CHAPTER 1

INTRODUCTION


(Accessed from http://www.theplantlist.org/browse/A/Leguminosae/Glycyrrhiza/)

Amongst them *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi*, and *G. eurycarpa* are generally recognized as licorice because of their sweet taste (Nomura *et al.*, 2002).

**Scientific Classification**

**Kingdom:** Plantae  
**Division:** Angiospermae  
**Class:** Dicotyledoneae  
**Order:** Rosales  
**Family:** Fabaceae  
**Genus:** Glycyrrhiza  
**Species:** glabra Linn

The species are widely distributed in the world from 50W to 1000E longitude and 200 to 500N latitude (Fig 1.1). *Glycyrrhiza glabra* is geographically distributed from Southern
Europe to Western China, Medirerranean countries, Asia Minor, Egypt, Turkistan, Iran and in India, it is cultivated in Baramulla, Srinagar, Jammu, Dehradun, Delhi, Punjab and South India (Meena et al, 2010). *G. uralensis* Fisch, is found in Central Asia to Eastern China (Rauchensteiner et al, 2005). *Glycyrrhiza lepidota* is found throughout America (Foster, 2000).

![Geographical distribution of *Glycyrrhiza glabra*](image)

**Fig 1.1 Geographical distribution of *Glycyrrhiza glabra* (Dzyubenko & Dzyubenko, 2007)**

*Glycyrrhiza glabra* also known as Licorice or Liquorice or Sweet wood in English and Yashti-madhuh or Madhuka in Sanskrit (Sofia & Walter, 2009). It is a shrub attaining a height up to 6ft. Leaves are multifoliate and imparipinnate, leaflets 4-7 pairs, flowers are papilinaceous, spikes are lavender to violet in colour. The fruit is compressed pod usually containing 3-5 brown reniform seeds (Fig 1.2). *Glycyrrhiza* flowers in March and fruits in August (Sofia and Walter, 2009).

The licorice plant has an extensive root system with a main taproot which is harvested for medicinal use. The tap root is upto 1.5 cm long and subdivided into 3-5 subsidiary roots from which woody stolons aries. The dried, peeled or underground stems and roots constitute the
drug, known in the trade as Liquorice (Wang, 2001 and Khan et al, 2009). The root extract of G. glabra contain 6 to 8 % glycyrrhizin (Kovalenko et al, 2002). Owing to immense medicinal properties roots of Glycyrrhiza are also used as chewing sticks. Liquorice has been used in medicine for more than 4000 years (Sofia and Walter, 2009).

The earliest record of its use in medicine is found in ‘Code Humnubari’ (2100 BC). Hippocrates (400BC) mentioned its use as a remedy for ulcers and quenching of thirst. The drug was also mentioned by Theophrastus and Dioscorides. In traditional Siddha system of medicine, Liquorice is used as a demulcent, expectorant, anti-tussive, laxative and sweetener (Sofia and Walter, 2009). The main ingredient in the root is a saponin like glycoside called Glycyrrhizin. It is more than fifty times sweeter than sugar (Tamir et al, 2001 and Treven et al, 2005). It is otherwise known as glycyrrhizic acid and is commercially available as ammoniated glycyrrhizin. Glycyrrhizin is considered as primary active ingredient. Glycyrrhiza glabra roots sell for $3.35 to $25.60 per pound (Ib) dry weight. National Medicinal Plant Board has reported 40,000 tons/acre and 1,00,000 tons/hectare production of Glycyrrhiza glabra in India.
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Fig 1.2 Morphological view of *Glycyrrhiza glabra* plant growing in field

Chemical Constituents

Bioactive compounds currently extracted from plants are used as drugs, food additives, pigments, dyes, insecticides, cosmetics, perfumes and fine chemicals (Balandrin and Klocke, 1988). The major bioactive constituents in rhizomes of *G. glabra* are glycyrrhizin, glycyrrhizinic acid, glabrin A and B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols (Nomura *et al.*, 2002; Saxena, 2005). Glycyrrhizin compound represents a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid that varies within a 2-25 percent range. Glycyrrhizin is a molecule composed of a hydrophilic part, two molecules of glucuronic acid and a hydrophobic fragment, glycyrrhetic acid. The yellow color of liquorice is due to the flavonoid content of the plant, which includes liquiritin, isoliquiritin, and other compounds. Traditionally it has been used as the Tincture, decoction, syrup, tonic wine, to reduce fever in women after childbirth, for treating sores, toothaches (Kumar and Dora, 2012).

The Pharmacological activity of *G. glabra* is due to the presence of several alkaloids, maximum being in roots and extraction of these alkaloids will necessitate the uprooting of plants and deplete their natural resources as these plants are fast becoming extinct. The production of the wild *G. glabra* has sharply decreased due to excessive and indiscriminate uprooting.

*Glycyrrhiza glabra* is available only during a short period of growing season and not throughout the year. Commonly the plant is propagated by stolon division but this conventional method is slow. The demand of *G. glabra* and its important bioactive constituents is increasing because of its high therapeutic value (Tailing and Kharya, 2001). With the ever increasing demand, there should be optimum availability of these bioactive agents but also of right stage when the active principles are available in optimum quantities. Further *G. glabra* suffers from poor seed availability and poor seed viability (CIMAP Newsletter, 1995; Sawaengsak *et al.*, 2011). By keeping all these things in mind, efforts are now being directed to produce medicinally important substances of plant origin by cell culture or by using various phytohormones.

Micropropagation is a viable approach to fulfill the ever increasing demand of medicinal plant products and supply of raw material to pharmaceutical industry. These will continue to provide dividends for increased and efficient system for *in vitro* production of secondary
metabolites. Natural plants will remain the key sources for supply of raw materials for both pharmaceutical and aromatic industries in the next century. A preliminary report of in vitro multiplication of *Glycyrrhiza glabra* was published as early as 1980 by Shah and Dalal. In vitro regeneration via somatic embryogenesis is important for clonal propagation and is usually an integral part of genetic modification (Varisai *et al*., 2004; Huo *et al*., 2005).

*Agrobacterium rhizogenes*, the causative agent of hairy root disease, is a common soil organism capable of entering in a plant through a wound (Wordragen *et al*., 1992; Shirazi *et al*., 2012). Hairy roots are well established as experimental systems and most importantly, they have been characterized by a high growth rate and are able to synthesize root derived secondary metabolites (Giri and Narasu, 2000). This bacterium transfer a segment from its large root inducing (Ri) plasmid into the genome of the infected plant (Guillon *et al*., 2006). Four loci involved in root formation have been identified in the Ri plasmid and designated root loci A, B, C, D (Ayala-silva *et al*., 2007). If the *Agrobacterium rhizogenes* containing modified Ri plasmid is used to infect the cells, it is possible to transfer the target genes to the plant tissues and to induce regeneration of transgenic plants. Therefore, *Agrobacterium rhizogenes* can be used in genetic manipulations of higher plants for improving plant resistance, quality and possible industrial applications. The hairy root system is stable and high productive under hormone-free culture conditions (Doren, 2006). The greatest advantage of hairy root is in its greater biosynthesis capacity for secondary metabolite production (Kim *et al*., 2002). Keeping in view all these points, the present investigation “Micropropagation of *Glycyrrhiza glabra* Linn. and induction of Glycyrrhizin through in vitro manipulations” was carried out with following objectives:-

- **Optimization of micropropagation protocol for *Glycyrrhiza glabra***

- **Studying the effect of physico-chemical modification of the culture medium on productivity of root alkaloids of *G. glabra***

- **Optimization of transformation protocol of *G. glabra* with *Agrobacterium rhizogenes* for stable transfer of Ri plasmid to induce rhizogenesis**

- **Quantification of glycyrrhizic acid/ glycyrrhizin in *in vitro* roots, callus and transformed cultures**