CHAPTER-VII

SUMMARY
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Indian systems of medicine are largely dependent on plant drugs for production of medicines. The Rigveda (5000 years B.C.) mentioned 67 medicinal plants, Yajurveda 81 and Atarvaveda (4500-2500 years B.C.), 290 species. Later, the Charak Samhita (700 B.C.) and Sushrut Samhita (200 years B.C.) have described properties and uses of 1100 and 1270 plants respectively. About 7000 species of plant have been reported to be used for medicinal purposes in India by practitioners of various systems of medicine viz. Ayurveda, Siddha, Unani and ethnic communities and most of the drugs have so far being gathered from the wild sources. Due to destruction of forests and over exploitation many valuable medicinal plants along with several others, which would have been useful, have already been threatened and many of the remaining ones are not available in sufficient quantity. If such conditions continue, it is feared that in coming decades there is going to be an acute shortage of drugs. Therefore for conservation, to meet the ever-increasing requirements of medicinal plants, to make available fresh, genuine and quality raw material for manufacturing of standardised and efficacious drugs, to check the use of spurious substitution and adulterants and to minimise import and maximise export, it is necessary to undertake mass scale cultivation of commercially important medicinal plants (Pal and Jain, 1988; Gupta and Chadha, 1995; D'Souza et al., 1998). For the purpose, it is necessary to develop agrotechniques, as at present cultivation practices of very few medicinal species are fully developed. Considering this aspect studies related to propagation and cultivation including tissue culture of the selected
medicinal plants were undertaken. The studies carried out are summarised along with significant findings.

1) *Desmodium gangeticum* (L.) DC. (Family-Fabaceae).

It is an important Ayurvedic medicinal plant known as 'Shalaparni'. It grows wild in lower Himalayan regions and throughout the plains of India. It is a bitter tonic, febrifuge, digestive, anticatarrhal and alterative. The roots of *D. gangeticum* (L.) DC. are used as one of the ingredients of famous Ayurvedic preparations 'Dashamula Kwatha' and 'Dashamoolarishta' (Anonymous, 1952).

The drug, *Desmodium gangeticum* (L.) DC., is mostly collected from wild sources to meet the requirement of pharmaceutical industries, as such no efforts have yet been made towards its cultivation, except some preliminary investigations by Dhan Prakash *et al.* (2000). Department of Indian Systems of Medicine and Homeopathy of the Ministry of Health and Family Welfare, Government of India has formulated a central scheme for cultivation and development of medicinal plants. They have included *Desmodium gangeticum* (L.) DC. in the list of plants identified for promoting their cultivation in order to reduce pressure on their natural habitat and to meet the shortage against the demand of the industry (Rawat and Sharma, 1998). Tissue culture studies are reported only on *D. Heterocarpon* (L.) DC. and *D. ovalifolium* Wall. (Wofford *et al.*, 1992). Therefore, in order to domesticate the plant and to evolve techniques for cultivation and propagation trials were made to overcome the dormancy of seeds, to evolve viability of seeds, to propagate the plant by stem cuttings and through tissue culture, using cotyledonary nodal explant. Studies were also carried out to evaluate the effect of cowdung
manure, GA₃, and different soil samples on growth and alkaloid content in root of *D. gangeticum* (L.) DC.

2) *Viola serpens* Wall. (Family-Violaceae)

This plant is distributed in hilly districts throughout India. It is considered as antiseptic, diaphoretic, expectorant and febrifuge and used in cough, fever and respiratory diseases. In traditional medicine, it is extensively used in respiratory diseases (Anonymous, 1976). This species also yields 'Banafsha' of the bazaars and is considered to have medicinal properties similar to those of *Viola odorata* L. A medicinal oil called 'Raughan-i-banafsha' is prepared from it (Kirtikar and Basu, 1935). In the Unani system, this plant as the main ingredient of 'Joshanda' consisting of a mixture of drugs, is used mainly for cough and cold in the form of decoction. A decoction of the flowers is administered for improving the complexion (Anonymous, 1976).

The areas which are rich in *V. serpens* Wall. have been too heavily exploited and this has adversely affected the natural regeneration, The result being that the plant is facing depletion in nature. But commercial cultivation of this species has not been taken up so far (Gupta, 1971; Anonymous, 1976; Chauhan, 1999). Therefore, it is necessary to provide protection to these areas and to permit natural regeneration and simultaneously to bring out the plant under cultivation. Department of Indian Systems of Medicine and Homeopathy of the Ministry of Health and Family Welfare, Government of India has formulated a scheme for cultivation and development of medicinal plants wherein they have identified some species, *Viola serpens* Wall. being one of them, for promoting their cultivation in order to reduce pressure on their
natural habitat and to meet the demand of the industry (Rawat and Sharma, 1998).

There is no report on tissue culture studies of *Viola serpens* Wall., though such studies on other species of *Viola* have been reported, such as *Viola odorata* L. (Sakai Shingo et al., 1991; Van Canegnem, 1997), *Viola tricolor* L. (Sharma and Babbar, 1991) and *Viola patrinii* DC. (Tadahiko Sato et al., 1995). Therefore efforts were made to develop methodology for *in vitro* propagation of *Viola serpens* Wall.

The significant findings of the studies carried out on the aforesaid two plants are summarised below.

Seeds of *Desmodium gangeticum* (L.) DC. showed very poor germination due to hard seed coat. Therefore, to overcome the hard seed coat dormancy and to achieve maximum germination percentage, seeds were subjected to various presowing treatments viz., soaking in distilled water and GA₃, acid scarification and mechanical scarification, and incubated at 15°C, 25°C and 35°C temperature and 92% humidity. Treatment with concentrated H₂SO₄ for 15 minutes and incubation at 25°C with 92% humidity proved to be the most suitable methodology, giving 96% germination, which is about 12 times more as compared to control group. It also helped to enhance the germination index and vigour index 23 and 13 folds respectively. The mechanical scarification and hot water treatments were found useful to some extent in enhancing the seed germination. Other treatments *i.e.* soaking of seeds in cold water, GA₃ and HCl failed to made any impact.

Successful regeneration of plants depends on the quality of seeds, their viability and vigour, which ultimately depend on storage system and
period of storage. Therefore to assess the effect of storage period on germinability and viability of seeds of *Desmodium gangeticum* (L.) DC., fresh as well as seeds stored at room temperature for 1, 3 and 5 years were subjected to germination and viability test. Fresh and one year old seeds showed highest germination percentage (96.66%) and viability percentage (100%). The viability decreased with the increase in storage period beyond one year.

To find out the suitable medium for seed germination of *Desmodium gangeticum* (L.) DC, seeds were sown in different compositions of black cotton soil, garden soil, river sand and cowdung manure. Black cotton soil: cowdung manure (1:1) composition was found to be the most suitable for germination of seeds of *Desmodium gangeticum* (L.) DC.

To standardise the method of vegetative propagation by stem cuttings of *D. gangeticum* (L.) DC., trials were made. Stem cuttings were treated with different concentrations of IAA and IBA for 15 seconds, 1 minute, 3 minutes, 5 minutes, 10 minutes and 15 minutes. Among different treatments used, treatment of 4000 ppm IAA and 10000 ppm IBA for 15 minutes were found to be highly beneficial for vegetative propagation of *Desmodium gangeticum* (L.) DC., giving 100% rooting response as compared to 30% in control. However, amongst the two, IBA was found better as the number of roots were higher in the cuttings treated with it.

To study the effect of cowdung manure, urea, GA₃ and different soil samples on growth and total alkaloid content in roots of *Desmodium gangeticum* (L.) DC., four experiments were conducted. In the first experiment four different doses of cowdung manure were given @ 250 gm, 500 gm, 750
gm and 1000 gm per pot (plant). In the second experiment plants were treated with foliar spray of aqueous solution of GA₃ (in concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) for one year at an interval of every 15 days. In the third experiment the plants were treated with foliar spray of aqueous solution of urea (0.5%, 1.0 %, 1.5%, 2.0% and 2.5%). for one year at an interval of every 15 days. In the fourth experiment seedlings were planted in earthen pots containing garden soil and different combinations of black cotton soil and river sand. The cowdung manure @ 750 gm/ pot (plant), foliar application of GA₃ 300 ppm and urea 0.5% were found to be highly beneficial as they showed significant increase in overall growth and total alkaloid content in roots. Among different soil samples used, black cotton soil: river sand (1:1) was found to be better for growth of *D. gangeticum* (L.) DC.

In order to develop methodology for *in vitro* propagation of *Desmodium gangeticum* (L.) DC. experiments were conducted. The cotyledonal nodal explants were inoculated on MS medium supplemented with different concentrations of BAP, Kn, IAA and NAA singly and in combinations, to assess their influence on shoot regeneration. BAP was found more effective than Kn when used either singly or in combination with IAA and NAA. Highest number of shoots *i.e.*, 9.2 (average) were obtained in explants inoculated on MS+ BAP (2.0 mg/l) + NAA (4.0 mg/l). To obtain complete plantlets, *in vitro* grown shoots were subcultured on full strength and half strength MS media, supplemented with different concentrations of IAA and IBA, singly. Root initiation was observed in shoots inoculated on both, full strength and ½ strength MS media supplemented with IAA and IBA. However the half
strength MS medium supplemented with IAA (3 mg/l) was found better, as there was higher rooting response, with maximum number of roots.

Experiments were conducted to develop methodology for *in vitro* propagation and rapid multiplication of *Viola serpens* Wall through tissue culture. The petiole explants were inoculated on MS medium supplemented with different concentrations of BAP, Kn, IAA, NAA and 2,4-D, singly and in combinations. The MS medium supplemented with 2,4-D (1.5 mg/l) was found most suitable for callus induction and growth. The formation of active green callus and regeneration of multiple shoots were observed on MS+BAP (1.5, 2.5 and 3.5 mg/l) and MS+Kn (1.5, 2.5 and 3.5 mg/l) media. But the best growth response and higher rate of shoot regeneration was observed on MS medium containing BAP (2.5 mg/l) as the number of shoots could be increased to 35-40 on 4th successive subculturing. Multiple shoots when subcultured on MS medium supplemented with BAP (1.5, 2.5 and 3.5 mg/l) showed *in vitro* flowering at all concentrations tried under 8 hours photoperiod. But no flowering was noticed on hormone free MS medium and MS medium supplemented with Kn. MS medium supplemented with BAP (2.5 mg/l) was found to be better for *in vitro* flower induction. Root initiation was observed in shoots inoculated on both full strength and half strength MS media supplemented with IAA and IBA, but the half strength MS medium supplemented with IBA (4 mg/l) was found better as there was higher rooting response with larger number of roots.