INTRODUCTION

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Chapter 1

INTRODUCTION
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Medicinal and aromatic plants (MAPs) have been the focus of study in terms of their conservation and traditional usage in herbal medicines (Malabadi and Kumar, 2007). Throughout the history of mankind, many infectious diseases have been treated with herbs. Medicinal and aromatic plants possess aromatic compounds mainly the oils, which are volatile at the room temperature and the specific property (s) found among them act as a cure for several diseases. These plants have traditionally been used as raw materials for extraction of essential oils, as well as source of spices and other natural products such as traditional herbal medicines, pharmaceuticals, cosmetics, botanical pesticides, insect repellents, other herbal products etc. (Dhar et al., 2002; Ravindran and Balachandran, 2004). More than 30% of the entire plant species, at one time or other was used for medicinal purposes (Joy et al., 1998). It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in the developing countries such as China and India, the contribution is as high as 80% (Joy et al., 1998). Thus, the economic importance of medicinal plants is much more to countries such as India than the rest of the world. The Indian subcontinent constitutes a rich repository of medicinal plants that are used by various indigenous health care systems. As per the estimate, over 7000 species of medicinal plants are used for medicinal purposes (Umashanker and Ganeshaiah, 1997). Here the drugs of herbal origin have been used in customary systems of medicines such as Siddha, Unani, Folk (Tribal) and Ayurveda since ancient times. Among these systems, Ayurveda is most developed and widely practiced in India.
Due to indiscriminate use of medicinal plant resources over time and fragmentation of their habitats, many of these species are increasingly threatened and face the risk of genetically impoverished (Umashanker and Ganeshaiah, 1997). Therefore, there is need to conserve and exploit potential of the various medicinal plant species judiciously and sustainably in the field of medicinal and pharmaceutical sciences for novel and fruitful applications. In this context, *Rauwolfia serpentina* (Figure 1) is one of the highly valued medicinal plant species, which also requires conservation and utilization efforts for overall development and application in medicinal and pharmaceutical science.

1.1. *Rauwolfia serpentina*

*Rauwolfia serpentina* is an important medicinal plant of the world. There is a great demand for its roots, which is the prime cause of its indiscriminate uprooting from wild sources where it grows abundantly. Its intensive collection brought the plant to the verge of extinction. Presently all supplies of *Rauwolfia serpentina* roots are being met from the natural resource, which is declining due to overexploitation by tribal and local collectors (Rajendra and D’souza, 1999). This has led to listing of this species as “endangered” by the International Union for Conservation of Nature and Natural Resources (IUCN) (Jain *et al.*, 2003). In India, it has also become an endangered species due to overexploitation and Government of India has prohibited the collection of plants growing in wild in forests and its export since 1969. In this scenario, there is urgent need for developing its conservation strategy.
Fig. 1. Photographs showing *Rauwolfia serpentina* L. (a), root sample 18 months old (b).
1.1.1. Species description

*Rauwolfia serpentina* (L.) Benth. ex Kurz. (2n=22), an important medicinal plant under shrub, belongs to *Apocynaceae* family of dicots. It is indigenous to India and other tropical countries of Asia and is pantropic in distribution. There are approximately 85 species in the genus *Rauwolfia* mainly found in tropical regions. *Rauwolfia serpentina, R. caffra, R. canescens, R. micrantha, R. tetraphylla* and *R. vomitoria* are some of the most important species of the genus. Reserpine content in the root of *Rauwolfia serpentina* has attracted worldwide attention for drug development. More than 50 alkaloids have been reported from *Rauwolfia serpentina* including the therapeutically important reserpine, rescinnamine, deserpidine, ajamalacine, ajmaline, neoajmalin, serpentine, and yohimbine.

1.1.2. Geographical distribution

Several species of *Rauwolfia* are observed growing under varying edapho-climatic conditions in the humid tropics of India, Nepal, Burma, Thailand, Bangladesh, Indonesia, Cambodia, Philippines and Sri Lanka. It is indigenous to the moist, deciduous forests of South East Asia including Burma, Bangladesh, Sri Lanka, Malaysia, the Andaman Islands and Indonesia. In India, it is found in the central region, i.e. between Sirmor and the Gorakhpur districts of Uttar Pradesh in shady, moist or sometimes swampy localities. In the east in Bihar, North Bengal and Assam as well as in Khasi, Jaintia and Gharo Hills, the plant is encountered more commonly on the forest margin of mixed deciduous forests. In the Western Ghats, *Rauwolfia serpentina* occurs more frequently in Shigoni, Pisone, Mollem in Goa, Coorg in North Kanara and Shimoga districts of Karnataka and Anjanakund, Odakkali, Palghat, Calicut and Trichur in Kerala. In Orissa and Andhra Pradesh different areas including
the catchments of the river Godavari are the richest in its occurrence, and in the foothills of Himalayan range, the species is available up to the elevation of 1300-1400 m. The plant is mainly associated with sal (*Shorea robusta*) forests as well as bamboo brakes.

### 1.1.3. Botany

*Rauwolfia serpentina* is an evergreen, perennial, glabrous and erect undershrub. Generally it grows up to 15-45 cm in height, but may grow up to 90 cm under very favourable conditions. Leaves grow in whorls of 3-4, are deciduous, elliptic-lanceolate or obovate, pointed, green on the upper surface, pale-green underneath, 7.5 cm long and 3.5 - 5 cm broad in size. Its flowers are in irregular corymbose cymes, white, often tinged with pink to violet. The flowering time is from March to May and July to November especially in central to west-south regions of India. Flowers are numerous, borne in terminal or auxiliary part of stem, long-stocked clusters, tubular, with 5 - lobed, 1 - 3 cm long, whitish pink in colour. Stamens are 5 in number and epipetalous. Carpel is 2 in number, connate, style filiform type with large stigma. At the onset of fruit development calyx, pedicel and peduncles become bright red. The inflorescence is characterized with red colour pedicels and calyx, and white corolla. Fruits are drupe, single or generally didymous, obliquely ovate, 7.5 mm in size, purple bluish to black when ripe, containing 1 or 2 stony seeds. The root system consists of a prominent, tuberous, nearly vertical, tap root up to 15 cm thick at the crown and long giving a serpent-like appearance occasionally branched or tortuous developing small fibrous roots, reaching a length of 30-50 cm in 2 year old plant. Roots greenish-yellow externally and pale-yellow inside, its diameter at the thickest portion varies from
1.2 to 2.5 cm. The root-bark constitutes 40-60% of the whole root and about 90% of alkaloids. The fresh roots emit a characteristic acrid aroma and are very bitter in taste.

1.1.4. Therapeutic value

*Rauwolfia serpentina* plant commonly known as Sarpaghandha is widely used medicinally both in the modern medical system and also in Ayurveda, unani and folk medicine. Roots are main source of drug. *Rauwolfia serpentina* is a rich source of indole alkaloids of medicinal value such as reserpine, ajmaline, ajmalicine and serpentine which are used in the treatment of circulatory disorders (Tyler et al., 1981). It helps to reduce blood pressure by dilating blood-vessels, depresses activity of central nervous system and acts as a hypnotic. In Ayurveda, its roots and whole plants are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and leaves are used in removal of opacities of cornea (Joshi and Kumar, 2000; Manuchair, 2002). It is used in traditional medicine in India, China, Africa and many other countries. In India and Nepal, it is a common treatment for hypertension and insomnia. But, its prolonged usage stimulates release of prolactin and causes breast cancer. Deserpidine and rescinnamine are also used as hypotensive and tranquillizer. Because of toxic nature of reserpine, recent trend has been to use total extract and powdered root extract as therapeutic agents in various countries as a hypotensive as well as sedative.

1.1.5. Phytochemical constituents

Root of *Rauwolfia serpentina* is bitter, acrid, laxative, anthelmintic, thermogenic, diuretic and sedative. The root barks has more than 90% of the total alkaloids in roots. The alkaloids are accumulated in the roots over a period of 1-3 years and total content varies from 1.0-3.0% of the dried roots (Virmani et al., 1979).
More than 50 alkaloids have been reported from *Rauwolfia serpentina*. The alkaloids are classified into 3 groups, viz, reserpine (Figure 2), ajmaline and serpentine groups. Reserpine group comprises of reserpine, rescinnamine, deserpine etc. Ajmaline, ajmalicine, ajmalinine, iso-ajmaline etc. are of the ajmaline group. Whereas, serpentine group includes serpentine, sepentinine, alstonine etc. (Iyengar, 1985). Quantitative analysis of reserpine–rescinnamine group of alkaloids is performed by spectrometric analysis or by high-performance liquid chromatography. The roots also contain ophioxylin, resin, starch and wax.

1.1.6. **Propagation**

The plant can be propagated through seed or vegetatively by root or stem cuttings as well as micro-propagation by *in-vitro* technique. However, for commercial purpose, multiplication through seed is advisable. Vegetative multiplication can be used where there is a shortage of planting material and where it is desirable to multiply a particular clone or variety.

1.1.7. **Cultivation economics**

There is a great demand for the alkaloids as well as for the raw drug in the international market. The annual world requirement of dried *Rauwolfia* roots is around 20,000 tons. The annual requirement of roots in the country for the preparation of *Rauwolfia* extracts, have been estimated at about 650 t against the present annual supply of 30 t from all the sources. The consumption of the raw drug is substantial in the indigenous drug market. Steps, therefore, needs to be taken to increase the present production to about 100-150 t/annum of dry roots. This is possible only if high yielding cultivars of the plant are brought under large-scale cultivation in agro-climatically suitable areas. The root bark constitutes 40-45% of the total weight of
Reserpine ($C_{33}H_{40}N_2O_9$)

[Systematic (IUPAC) name: methyl-11,17a-dimethoxy-18β-[3,4,5-trimethoxybenzoyl]]

Fig. 2. Chemical structure of reserpine compound found in *R. serpentina*
root and contributes more than 90% of the total alkaloids yield. On an average, root yield vary from 15 to 25 q/ha of dry weight under irrigation depending upon soil fertility, crop stand and management. In highly favourable condition average yield is 27 to 33 q dried roots/ha and 8–10 kg seed per hectare. A major part of the commercial supply of this drug, used in the USA and European countries originates from India, Pakistan, Sri Lanka, Myanmar (Burma) and Thailand, with India being the major supplier. In India, it is cultivated in the states of Uttar Pradesh, Bihar, Tamil Nadu, Orissa, Kerala, Assam, West Bengal and Madhya Pradesh (Dutta and Virmani, 1964). The present day commercial supplies of the roots of *Rauwolfia* are mostly from Uttar Pradesh, Bihar, Orissa, West Bengal, Assam, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Maharashtra and mostly obtained from wild sources.

1.2. **Reproductive biology and population genetic study**

Knowledge in reproductive biology is fundamental for systematic, evolutionary and conservation studies (Ornduff, 1969; Holsinger, 1991; Anderson, 1995). The study of reproductive biology of species, along with the analysis of its genetic variation provides data critical to their conservation and improvement efforts. This is especially true for endangered species when there is limited population available to supply propagules for future generation. Both types of information viz. reproductive biology as well as genetic variation are essential to design conservation programs which aim to preserve the genetic spectrum of endangered species.

Medicinal plants have received much less attention in genetic studies and species improvement program compared to agricultural crops. However, during the last decade valuable information on variation and inheritance pattern of some important medicinal plants has been published (Sharma *et al*., 1988; Kandalkar and
Nigam, 1993; Kandalkar et al., 1993; Padmesh et al., 1998; Lynrah et al., 1998 and Sabu et al., 2001). In case of Rauwolfia serpentina, little is known of its reproductive biology, breeding system and genetic variation, except some scatter information on floral biology, fruit setting and seed development (Mital and Issar, 1969; Mitra, 1975), pollen (Vaid, 1963; Nair and Kaul, 1965). There are limited data available on genetic variation (Bhagat et al., 1980), biomass and alkaloid estimation (Ahluwalia, 1961; Shahrear et al., 2002; Gupta et al., 2005), seed germination and variability (Miyazaki and Godaishi, 1962; Choudhury, 1963; Dutta, 1963; Torne, 1964), propagation and root yield (Badhwar et al., 1963; Rajkhowa, 1964; Trivedi, 1995).

1.2.1. Breeding system

The type of breeding system operating within a species has a major effect upon the variation pattern. Out crossing or open pollination system, which is common in most crop plant species usually produce highly variable (heterozygous) genetic populations. In out crossing systems, different genotypes cross successfully with each other and little successful crossing take place between male and female structures of the same plant, or with closely related individuals. Crossing has an advantage because it enables the creation of something entirely different by recombining the variability produced in nature in to a new “package,” the hybrid plant. Thus, it may be possible through crossing to create plant having characteristic of tolerance and sustainability for difficult environments, pest resistance, or especially desired products. Through, crossing, it is also possible to obtain and retain a broad genetic base and to combine desired characteristic of selected individuals, which have high economic values in a single individual.
1.2.2. Genetic variability

Variability in the population is the prerequisite for any successful breeding programme. Without the presence of sufficient genetic variability for any desired traits that are of economic interest, an attempt to use genetic principles to improve plants will be unsuccessful or a failure. Therefore, it is fundamental for any plant improvement program to determine the amount, cause and nature of the variation that is present in the species of interest. It is a difficult but essential task to find out what portion of the variation is genetically controlled so that a determination can be made about how best to exploit it in a plant improvement program, to produce better plants with higher quality products. One major advantage of genetic improvements in plants is that once a change is obtained, it can be kept over a number of generations.

1.2.3. Genotype x environment interaction

A condition that is pivotal in studying variation is commonly referred to as genotype x environment interactions. The term is used to describe the situation where, there is a change in the performance ranking of given population when grown in different environments. Strong genotype x environment interactions is more likely to occur when environments differ widely. This results in offset planting environments that is grossly different from those to which the species is best adapted. Such interaction must be known, if maximum progress in breeding is to be obtained. There are many causes for interaction, but it is generally considered that most of them are more closely related to edaphic than to climatic factors. Traditionally, breeders tend to select genotypes that show stable performance as defined by minimal genotype x environment interaction effects across a number of location and/or years. The trademark and challenge of plant breeder is the necessity to grow plants in a variety of
environments, some of which are greatly different from others. One more major objective of plant improvement program is to identify superior population and locations that best represent production environments. Keeping in view the above, present research was carried out with the following objectives:

1. To study reproductive biology and standardize hybridization techniques.

2. To study the pattern and magnitude of genetic variation amongst the populations in morphological and chemical traits.

3. To study interrelationship among yield and its attributes using path analysis.

4. To identify the promising selections with high root yield and alkaloid contents.

5. To study the genetic divergence amongst different populations.