7. SUMMARY

The angiospermic climber *Clitoria ternatea* belongs to the family of Fabaceae. It is an ornamental perennial climber with conspicuous blue or white flowers. It has got several medicinal properties including promoting memory and intelligence and curing neurological disorder. The roots are bitter, refrigerant, and laxative, diuretic antihelmintic and tonic and useful in dementia, hemicranias, leprosy, inflammation, asthma, pulmonary tuberculosis and hepatopathy.

7.1 *In vitro* Propagation of Medicinally Important Plant *Clitoria ternatea*.

Methods for mass clonal propagation are essential to fulfill cultivars demand. The conventional methods for field propagation are unsuitable for generating large quantities needed. *In vitro* technique has found application in a number of areas impacting on the conservation and management of rare and endangered plant species. The development of micropropagation method had allowed efficient exploitation of medicinal plant species. The present investigation was carried out in order to develop a successful *in vitro* regeneration of medicinally important plant *C. ternatea*.

The actively growing young shoots were collected, defoliated and cut into pieces of 5-7 cm in length. The explants were surface sterilized by keeping under running tap water for 10 min. followed by treating with sodium hypochlorite and tween 20 for 5 min; 70% ethanol for 1 min; 0.1% fytolan with tween 20 for 5 min and 0.1% mercuric chloride with tween 20 for 3 min and the explants were rinsed thoroughly with sterile distilled water for 4-5 times. While, following disinfection, the nodes and internodes were carefully cut aseptically and placed *in vitro* both horizontally as well as vertically on MS medium supplemented with different concentrations and combinations of BA, KN, NAA, IAA and 2,4-D. The explants were subcultured in fresh media after ten days of inoculation. The nodal segments inoculated onto MS medium supplemented with BA (2.0 mg/L) in combination with NAA (0.2 mg/L) produced maximum number of multiple shoots and maximum shoot length.

Internode explants were inoculated on MS medium fortified with various concentrations of 2, 4-D, 2, 4, 5-T, NAA and IAA for callus induction. Of the various concentrations of auxin used, the maximum percentage of callus proliferation (72.3%) was observed from the internode explants when they were inoculated onto medium containing
IAA (1.5 mg/L). Callus proliferation was also observed in the MS medium augmented with 2, 4-D (1.5 mg/L).

The *in vitro* raised shootlets were inoculated onto full strength as well as half strength MS medium containing 2% (w/v) sucrose with different concentration of IBA and IAA for root induction. The maximum percentage of root formation (75%) was observed in MS medium supplemented with IAA (1.0 mg/L) (T49). The maximum number of roots per shootlet was observed in the same medium and the maximum root length was observed in MS medium supplemented with IBA 2.0 mg/L (T42).

The 6-8 week old regenerated plantlets were washed thoroughly in the tap water to remove traces of agar and transferred to polycups containing the mixture of the garden soil and sand. The plants were irrigated with 10X diluted MS liquid medium once in a week. The plantlets were kept in the culture room for the first fifteen days and 70% of the plants succeeded in hardening process. Those plants were transferred to pots under greenhouse. 70% of them got established in the field.

*Clitoria ternatea* seeds were submitted to temperature, chemical, physical and mechanical treatments to break seed dormancy. The speed of germination was faster at 30°C and 28°C followed by 16°C. Sulphuric acid treatment for 5 min was effective in promoting germination (69.7%) in *Clitoria ternatea* but only at the highest concentration (100%). The highest germination was obtained in seeds treated with 100 ppm GA₃ (42.63%). Soaking in hot water poorly removed coat-imposed dormancy in *Clitoria ternatea*. The thermal increase from 60°C to 100°C gradually, decreased germination from 39.98% to 2.85%. Scarification in Sulphuric acid followed by soaking in GA₃ (100ppm) for overnight clearly resulted in high percentage of germination (71.8%).

### 7.2 Studies on Antibacterial Activity of Different Extracts of *Clitoria ternatea*

In the present investigation, antimicrobial activity of both the plant extracts against four microbial species namely, *B. subtilis*, *E. coli*, *K. pneumoniae*, and *P. vulgaris*, were recorded. Considerable antimicrobial activity was detected in Ethyl Acetate and Acetone, extracts of *C. ternatea* (Blue) and Petroleum Ether, Acetone and Ethyl Acetate extract of *C. ternatea* (white).
The Ethyl Acetate and Acetone extracts of *C. ternatea* showed better inhibition for all the four pathogens. The highest degree of inhibition was exerted on *B. subtilis*, *K. pneumoniae*, *E. coli* and *P. vulgaris*. All the four pathogens exhibited resistance to the Ethanol and Aqueous extracts of *C. ternatea*.

In conclusion, our results revealed that Marmesin from the leaf extract of *C. ternatea* (Blue and White) possessed a broad-spectrum of antibacterial activity against a panel of bacteria responsible for the most common bacterial diseases. Hence this compound can be used for the treatment of various diseases.

**7.3 Isoenzyme Analysis of *in vitro* propagated and Mother Plants of *Clitoria ternatea* for Genetic Confirmation**

The term isozyme is used to refer to multiple form of an enzyme with similar or identical catalytic activities occurring within the same plant. Isozymes may differ in their primary structure, because they are encoded in different genes. The genetics of isozyme is well understood and thus can be used as an effective marker in the developmental genetics and differentiation. They are valuable tools to study the genetic variability within the population of plants.

The present experiment was carried out to study the genetic stability between the *in vitro* raised plantlet and mother plant of *C. ternatea* using four isoenzyme systems namely, Peroxidase, Esterase, Alkaline Phosphatase and Polyphenol Oxidase.

The young leaves of *C. ternatea* were collected from the mother plant and *in vitro* raised plant species and mashed in a pre-chilled pestle and mortar by using respective extracting buffer (0.1 M Sodium phosphate buffer, pH 7.0 for Peroxidase and Esterase; 0.01 M Potassium phosphate buffer, pH 7.0, for Polyphenol Oxidase; 50 mM Citrate buffer, pH 9.0, for Alkaline Phosphatase). The slurry was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was collected and used as the enzyme source for the study. Each isoenzyme band was characterized by its Rf value and their similarity index was calculated.

**7.3.1 Peroxidase**

The Peroxidase isozyme pattern showed no variation between the regenerated plantlets and the mother plant with the Rf value 0.487 for *C. ternatea* (Blue) and 0.533 for *C. ternatea* (White) whereas variation was observed in the callus, which showed an Rf value of 0.533 for *C. ternatea* (Blue). But in *C. ternatea*, the callus showed 100% similarity.
7.3.2 Esterase

Comparison of the Esterase isoenzyme system between the mother plant and in vitro raised plantlet showed 91.4% similarity for *C. ternatea* (Blue) and 84.8% for *C. ternatea* (White). Callus showed more variations 41.4% and 45.7% respectively in *C. ternatea* (Blue and White).

7.3.3 Alkaline Phosphatase

The Alkaline Phosphatase isoenzyme banding pattern of *C. ternatea* (Blue) showed 40% of similarity in the mother plant, in vitro raised plantlet and callus; whereas, in *C. ternatea* (White) the in vitro raised plantlet and callus showed 100% similarity towards the mother plant.

7.3.4 Polyphenol Oxidase

The Polyphenol Oxidase isoenzyme banding pattern revealed that, there was 100% pairing affinity between the mother plant and in vitro raised plantlet of *C. ternatea* (Blue), but the callus exhibited variation. In *C. ternatea* (White), the in vitro raised plantlets showed 66.7% similarity, and the callus had 100% similarity with the mother plants.

7.3.5 Total Soluble Proteins

The tissue homogenate of mother plant leaves, in vitro raised plantlet leaves and callus of both *C. ternatea* (Blue and White) was used to determine the position of the protein bands and their relative molecular weight by SDS-PAGE. It was found that the callus and in vitro raised plantlet of *C. ternatea* (Blue) produced maximum number of bands. The protein bands of *C. ternatea* (Blue) had the molecular weight ranging from 60 KD to 15.4 KD and 60 KD to 7.2 KD in *C. ternatea* (White).

The genetic conformity experiments proved that in vitro raised plantlets had more similarity to the mother plant. The callus of *C. ternatea* (Blue) and of *C. ternatea* (White) illustrated variations from their mother plant. This might be either due to the dedifferentiated nature of the callus, in which the gene expression may vary from that of the differentiated tissues or by the somaclonal variations. This type of variations can be used for the improvement of plant breeding in future.
7.4 Analysis and Identification of Different Bioactive Compounds of Medicinally Important Plant *Clitoria ternatea* L by using HPTLC.

The results of the present study confirm the folkloric usage and pharmacological studies of the medicinally important plant *C. ternatea* and suggest that some of the plant extracts possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases. A recent review shows that the HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmacy, chemistry, biochemistry and toxicology. In the present study, we identified the alkaloids profile of the medicinally important plant using HPTLC. HPTLC has been recommended for identification of the medicinal plants. In the present study we also produced the alkaloids, flavinoids and terpenoids profile using HPTLC of the different parts of *Clitoria ternatea*. This profile can be used for the identification of the medicinally important plants from the adulterant. Further, separation and characterization of the bioactive compound (principles) from the selected plants to be evaluated and reported in near future.

7.5 Identification of Bioactive Components of *Clitoria ternatea* (Linn.) by GC–MS Analysis

The bioactive components of ethanol leaf, extract of *Clitoria ternatea* (Blue and White) were evaluated by GC-MS analysis. Ethanol leaf extract of *Clitoria ternatea* (Blue) showed the presence of twenty eight Phytocompounds, Ethanol leaf extract of *C. ternatea* (White) showed the presence of twenty four phytocompounds.

7.6 RAPD Analysis

Good quality DNA was extracted from fresh leaves of four different varieties of *C. ternatea* (Blue and White). The DNA was extracted by CTAB method suggested by Porebski *et al.*, (1997) with slight modification of 2% PVP with fresh leaves. The isolated DNA was quantified using “Hoefer’s DyNA Quant”. Further, the quality of DNA was confirmed by 1% Agarose gel electrophoresis.
The PCR reaction was carried out with the slight modification in dNTP’s and template DNA concentration. The varieties *C. ternatea* (Blue and White) exhibited clear banding pattern with 3-8 bands at molecular weight about 500 bp to 1 kb. The genetic distance distributed between *C. ternatea* (Blue and White) varieties (*C. ternatea* (KL blue) *C. ternatea* (KL white) *C. ternatea* (TN blue) *C. ternatea* (TN white)) was obtained by Jacarrd’s coefficient distance method. Thus the polymorphisms among the varieties were detected by the RAPD analysis.

Genetic diversity noticed among the varieties would be useful in breeding programmes and in the analysis of the pros and cons of their genetic relatedness. Earnest attempts should be made to establish a large reservoir of germplasm and employ selected lines in breeding and crop improvement works. Gene transfer through molecular engineering provides another route to improve upon yield which is yet to be explored in aromatic crops.