



Research Article

MASS CULTIVATION OF SARPAGANDHA (*RAUWOLFIA SERPENTINA* BENTH. EX KURZ) IN CONSIDERATION WITH ENVIRONMENTAL FACTORS AND CULTIVATION TECHNIQUES

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ABSTRACT

Sarpagandha (*Rauwolfia serpentina* Benth. Ex Kurz.) A perennial medicinal plant of family Apocynaceae is well known as *Serpentina* root, the natural source of alkaloid reserpine used for the treatment of Hypertension, neuro disorders like psychosis, schizophrenia, insanity, insomnia and epilepsy. It contains alkaloids reserpine, yohimbine, ajmaline, ophioxylon, resin, wax, starch are used as a sedative and tranquilizing agent. Main Ayurvedic products of *Sarpagandha* are *Sarpagandha Ghanvati*, *Sarpagandha Yoga*, *Sarpagandha Churna* and *Mahesvari Vati*. The world requirement for dry *Sarpagandha* roots is around 20,000 t per annum which is a great demand because of its high medicinal value. The internal consumption of dried roots is ≈150 t per annum. It is the red listed plant and as its germination rate is low and high demand in national and international market, it is necessary to increase its commercial production by using various effective and low cost techniques. This paper is going to through light upon various effective cultivation techniques and environmental factors responsible for growth of plants so that cultivar's interest in *Sarpagandha* cultivation would increase on commercial basis and it will become again a boon for Ayurveda.

KEYWORDS: *Sarpagandha*, *Rauwolfia serpentina*, Cultivation techniques, Low cost, Mass cultivation.

INTRODUCTION

Rauwolfia serpentina Benth ex. Kurtz. is an endangered medicinal plant, of family Apocynaceae is a woody perennial shrub, commonly known with different names; *Sarpagandha*, snake root plant, chotachand, chandrika etc.^[1] now attained worldwide popularity. It is an erect, evergreen perennial shrub with a long, irregular, nodular, yellowish root stock, growing to a height of 60-90cm. Its leaves are simple, 7.5-10cm long and 3.5-5cm broad, elliptic or lanceolate, glabrous, bright green above and pale green beneath, pointed and occurring in whorls of 3-5. The fruit is drupe, 0.5cm in diameter and shiny black when fully ripe. The root system consist of prominent, tuberous, soft tap root reaching a length of 30-50cm in 2 year old plant with diameter 1.2-2.5cm.^[2]

The roots of this shrub have a great medicinal value since many centuries. This shrub is greatly effective in treating *Uchha raktachap* (high blood pressure) and in mental disorders like insanity, hysteria, mental illness and traumas. Its sedative properties greatly help to use it in insomnia. As long time, its roots used to treat mental illness and snakebite, commonly known as "Pagal ki buti" or "insanity herb"^[3]. Useful parts are root and leaves.^[4] Rapid growth of world population, increasing anthropogenic activities, rapidly eroding natural ecosystem etc, resulted in decreasing the natural habitat of this significant shrub *Rauwolfia*. It is now an endangered species in India due to indiscriminate harvesting of roots and over exploitation of natural resources to meet the demand of pharmaceutical industry, coupled with limited cultivation. The annual requirement of roots in the country for the manufactured rauwolfia is estimated at about 650

tones. The world requirement of dried *rauwolfia* is around 20000 t/annum.^[5] To reduce the pressure on natural resources a profitable cultivation technique to obtain higher root yield and total alkaloid content is essential as well as the rate of plant propagation is important for commercial cultivation to meet the pharmaceutical demand for reserpine. Chemical synthesis of reserpine is possible but due to its high cost process as compared to extraction from natural resources, it is not adopted^[6]. So, Objectives of this study is to conserve and improve the quality of *Rauwolfia serpentina* (*Sarpagandha*) by using various low and effective cultivation techniques with considering environmental factors.

MATERIALS AND METHODS

Material for Data collection of this article is mainly ancient literature, database, all recent editions of *Dravayaguna* and flora of medicinal plants. Other sources are Internet; all published articles in authentic journals.

OBSERVATION AND RESULTS

Mass scale cultivation of *Sarpagandha* will occur by considering various factors like soil, climate, altitude, rainfall and other conditions responsible for growth of plants and various techniques. Manures and fertilizers also plays important role in rapid growth of plants and pest management is also necessary. Environmental factors affect the production of secondary metabolites will be of great importance for the conservation of medicinal plants.

Soil

The plant prefers soil with plenty of humus and rich in nitrogenous and organic matter with good drainage.

Alkaline soils are not suitable for commercial cultivation.^[7] *Rauwolfia* grows in wide variety of soils alluvial loam to red lateritic loam or stiff dark loam. In its natural habitat it prefers clay or clayey loam with a large percentage of humus and it does not grow well in soil having pH 8 or above. The ideal pH for this crop is from 4.6-6.2. Soils containing large quantities of sand retard the growth of plants and make them more susceptible to root and leaf diseases. It grows well in frost free tropical to sub-tropical situations under irrigation.^[8]

Climate

R.serpentina grows in wide range of climate conditions but flourishes well under hot, humid tropical climate open or partial shade. It prefers a tropical or subtropical belt, having the benefit of monsoon rains. Elevation of 1300 m having a temperature range of 10-38°C and annual rainfall of 2500 mm are suitable for this species. Good yield is obtained in areas less prone to frost and having less severe water.^[9]

Cultivation Techniques

Propagation: *Rauwolfia* is propagated by seeds and also by vegetative means like root-cuttings, root-stumps, stem-cuttings, and leaf-cuttings. The plant is usually propagated by seeds. The percentage of germination of seeds is very poor and variable(25%-50%).^[10] The rate of germination depend on the percentage of fully matured, heavy seeds in a particular lot. Fresh seed, collected from ripe fruits and immediately sown, show higher percentage of germination. The collection of mature seeds is usually done from September-February. Fruits mature between July-November. The germination gradual and growth of the seedling is slow. Germination starts after 15-20 days and continuous up to 40-50 days after sowing. the nursery should be kept moist throughout the germination period. Growth Time period is equally important to get higher amount of alkaloid contents.

Table 1: Growth characteristics of *R. serpentina* plants raised by different propagation methods^[11]

Treatments Growth characteristics of <i>R. serpentina</i> plants									
	6 months			12 months			18 months		
	Height (cm)	Collar diameter (mm)	No. of Branches	Height (cm)	Collar diameter (mm)	No. of Branches	Height (cm)	Collar diameter (mm)	No. of Branches
Seeds	22.33	4.24	2.56	32.67	5.20	3.52	45.67	7.42	5.12
Root cuttings	18.67	4.25	2.25	32.33	5.78	3.84	42.33	8.71	4.82
Stem cuttings	17.00	4.50	2.15	30.00	9.14	3.25	40.38	9.04	4.54
CD (5%)	NS	NS	NS	NS	NS	NS	NS	NS	NS

From above table, there was not much difference in the growth pattern of the plants raised through various propagation methods. However, the plants raised through vegetative means (root and stem cuttings) started flowering very early i.e. two months after planting.

Table 2: Root biomass and alkaloid content of *R. serpentina* roots raised by different propagation methods.^[12]

	Treatments Harvesting time of <i>R. serpentina</i> roots								
	6 months			12 months			18 months		
	Fresh weight (gms)	Dry weights (gms)	Total alkaloid content	Fresh weight (gms)	Dry weights (gms)	Total alkaloid content	Fresh weight (gms)	Dry weights (gms)	Total alkaloid content
Seeds	20.33	7.67	0.41	41.46	13.52	0.69	58.73	25.23	1.97
Root cuttings	18.33	6.27	0.35	40.05	12.31	0.59	48.33	21.48	1.71
Stem cuttings	18.38	6.31	0.38	37.33	11.30	0.56	47.66	19.77	1.63
CD (5%)	NS	NS	NS	NS	NS	NS	3.07	NS	NS

Maximum alkaloid contents (1.97%) were found in the roots harvested after maturity i.e. in the month of December after 18 months of planting.

DISCUSSION

Various propagation techniques and other important environmental factors can be used to increase the cultivation rate of *Sarpagandha*.

Transplanting: Seedlings of 40-50 days which have 4-6 leaves are ready for transplanting. The seedlings are carefully dug out and tap root should be cut. Then these are dipped in a 0.1% solution of emisan fungicide before planting, to protect them against soil-borne fungus causing damping off disease.^[13] The field is then divided in small plots for irrigation. The seedlings are transplanted in to

the furrows, by making holes large enough to receive the seedlings along the accompanying clump of earth. A spacing of 30cm between the plants should be maintained.

Vegetative propagation: As collection of seeds from wild sources is both laborious and costly. Vegetative propagation by root or shoot cutting is advocated for raising plantations to collect the seeds as well as to quickly multiply the genetically superior clones.^[14]

By root-cuttings: Large tap root s with few lateral secondary rootlets are used. Cuttings of 2.5-5.0cm length are planted in holes at the beginning of the monsoon and are completely covered with earth. About 100 kg of root cuttings are required to plant 1 hectare.^[14]

By root stumps: This propagation is done by using about 5 cm of root with a portion of stem above the collar. This method gives about 90-95% success. Such plant transplanted in May-July in irrigated fields become well established by the end of September.^[14]

By stem-cuttings: Hard wood cuttings are found to be better than soft wood cutting. Cuttings of 15-22 cm length with three internodes are the most suitable. Stem cutting planted in the nursery during the early monsoon or June and kept moist until they give about 40-65% success rate.^[14]

Genetic transformation

The gram-negative soil bacteria Agro bacterium rhizogenes used for genetic transformation of many medicinal plants successfully. Genetic transformation would be a powerful tool for enhancing the productivity of novel secondary metabolites of limited yield. Plant infection with this bacterium induces the formation of proliferative multi branched adventitious roots at the site of infection followed by transfer of a portion of DNA i.e.-DNA to the plant cell chromosomal DNA.^[15]

Micro propagation (Tissue culture)

Tissue culture techniques for the mass propagation are highly desirable. Endophytic micro flora is a major cause of contamination for in vitro culture of *R.serpentina*. Hence, a proper sterilization was needed for successful culture. In vitro propagation has become the boon to satisfy the commercial demand for production of *Sarpagandha*. It uses shoots and leaves as explants and cultured on MS media supplemented with phytohormones 2, 4-D, IBA, BAP. It includes callus induction from leaf and stem tissues and direct regeneration from apical and nodal tissues of field grown *R.serpentina*.^[16]

Manures and Fertilizers

The use of organic manure, leaf mould and compost is recommended to increase the quantity of nutrients in the soil and improve the drainage. Nitrogenous fertilizers induce more vegetative growth, followed by organic manure. Application of phosphates induces more growth than nitrogen alone. It is better to apply 25-30 t of well rotted FYM at the time of land preparation and 10 kg N, 60 kg P2O5 and 30 kg K2O per hectare in moist soil.^[17]

Irrigation

The crop is irrigated fortnightly in the hot dry season and about once a month in winter. The crop is cultivated under rain fed conditions also but yield is considerably poorer.

Weeding and Intercultural operation

About 2 weeding are necessary during the monsoon and one hoeing at the end of growing season or December.

Pest and Diseases

Insect pests

- Root knots appear as galls of various sizes, covering the root system. Application of 25 kg of 3 G carbofuran or 20 kg of 10 G phorate granules per hectare will control them.
- A pyralid caterpillar causes appreciable damage the leaves. These are controlled by spraying 0.2% Rogor.

- Cockchafer grubs attack the seedling about 2 cm below the hypocotyls, resulting in their drying up. To control the attack of grubs, mix phorate granules with the soil at the time of nursery preparation.

Diseases

- Leaf spot caused by *Cercospora rauwolfiae* manifests as dark-brown colored spots on the upper surface of the leaf and yellowish-brown on lower surface. To control this disease Dithane Z-78 or M-45 @ 0.2% is to be sprayed in early June, before the monsoons and repeated at monthly intervals until November.
- Alternaria tenuis* attacks the leaves, resulting in minute, brownish or dark-coloured circular spots with a yellowish margin on ventral side of leaves. This fungus also affects the flowers and fruits. To avoid this problem crop is sprayed with 30 g Blitox in 10 liters of water.
- Mosaic is common disease which is avoided by selecting proper seeds.^[18]

Harvesting, processing and storage

Highest root yield and alkaloid content was obtained in the crop raised through seeds harvested after 18 months of planting. December was found ideal time to harvest the crop as it yielded higher alkaloid content. The roots dug out in winter, when the plants have shed their leaves are richer in total alkaloid content than the roots harvested in August. A light irrigation is given in advance to facilitate easy digging of roots. The root are freed from adhering soil, washed, cleaned and dried in shade till they become brittle and packed in gunny bags. Care was taken not to damage outer bark of roots while uprooting as it contains maximum amount of alkaloid. They are stored in cool, dry place to prevent mould.^[19]

Yields

The optimum yield of roots is obtained by propagation through seeds. The yield of fresh roots per plant varies widely from 1-4 kg. The total yield of roots in the case of plants raised from seeds is about 1175 kg/ha on air dried bases as compare to 175 kg/ha in case of plants raised from stem cuttings and 345 kg/ha in case of root cuttings. Under irrigated conditions, dry root yeild varies from 15-25q/ha. According to cultivator's report, average yield is 2700-3300kg dried roots/ha and 8-10 kg of seeds. According to estimates, 400-500 tons of roots are being collected annually mostly from forests in India, Bangladesh, Shrilanka, Thailand.^[20]

The rate of plant propagation is important for commercial cultivation to meet the pharmaceutical demand for reserpine. It grows well in tropical to sub tropical climate receiving heavy rains between June and august. *Serpentina* roots prefers soil with plenty of humus and rich in nitrogenous and organic matter with good drainage. Alkaline soils are not suitable for commercial cultivation. Growth characteristics (height, collar diameter and number of branches) of *R. serpentina* plants grown through different propagation techniques were recorded and depicted in Table 1. Height of the *R. serpentina* plants after 18 months was 45.67 cm in plants raised through seeds, 42.33cm in the plants raised through root cuttings and 40.38 cm in the plants raised through stem cuttings. The

collar diameter of 18 months old plants raised through seeds, root cuttings and stem cuttings were 7.42 mm, 8.71 mm and 9.04 mm respectively. There was not much difference in the growth pattern of the plants raised through various propagation methods. However, the plants raised through vegetative means (root and stem cuttings) started flowering very early i.e. two months after planting.

Fresh root biomass of the roots obtained from 18 months crop raised through seeds was maximum (58.73gm) followed by crop raised through root cuttings (48.33 gm) and stem cuttings (47.66 gm). However, the dry weight of the roots of 18 months old crop raised through seeds, root cuttings and stem cuttings were 25.23 gm, 21.48 gm and 19.77 gm respectively. Data revealed that crop raised through seeds yielded more roots in comparison to crop raised by vegetative means i.e. root and stem cuttings. The roots obtained from the crop raised through seeds and harvested after 18 months of planting possesses higher alkaloid content (1.97%) compared to roots obtained by the plants harvested after 12 months (0.69%) and 6 months (0.41%) after planting.

Advantages, Disadvantages, Limitations of Various Cultivation Techniques

Asexual method of propagation (root cuttings, stem cuttings, root stumps) has certain advantages, it contains same features as mother plant and number of plants can grow with little quantity. Chemical analysis showed that there was not much difference in the total alkaloid content in the crops propagated through seeds, root and stem cuttings. And flowering comes earlier to plants which raised asexually, so seeds can be collected for seed bank. And disadvantages are, it has less reserpine content as compared propagation by seeds. Cultivation by seed is the best technique for cultivation but its germination rate is variable according to quality of seeds (heavy seeds, light seeds). So limitations for these techniques are as this plant is slow growing plant gives benefit after a period of 3-4 years and if harvested before 18 months, it doesn't give benefit of required reserpine content.

CONCLUSION

Transplantation of seedlings through seeds is better method of cultivation. To fulfill the demand of *Rauwolfia serpentina* in national and international market, techniques like in vitro propagation, genetic transformation should be used and there is a need a farmers friendly cultivation package by which good quality of roots will be obtained. Higher root yield and higher alkaloid content is possible only if the plant is brought under large scale cultivation in agro-climatically suitable areas by using various effective advanced techniques and by raising crop through seeds harvested after 18 months of planting in the month of December. To increase cultivation rate, Cultivars should be educated with proper propagation methods, knowledge about soil, climate, harvesting time and pest control so as to increase high yield and alkaloid content.

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