of 100–1000 m (Farooqui and Sreeramu, 2004).

1.2 Soil and Climate

1.3 Description of Plant Botany

Gymnema sylvestre (Retz.) R. Br., syn. Asclepias germinata Roxb., belonging to the family Apocynaceae, having the chromosome number 2n=22, which is a perennial, woody, medicinal vine, It is slow-growing, with many branches, and it also needs support to grow with pubescent young parts. Leaves are simple, opposite, elliptic or ovate, pubescent on both sides, and base-rounded or cordate. Flowers are small, yellow, and arranged in umbellate cymes, which are solitary, small, numerous flowers in axillary position on the stem, bell-shaped. The umbel of flowers is made up of 5 sepals, which are long, oval, thick, and velvety, and the corona is long. The stigma (corolla) is attached to the inflorescence by a single, long peduncle. The stigma is curled, thickly lobed, and often also covered with buds. The style is often longer than the stamen. Flowering occurs in October–January, while fruits mature from March to May. Fruits are slender, and follicles are up to 7.5cm long. Seeds are about 1.3 cm long, narrowly ovoid–oblong, flat, with a thin, broad, brown, and glabrous marginal wing. (Warrier et al., 1995). Two allied species, G. hirsutum, found in Bundelkh and Bihar and the Western Ghats, and G. montanum, growing wild in the Eastern Ghats and Konkan, are also used for the same purpose and are also called "Gurmar" (Thakur et al., 1989).

Table: 2. Taxonomy of Gymnema sylvestre (Kirtikar and Basu, 1987).

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales
Family	Apocynaceae
Sub-family	Asclepiadaceae
Genus	Gymnema
Species	Gymnema sylvestre (Retz.) R. Br.ex Sm

1.4 Phytochemistry of Gymnema. sylvestre

G. sylvestre leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentriacontane, pentatriacontane, α and β-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives (Dateo and Long, 1973). The drug had composed different medication in it formaulation like Ayaskrti, Varunadi Kasya, Varunadighrtam, Mahakalyanakaghrtam, etc. Triterpines and saponins found in the plant's leaves are thought to be responsible for its anti-diabetic properties. Gymnemic acids A, B, C, and D, which contain Gymnema genin and gymnestrogenins, have been designated these names. Additionally, nonacosane and hentria contane, which were extracted from leaves using hexane, are found in leaves. A trace component known as gymnamine, an alkaloid, has been isolated and identified (Farooqui and Sreeramu, 2004).

1.5 Nutraceutical application

This climber is extensively used in almost all the Indian systems of medicine to cure pitta, kapha, diabetic, ulcers, cough, dyspnea, and eye pain. Inflammations, hepatosplenomegaly, dyspepsia, constipation, jaundice, haemorrhoids, stranguria, renal and vesical calculi, helminthiasis, cardiopathy, cough, asthma, bronchitis, intermittent fever, amenorrhea, cataracts, and leucoderma might all benefit from the plant. Chewing on the fresh leaves has the unique effect of permanently paralysing the sense of taste for both sweet and bitter foods (Warrier et al., 1995). The medication is referred to as a destroyer of glycosuria (madhumeha) and other urinary diseases. Root has a long history of being used as a snakebite treatment. Castor oil and crushed leaves are applied externally to swollen glands and the growth of internal viscera like the liver and spleen (Nadkarni, 1954). The Nutraceutical is used to improve heart health, treat jaundice, piles, urinary calculi, problematic urination, and irregular fevers (Sharma, 1983).

2. Biological diversity

2.1 Exploration and collection of germplasm

Survey and exploration were carried out from Belgaum, Gadag, Uttara Kannada, Udupi and Shimoga districts of Karnataka in the Western Ghats for the collection of Gymnema sylvestre. Wild habitats of the targeted species, which included plain forest areas, tribal hilly areas, etc were thoroughly explored and a total of 30 accessions of G.sylvestre was collected. During the exploration, two types of variants of the species were identified i.e., narrow leaf type and broad leaf type. In a second exploration trip, Jabalpur, Damoh and Sagar districts of Madhya Pradesh were surveyed with the help of traditional healers and forest range officers

and old persons of the local society. In wild areas of Damoh and Sagar, the variability was observed in the case of fruits traits. Two types of fruits were observed i.e., fruit of short length and fruits of longer length. A total of 14 accessions of G. sylvestre were collected from the area. It was noticed that the tribal people at Dhamoni use Gymnema leaves for eye problems, locally known as "Phoolkat" in addition to the popular use for the treatment of diabetes. A third exploration of Gymnema was taken up at Tumkur districts of Karnataka. The Siddarabetta of Tumkur is a hot spot for various medicinal and aromatic plants. In this area, a total of 15 accessions of Gymnema was collected with the help of local people. Another exploration conducted at Ranjendrangar, Hyderabad in the Telangana state and five accessions were collected from the wild source. Thus, altogether a total of 65 accessions were collected during the current year from diverse area of Madhya Pradesh, Karnataka and Telangana. The collected genetic resources of Gymneam sylvestre are now maintained in the field gene bank under nursery for the future multiplication, characterization and evaluation for growth, yield and quality traits to select elite accessions for specific trait of interest (ICAR – DMAPR, 2018-19).

2.2 Collection, characterization, evaluation, and maintenance of germplasm

TNAU, Coimbatore: Sixty-six accessions of Gymnema sylvestre are maintained in the field at Department of Medicinal and Aromatic Crops, Coimbatore and the morphological, yield and quality characters were recorded. Among the 66 accessions, variations were observed for leaf shape, leaf base, leaf tip, leaf colour and leaf pubescence. Based on leaf shape, the accessions were grouped into ten different sets viz., elliptic (15 accessions), ovate (25 accessions), lanceolate (15 accessions), oblanceolate (1 accession), elliptic-ovate (1 accession), elliptic-lanceolate (1 accession), ovate-elliptic (1 accession), ovate-lanceolate (1 accession), oblong-ovate (1 accession) and ovate-oblong (1 accession). The leaf shape was oblong in three accessions; elliptic in16; cordate in 2; ovate in 45 accessions. Based on leaf tip nature, the accessions were grouped into three viz., acute (40 accessions), acuminate (13 accessions) and attenuate (3 accessions). Accessions were grouped into four based on leaf base viz., round (51 accessions), cordate (7 accessions), obtuse (7 accessions) cuneate (3 accessions). Leaf pubescence was present in 56 accessions and absent in 10 accessions. Mid-rib pubescence was present in 49 accessions and absent in seven accessions. Variations were also observed for leaf length, breadth, petiole length and intermodal length.

Pooled mean for two years data revealed that leaf length varied from 2.40 to 4.58 cm and the accession Kolli hills local-1 recorded the highest length (4.98 cm); leaf breadth varied from 1.57 to 2.90 cm and the accession Anaikatti local-2 recorded the highest leaf breadth (2.90 cm); petiole length varied from 0.47 to 1.37 cm, the accession Kolli hills (5) recorded the highest petiole length (1.37 cm); internodal length varied from 1.07 to 2.74 cm, the accession Kolli hills local-1 recorded the highest intermodal length (2.74 cm). The yield character viz., dry leaf weight of leaves ranged from 0.08 to 0.75 kg plant-1.Gs14 (Yercaud local 5) recorded highest leaf dry weight. The gymnemagenin content of the accessions ranged from 0.48 to 1.54% and the accession Gs34 (Sirumalai local 4) recorded the highest gymnemagenin content (1.54%).

Among the 66 genotypes, 14 genotypes were identified for high yield and gymnemagenin content. Of the 14 genotypes, leaf dry weight ranged from 0.75 kg plant-1 to 0.49 kg plant-1. Gymnemagenin content ranged from 1.54% to 0.72%. Based on the average leaf dry weight 0.62 kg plant-1 and gymnemagenin content (1.13%), they were classified as genotypes with high biomass and low gymnemagenin content, genotypes with high gymnemagenin and low bio-mass and genotypes with low biomass and low gymnemagenin content. Pooled data for two years revealed that the accession Gs14 (Yercaud local 5) recorded highest leaf dry weight (0.75 kg plant 1) and the accession Gs34 (Sirumalai local 4) recorded the highest gymnemagenin content (1.54%). The grouping of genotypes revealed that the accession GSy-14 (Yercaud local-5) recorded the highest leaf dry weight (0.75 kg plant-1). The gymnemegenin content of the genotype is 0.72 % (ICAR – DMAPR, 2018-19).

Madhunashini creeper can be classified into two types based on the leaf size

Small leaved type: Leaves are oval, measuring 1.0-3.5 cm in length and 1.5-2.5 cm in width, and very soft, found in dry regions.

Broad and pubescent type: The leaves are also oval, measuring 3-6 cm in length and 3.5-5.0 cm in width. Leaves are dark green compared to small leaved type and are pubescent

3 Propagation

Madhunashini can be multiplied either by seeds or vegetative propagation methods, which are used for commercial cultivation.

3.1 Seed treatment and Germination percent

The study conducted by (Pandey., 2012) stated that the germination was initially poor, but it was significantly affected by pretreatments which varied from 28.50 - 42.50 percent. Highest germination was obtained when seeds were soaked in cold water for 24 hours. Propagation of G. sylvestre through seed is not easy due to difficulty in seed availability and prevailing dormancy problem (Arunakumara and Subasinghe, 2004). Also reported that dynamics of seed germination of G. sylvestre and availability of high moisture content increases germination response (Harakumar et. al., 2000).

3.1.1 Seeds

The shrub produces fruit from February to April. The seeds of freshly picked fruits are collected and soaked in water overnight, the seeds are planted the following day in a seed pan that contains soil mixed with sand to support germination. Daily watering of the seed trays results in the seeds germinating after about 15 days. 40 to 50 days after sowing trays are transferred to polythene bags holding a mixture of soil, sand, and FYM mixed in equal proportions. Whereas seeds are sown in mixed soil using vermicompost instead of farmyard manure, the maximum germination percentage is up to 50%-55%. The plants are cared and constantly watered until they are transplanted. (Farooqui and Sreeramu, 2004)

3.1.2 Vegetative propagation

Semi-hardwood cuttings of terminal shoots 15 to 20 cm in length are used for vegetative propagation. G. sylvestre cuttings are planted in polybags filled with soil, sand and FYM in 1:2:1 ratio. Under North Indian conditions, February to March is the best time to take cuttings in the nursery. The cuttings adapt to humid conditions in shade houses or mist chambers for good root development and within a month, root formation begins. Before planting in polybag or nursery to promote rooting. The cutting is dipped in a 100-ppm solution of indole butyric acid (IBA) for six minutes (Farooqui and Sreeramu, 2004). The cuttings are also administered 1% Bavistin treatment prior to planting in the polythene bags to prevent root infectious diseases. Planted cuttings are regularly irrigated. After 90 days of planting, roots begin to emerge and about 6 months old, rooted cuttings are ready for transplanting in the main field. The studies from Pandey., 2012 revealed higher rooting success (52%) in hardwood cuttings followed by semi hardwood and soft wood cuttings (26% and 15% respectively) without any hormonal treatment. The propagation of G. sylvestre through rooted cuttings is preferred due to short supply and dormancy problems in seeds (Harakumar et al., 2000). Hardwood cuttings had higher potential of root production and success rate is high in rooting of G. sylvestre when stem cuttings are placed with at least one node inside the planting medium (Singh et.al., 2008).

3.2 Micropropagation

Micropropagation is a well-established process for mass-scale production of plants with clonal stability. In vitro multiplication of Gymnema was first attempted by Bahadur et al., (2007). Maximum number of shoots were achieved on MS medium containing BAP (5 mg l−1) and NAA (0.2 mg l−1). Multiplied shoots were brought to rooting on ½ strength MS (Murashige and Skoog, 1962) medium without adding any plant growth regulator. Komalavalli and Rao (2000) investigated the effect of various factors which remarkably affected in vitro regeneration of Gymnema that were; seedling age, the nature of the explant, basal medium, plant hormones, antioxidants (activated charcoal, ascorbic acid, citric acid and poly vinyl pyrrolidone) and undefined supplements (coconut milk, yeast extract, casein

hydrolysate and malt extract). A maximum of 57.2 shoot were induced from 30 day old seedling axillary node explants incubated on MS medium supplemented with 0.1 mg l−1 NAA, 1.0 mgl-1 BA, 0.5 mg l−1Kn, 100 mg l−1 citric acid and 100 mg l−1 malt extract. Best root regeneration was observed on shoots derived from axillary nodal explant (50%) on ½ MS medium supplemented with 3.0 mg l−1 IBA. The rooted shoots were subjected to hardening in soil and successfully acclimatized to natural conditions. Karthic and Seshadri (2009) developed a cost-effective mass multiplication of G sylvestre in hydroponic system. Effect of media and moisture on rooting of¬¬ Gymnema sylvestre stem cuttings was also studied by Arunakumara et al., (2013). Subathra and Srinivasan (2008) concluded that the requirement of MS mediums for shoot bud activation and propagation confirms the requirement of rich salts for the regeneration of Gymnema sylvestre. Influence of various growth hormones like; 2, 4-D, IAA, BAP, and Kinetin on the breaking of axillary bud dormancy was studied and synergistic action of vitamin B2 in relation to these plant growth regulators was also worked out. To minimize phenolic release by explants different antioxidants; activated charcoal, ascorbic acid and citric acid were also added in culture medium. Citric acid at a concentration of 100 mgl−1 prevented blackening of medium and enhanced the number of healthy micro propagated shoots in Gymnema. Both qualitative and quantitative improvement on rooting was obtained on ½ strength MS medium, where 53 % shoots were induced to root within 45 days. MS medium supplemented with 1.0 mgl-1 BA+0.5 mgl−1 IAA+100 mgl−1 vitamin B2+100 mgl−1 citric acid was best for shoot proliferation and ½ strength MS medium with 3.0 mgl−1 IBA was best for root induction. Solanki and Gupta (2013a) reported highly reproducible plant regeneration protocol from young shoots of mature plant. Highest multiple shoots (80%) were observed on MS medium supplemented with 5.0 mgl-1 BAP with Mean shoot length of 2.57+ 1.91 cm.

3.3 Somatic Embryogenesis

Somatic embryos are induced in somatic tissue of plants by giving proper stimulus of a plant growth regulator in vitro. Applications of this technique include large-scale production of clones, virus elimination, providing source tissue for gene transfer, and production of synthetic seeds. Somatic embryos can be obtained on explant either directly or by the intervention of callus phase. It depends on the presence of competent cells in explant. A proper stimulus by a specific growth regulator induces cells of explant to form an embryo. Whole plant regeneration by somatic embryo formation has been achieved by callus obtained from hypocotyl, cotyledon and leaf explants excised from in vitro raised seedlings of Gymnema (Gupta and Solanki, 2015). MS medium containing (0.5–5.0 μM) 2,4-D + (0.5–2.0 μM) BA and 2.0 percent (w/v) sucrose, induced embryogenic callus within 6–8 weeks after initiation of culture. On this medium globular and heart shape stage embryo were obtained, which further developed into a torpedo and cotyledonary stage in a medium supplemented with MS salts, B5 vitamins, 0.5μM BA and 2.0% sucrose. Subculturing on the same medium resulted in embryo germination and formation of plantlets, which were successfully adapted to greenhouse conditions (Kumar et al., 2002). Ahmed et al., (2009a) standardized a protocol for the formation of somatic embryos by suspension culture of Gymnema. Callus cultures were induced on MS medium with growth regulators 0.5 mgl-1 2,4 -D (or) 1.0 mgl-1 NAA

and 10 percent coconut water. They were transferred into an MS liquid medium containing 1.0 mgl-1 NAA, 1.0 mgl-1 BA, 3.0 percent sucrose (w/v), 10 percent coconut water, citric acid 1.0 mg/l and glutamine 10 mg/l for induction of somatic embryos from callus. Various stages of somatic embryo development like; globular, heart, torpedo and cotyledonary were identified in suspension cultures within 8 weeks. The maturation of embryos was found to be considerably influenced by plant growth regulators and length of light and dark cycles. Plantlets were germinated from 5-7 % of embryos induced on semisolid MS salts with B5 vitamins, 3.0 percent sucrose and 0.8 percent agar (w/v). After transferring in field plantlets have shown similar traits as that of source plant. The various factors affecting callus production in Gymnema have been investigated in detail, including the type and age of explant, media, carbon source and antioxidants. Leaves and stem cuttings from young plant were tested for their regeneration potential by inoculating on various concentrations of different combinations of auxins and cytokinins added in MS and B5 medium. Callus induction was shown by 100% explants on all the levels of 2,4-D (0.5-5.0 mg/l) within 3-4 weeks. Callus obtained on 2,4-D was pale yellow and friable but on NAA compact callus was formed. On combinations of BAP and 2,4-D only leaf explants responded to form compact yellow, green callus within 25-30 days. Among the different media tried callus was obtained only on MS medium (Solanki and Gupta 2013). Among different types of antioxidants (adenine sulfate, ascorbic acid and citric acid) and carbon source (glucose, maltose and sucrose) tested, citric acid at 30 mg-1 concentration and sucrose at 3% concentration produced the highest amount of light green, compact callus from leaves in 9-10 weeks after inoculation. (Solanki and Gupta 2013c)

3.4 Mass multiplication of Gymnema sylvestre in hydroponic system

Due to indiscriminate collection and over exploitation, natural stands of G. sylvestre are fast disappearing and threatened. Hence to avoid its disappearance, it requires cultivation in farmlands and this approach offers excellent scope for cultivation in the subtropical regions in southern India. Conventional propagation of this plant is hampered due to poor seed viability, low rate of germination, poor rooting ability of vegetative cuttings and low multiplication rate even in tissue culture (Lee et al., 2008). It was observed that rooting of G. sylvestre using IBA in tissue culture technique was also very low (Bahadur et al., 2007, Komalavalli, and Rao, 2000). Hydroponics offers opportunities to provide optimal conditions for plant growth and enables the growers to manage the supply of essential nutrients to crops more efficiently and accurately than traditional field systems (Jones, 1997). Hydroponic system has been developed for growing several plants (Butiner and Barzvi, 2003, Leontovich and Bobro, 2007). Therefore, hydroponic system was looked at as a potential alternative for rooting and mass multiplication of G. sylvestre. MCRCGY1, an accession collected from Muniyankudisai Village, Tamilnadu was micro propagated using MS basal medium supplemented with Benzyl aminopurine (BAP - 3.5 mg/L), Kinetin (KN 1.0 mg/L), Napthaleneacetic acid (NAA 0.2 mg/L) and the grown plantlets were established at Sri AMM Arunachalam Technology Resource Centre, Vadakadambadi, Tamilnadu (Karthic and Seshadri., 2009).

Preliminary studies showed that the explants with an actively growing side branches responding better than those actively growing single shoots and leaf less stem cuttings. According to research, IBA concentration and planting material size both have a significant impact on the development of roots in G. sylvestre. IBA supplementation of 1/10 MS basal salts medium resulted in greater root growth than the control. Within a week of incubation, root initiation was shown, and from the second week on, it became more apparent. The 30 cm long explants showed the highest root induction (93% rooting, 17.8 ± 1.1 root numbers, and 15.4 ± 0.8 cm root length) on 1/10 MS salts supplemented with 0.5 mg/L IBA, followed by 1.0 and 2.5 mg/L IBA and control. IBA and 1/10 MS media have been shown to reduce leaf fall and encourage shoot growth (Maene and Debergh, 1985). Plantlets were hardened in poly houses using red soil, river sand, and farmyard manure (1:1:1), with a 96% success rate for transplantation. This technique allows to produce more plantlets in just 21 days. The use of plastic tanks with nutrient solutions for mass multiplication of G. sylvestre stem cuttings using a hydroponic system is the first study on the soilless culture, and it is a highly appealing approach (Karthic and Seshadri, 2009).

3.5 In Vitro Response of Gymnema Sylvestre

Gymnema is a plant that propagates itself in nature by producing seeds, but these seeds have very short viability times and lack endosperm, which results in very low germination rates under natural environmental conditions. This plant has undergone traditional methods of propagation. The factors that can enhance seed germination, vegetative propagation through stem cutting, and rooting of stem cuttings have been optimized to grow plants in the field and in vitro condition (Singh et al., 2005; Arunakumara et al., 2013). Conventional propagation techniques do have drawbacks, however, such as being dependent on the environment, season, etc. For the extraction of gymnemic acid at commercial scales, it is now essential to multiply this valuable medicinal vine using tissue culture techniques. The review focuses on the ability of Gymnema to regenerate in vitro as well as the in vitro production of gymnemic acid, the chemical's principal anti-diabetic component. Gymnemic acid is found in plants only in trace amounts. A commercially viable production system is a major necessity to exploit its antidiabetic potential as a drug. In vitro regeneration of callus and maintenance of cell suspension culture allows the opportunity to not only produce gymnemic acid in large quantities but also maximize its production at the commercial level by manipulating cultural conditions (Lee et al., 2006).

4 Argo- technique of cultivation

4.1 Land Preparation and Planting

The best time for planting is from June to August. Waterlogged conditions will adversely affect the establishment of plants and therefore, transplanting in such areas should be avoided. 50 cm3 sized pits are dug after the soil has been ploughed and levelled, spacing of 2.5 m between rows and 1.8 m between plants (within the row) is followed. The pits are opened 15 days before planting, filled with topsoil and green leaves, and 10 kg of well-rotten manure is put to each pit. If there is no rain, irrigate the pits for one week before planting the

rooted cuttings and giving them irrigation. An optimum spacing of 1 m × 1.5 m is recommended for a crop stand of about 6700 plants per hectare. If the plant survival rate is 80%, then a total of 8400 plants will be required for one hectare area. The rooted cuttings/seedlings may be planted by crowbar method.

4.2 Effect of planting time and spacing on growth and yield traits

BAU, Ranchi, conducted a research experiment with three transplanting times (mid-July, mid-August, and mid-September) and three spacings (40×30 cm, 50×40 cm, and 50×50 cm). The maximum fresh biomass yield (0.98 kg plant-1) was recorded in a crop planted in mid-July at a spacing of 50 cm×50 cm and the minimum (0.66 kg plant-1) was recorded in a mid-August planting at a spacing of 50 cm× 50 cm with the grand mean of 0.80 kg plant-1. The maximum dry leaf yield (0.16 kg plant-1) was recorded in mid-July planting at 30 cm × 40 cm spacing, and the minimum (0.10 kg) was in mid-September planting at a spacing of 50 cm × 50 cm. Maximum dry biomass yield (0.49 kg plant-1) was recorded in mid-July planting with 50 cm × 50 cm spacing, and the minimum (0.33 kg plant-1) was in mid-August planting at 50 cm × 50 cm spacing (ICAR – DMAPR, 2018-19)

4.3 Effect of Phytohormone

A fresh 12 to 15 cm long cutting with 2-4 nodes having a diameter of 11 to15 mm was prepared. IBA rooting hormones were applied to the lower ends of cuttings for 30 minutes. The upper end of the cuttings was sealed with paraffin wax. The treated cuttings were planted with at least one node below the sand surface in trays filled with cleaned riverbed sand. The trays were kept moist by providing regular watering. After 3 months, the plantlets raised in trays were transferred to polybags 16 cm x 9 cm in size, filled with soil, sand, and FYM in a 1:3:1 ratio. The study found that cuttings dipped in 1000 ppm IBA solution for 30 minutes had showed the highest sprouting (77.87%), but the lowest rooting (52.50%) and survival (40.67%) by (Pandey., 2012). Similarly, study suggested that 2500 ppm IBA treatment to improve the rooting ability of apical shoot cuttings in G. sylvestre (Karoshi and Hedge, 2001).

The effect of plant growth regulators on rooting and sprouting in stem cuttings was studied at BCKV, Kalyani. The experiment was conducted in November 2018 with two growth hormones each at three levels (1000 ppm, 5000 ppm and 100 ppm) and pure honey, and compared with a control. After treatment, the cuttings were planted in sand in flat trays. Optimal watering was done. Cuttings treated with higher doses of PGR (1000 ppm and 5000 ppm) sprouted less, even less than those in the control. A higher dose had a detrimental effect on the sprouting of the stem cutting. Stem cuttings treated with a lower dose of PGR gave good sprouting results (58–66%). Stem cuttings treated with pure honey also gave a moderately better result (46% sprouting) (ICAR – DMAPR, 2018-19).

4.4 Effect of seasons and PGR on rooting

The experiment was conducted with the objective to identify a suitable season and plant growth regulator (PGR) doses for successful rooting in cuttings of madhunashini in JNKVV, Jabalpur. The cuttings were planted in three different seasons viz., July, August and September by treating with IBA 250, 500, 750 ppm and without IBA to study the rooting success and survivability percent. The sprouting percentage of cuttings was observed maximum (59.07%) in July planting, but maximum survivability (28-46%) was found in August month. The cuttings treated with 750 ppm IBA sprouted early (9.61 days) followed by 500 ppm IBA solution (11.20 days). The cuttings treated with 750 ppm IBA exhibited maximum sprouting (67.08%), sprouting length (2.91 cm) followed by 500 ppm treatment. Maximum survivability (33.03%) was recorded with 750 ppm IBA treated cutting. Considering the interaction effect, the maximum survivability (34.58%) was recorded in August month planting in 750 ppm IBA treated cuttings closely followed by 550 ppm IBA treatment (ICAR – DMAPR, 2018-19).

According to Vijay (2016), hardwood cuttings 15 cm long with 3-4 buds planted in July and August had the highest survivability. Among plant growth regulator (PGR) treatments, dipping cuttings in a 500 ppm IBA solution for 30 min was suitable for maximum survivability. The interaction between season and PGR revealed that 500 ppm IBA-treated cuttings planted in August had showed highest survivability.

4.5 Manures and Fertilizers

In the first year, in addition to the natural manure, the climber will grow and develop more quickly if 10 g of urea and 20 g of super phosphate are provided to each plant once before planting and then at intervals of one month. Fertilizers are applied in two split dosages beginning in the second year. During the first week of June, the crop is harvested, and the soil around the roots is loosened and treated with 40, 20, and 15 g of N, P2O5, and K2O per plant, or 90, 45, and 35 kg of NPK per hectare. To increase biomass production, a further equal dose may be added each year continued up to ten years. Application of FYM appeared to be the most promising, while vermicompost was found to be similarly efficient (Pandey., 2012). According to a previous study by Tanuprakash and Adholeya, (2004) the application of organic manures affected the biomass yield.

Padmapriya et al., 2010 reported that the treatment combination of FYM (25 t/ha) + recommended dose of fertilizer (90:45:35 kg/ha of NPK/ha) combined with foliar spraying of panchagavya and Manchurian mushroom extract each at 3% and humic acid at 0.3%, recorded highest plant height (227.53, 286.47, 300.1 and 334.54 cm), number of leaves (62.0, 70.0, 82.0 and 95.0) number of branches (36.048.055.058.0), leaf area (12.60, 15.52, 17.50 and 18.92 cm2), fresh biomass (2.55, 3.22, 3.88 and 4.10 kg/plant) and dry biomass (0.638, 0.782, 0.890 and 0.913 kg/plant) at 180, 240, 300 and 360 days after transplanting, respectively. Regarding the quality parameters, the treatment combination of FYM (25 t/ha) + Vermicompost (5 t/ha) + Neem Cake (250 kg) combined with foliar spraying of

panchagavya and Manchurian mushroom extracts each at 3% and humic acid at 0.3% registered the highest crude gymnemic acid content of 485.74 mg per 100 g -1 dry weight.

The experiment was conducted to standardize a suitable source and dose of nutrients for maximizing dry leaf yield in JNKVV, Jabalpur. The number of leaves per plant and fresh and dry herbage yields varied significantly with different organic treatments. However, maximum fresh leaf weight (95.20 g plant-1) was recorded in the treatment receiving RDF (NPK @ 50:25:50 kg ha-1), which was on par with the treatment receiving FYM at 10 t ha-1. The maximum dry weight (48.7 g plant-1) was recorded in the treatment receiving FYM at 10 t ha-1, which was on par with the treatment receiving FYM at 15 t ha-1. The economic analysis of various treatments revealed that treatment of FYM at 10 t ha-1 was most profitable with a B/C ratio of 2.87, followed by RDF with a B: C ratio of 2.11 (ICAR – DMAPR, 2018-19).

Bisht et al. 2019 observed that application of 1.2:0.8:0.4 kg NPK for 12, 18, and 24 MAT results showed the maximum plant height (188.96 cm, 293.29 cm, and 326.65 cm) and average number of branches per plant (7.85, 19.36 cm, and 22.46 cm) respectively. The highest dry leaf yield of 124.84 kg per acre was achieved by the application of 1.2:0.8:0.4 kg NPK in 12 MAT, while for 18 and 24 MAT, the highest yields of 348.84 and 381.44 kg were achieved by the application of 0.10:0.10:0.08 kg (Azospirillum, VAM, and PSB), respectively. Results was concluded that the combined application of bio-fertilizers (Azospirillum, VAM, and PSB) significantly improves the growth and yield of Gymnema sylvestre.

Madhavan and Sha, 2019 Observed that application of Vermicompost (1.0kg) + Azospirillum (Root isolate (10gm.) after four months of planting recorded highest plant height (264.36), Number of laterals/plant (52.19), Number of leaves/lateral (110.13), Leaf length (7.88cm), Leaf breath (5.87cm), Leaf area (17.55cm2), Single leaf weight (0.44gm.), 100 leaves weight (44.18gm.), Fresh leaves yield/plant (3.86kg.) and Dry leaves/plant (2.91kg.) which was followed by application of Decomposed coir pith (5.0kg) + Azospirillum (Root isolate 10 gm.)

4.6 Training

Gymnema is a climber, hence it needs to be trained on a 2 m stone pillar with a 'Y'-shaped iron structure bent at 60 degrees to the sides. The lower side of the iron structure needs to be connected, and 3 iron wires should also be connected to each of the bending ends, for a total of 7 wires. The two main stems are trained on the lower wires so that they develop in opposing directions; additionally, the produced laterals are trained on different top wires. The climbers must take care to avoid falling to the ground. These plants can also be trained to grow along a wire fence, which will serve two purposes (Farooqui and Sreeramu, 2004).

4.7 Irrigation

After harvest and manure application, irrigation is necessary. One irrigation is required immediately after transplanting for initial seedling establishment and growth. Thereafter,

once every 5–6 days of irrigation is sufficient. The frequency of irrigation needs to be raised during the summer based on the weather and soil conditions. The plants, however, no irrigation was provided during rainy season (Pandey, 2012) and (Kumar et al. 2002).

4.8 Drip irrigation

Irrigation through drip and drop-drop system is considered very successful. In a drip irrigation system, water is delivered through small diameter plastic pipes at a very low rate (2-20 litres/hour) to the plant roots through emitters or drippers outlets. The system consists of main pipeline, sub-level line and lateral pipelines. Water is delivered to the crop using a network of lines that have emission points along their length. Each dropper / emitter supplies water, along with nutrients and other growth substances, to the root zone of plants in a controlled manner. In this system, water is applied only to the root zone of the plants so that only that part of the soil gets wet which is the part of the roots. Unlike surface and sprinkler irrigation, which require wetting of the entire soil profile. In drip irrigation, water is applied more frequently (usually every 2-3 days) than in other methods, which maintains very favorable high moisture levels in the soil and improves plant growth and development (Kumar and Jnanesha, 2017). Similar results were reported by (Santosh and Maitra, 2022) in ginger and (Palada et al. 2000) in basil.

4.9 Weed management.

After transplanting in Gymnema Sylvester, the transplanted area should remain weed free during initial growth. Since the growth of its plants is very slow, during the initial development, gram crop can be grown as an intercrop. It is a creeper crop and alternatively, this crop can be raised under tree species. These tree species will also aid in its growth by acting as a staking for its branches. Apart from this, bamboo poles can also be used for staking (Farooqui and Sreeramu, 2004) and (Pandey, 2012).

4.10 Pests & Diseases management

The risk of diseases is very low in Madhunashini farming, but due to the outbreak of insects, the crop suffers a lot. Among the pests, mainly aphids (Aphis nerii), and mealybugs (Phenacoccus solenopsis) in southern India and papaya mealybugs (Paracoccus marginatus) in western India are very destructive. Leaf weber, leaf minor, and cutworm also cause damage in different environments and geographical conditions.

4.10.1 Aphids

The aphid is a small, orange-coloured sap-sucking insect and comes in the Aphididae family. The bodies of these insects are soft and oval, the heads are small; the trunk is jointed, the tentacles are of seven segments; and there are four transparent wings (when wings are present). Generally, aphids are the most destructive enemies of agricultural crops grown in temperate regions and cause great economic losses. Aphids are slow moving suck the sap of the plant so quickly and in such large quantities that most of the sap comes out of the body unchanged. This undigested food is in the form of a delicious, sugary liquid called honeydew.

Due to its presence, many times there is an outbreak of shooty mould on the plants, which is harmful for vegetative growth (Shivakumara et al. 2022) and (Prakash,2002).

4.10.2 Mealybug (Paracoccus marginatus)

They damage tender parts of Gymnema sylvestre by sucking the sap, which results in stunted plant growth. It damages various parts of the host plant, including the leaves, stem, and apical buds. It sucks the sap from the bark, leaves, tender twigs, and fruits of plants by inserting the stylet into the epidermis and injecting a toxic substance. This results in chlorosis, plant stunting, leaf deformity, and the loss of young leaves and fruits. The sap, which forms a honey-like substance, eventually becomes part of the plant. The formation of a thick white wax coat and death by heavy infection make the fruits inedible. Generally, 100% infestation of plants due to mealybugs has been observed. Its attack causes a lot of damage to the plants, along with a reduction in the quality and quantity of the leaves (Ravikumar et al. 2008).

4.10.3 Leaf webber (Spodoptera litura)

Gymnema sylvestre leaves are also attacked by leaf webber (Spodoptera litura). The female adult lays about 850–1000 eggs together in a group, and these are covered with light yellow-coloured hairs. It completes its life span in about 30 to 35 days. The larval stage of this insect infects the leaves of Gymnema and causes more damage. Newborn larvae are initially light green with a dark black colour, which should cover the colour of the larvae. Gymnema leaves are most severely affected by the insect's larvae. Newborn larvae are initially light green with a dark black colour, which later changes. Dark brown turns black with lines. In the beginning, it bites the leaves, and in cases of severe infection, only the skeletons of the leaves and shoots are left, which makes the crop 'almost unmarketable' and causes a lot of economic losses.

Keeping in view the importance and use of the crop, chemical pesticides should not be used in the madhunashini crop. Various types of suggestions have been recommended for its control. According to the recommendation of the Tamil Nadu Agricultural University, neem should be applied to the soil at a rate of 250 kg / hectare under the eco-friendly IPM module. Simultaneously, spray the first time with Neem, Seed Kernel Extract (NSKE) 5% at 2.5 ml. /Litre After this, a second spray of Beauveria bassiana (5 g/litre) and a third spray of Bacillus thuringiensis (5 g/litre) should be done. Release of 10000 grub/hectare of chrysoprala should be done, and cow pea should be grown as a border crop to encourage the activities of natural enemies. spray Fish oil resin soap @ 125g / 5L of water release Australian ladybird beetle, Cryptolaemus montrouzieri @ 10 beetles/tree, Band the trees with 20 cm wide degradable polythene sheets (150 gauge) for mealybugs. Organic practises include control measures using neem-based formulations; fish oil resin soap can be used to manage all sucking pests. Botanicals such as garlic, Vitex negundo, Lantana camera, Clerodendron inerme, and Calotropis gigantean extracts are frequently combined and sprayed on a regular basis to control pests (Mohan et al. 2010), (Antwi and Reddy, 2015) and (Ravikumar et al. 2008).

Powdery mildew and leaf spots are common diseases of this crop, and they can be controlled by spraying organic based sources trichoderma and pseudomona for leaf spot at intervals of 10 to 15 days based on the incidence level and crop rotation use of resistant varieties for powdery mildew (Mohan et al. 2010).

4.11 Harvesting and Yield

After one year of planting, the commercially important part of the harvest the leaves begin. When plants begin to bloom, the crop is ready for harvesting at the end of June or the first week of July. The plucking of leaves can be done manually or using a sickle or knife. The collected leaves are spread thinly on the cleared ground and allowed to dry under shade for about 7-8 days. Care should be taken to ensure that the leaves are not dried in the sun. It is preferable to stagger the harvesting to achieve optimum drying. (Padmapriya et al. 2016). The crop is only plucked once a year. At 3–4-year-old vine may yield 5–6 kg of dried leaves on average per plant, which equals to 9–10 tonnes of dried leaves per hectare. Under proper management, the crop can be grown for 10–15 years (Binnisha et al. 2021).

4.12 Packaging

To prevent spoilage, be sure to dry the leaves thoroughly. It is then packed in polythene bags when the moisture content is less than 8-9%. In excess of this moisture, mold can develop on the packed leaves. The risk of outbreak remains. Create the creative quality of the leaves. To keep it, it should not be dried in direct sunlight (Dhanani et al. 2015).

Conclusion

Gymnema is an important species used in traditional medicine, as well as a nutritional supplement, for a wide range of reported health benefits. With increasing demand for this ingredient in Southeast Asian pharmaceutical formulations, the plant is threatened. Bringing Gymnema into commercial cultivation with the improved agronomical practices will support conservation of wild populations and help meet market demands.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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