CHAPTER 5

DISCUSSION

Tinospora cordifolia is an important medicinal herb mentioned as “Rasayana” in Ayurvedic System of Medicine due to its rejuvenating effect on the body. Its root is reported to have anti-stress, anti-leprotic and antimalarial activities. The plant has antidiabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic and anti-allergic properties. The pharmaceutical significance of this plant is attributed mainly due to polysaccharides, alkaloids and clerodane diterpenoids. The aqueous extract of the plant is reported to have immunomodulatory activities against diverse experimentally induced infections which are mainly due to the presence of polysaccharides. Sixty accessions, collected from different eco-geographical regions spanning from the Himalayan region, Gangetic region, Coastal region and Arid region of India, were utilized for molecular and biochemical characterization of Tinospora cordifolia.

Tinospora cordifolia suffers from poor seed set and poor germination in its natural habitat. Stem cuttings, though useful for propagation, are dependent upon weather conditions for proper growth. Overexploitation has led to the acute scarcity of this plant to meet the present-day demand. Micropropagation may help in propagation and conservation of this important medicinal plant. Culture of shoot meristems, especially through enhanced axillary branching, permits rapid propagation of certain plants and a high degree of genetic uniformity of the progeny. Biochemical and molecular profiling of Indigenous germplasm will lead to identification of superior germplasm, further used for various improvement programs.

Considering all these aspects, the present investigation was designed for survey, exploration and collection assignment of giloy germplasm in eleven hot spot diversity rich Indian States (Jammu and Kashmir, Punjab, Himachal Pradesh, Rajasthan, Haryana, Gujarat, Uttar Pradesh, Orissa, Arunachal Pradesh, Tamilnadu, Kerala) and conserved germplasm from NBPGR (New Delhi), for analyzing the extent of genetic diversity and berberine content.

5.1 COLLECTION, ASSESSMENT, MANAGEMENT AND ETHNIC EXPLORATION OF TINOSPORA CORDIFOLIA

During the September 2010 to March 2011 survey and exploration of different locations was carried out and it reveals that the species has undergone heavy exploitation Characterization of Tinospora cordifolia (Willd.) Miers germplasm
Discussion

with exponential rise in demand which has paved way for quest of alternatives to balance the critical situation.

A total of 60 accessions, both wild and cultivated, were collected from diverse eco-geographical regions (eleven States and one National Capital Region) (Figure 3.1) and these accessions were established in the Green house by stem cutting method as shown in Figure 3.2. During the survey, sizable variations were recorded in morphological and geographical traits in different accessions (Table 4.1). The conventional methods cannot meet the increasing stipulate of this plant used as a raw material for the preparation of pharmaceutical products. Therefore, destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed severe threat to the endurance and accessibility of this highly useful plant. Secondly besides over harvesting (unscientific collection of underground part of medicinal use), several other regions of threats are – habitat destruction, livestock grazing, deforestation and fire. Problems related with its natural propagation and haphazard exploitation for medicinal purpose has pushed *Tinospora cordifolia* to the list of threatened plant species of India (Bhat *et al*. 2013). It is mainly propagated by cuttings and planting period is from early March to late August. During the field examination in Moti magri (Udaipur, Rajasthan), Pipli (Kurushtera, Haryana), and Hamirpur (Himachal Pradesh) predominantly house a large number of its plantation.

The medicinal applicability of the species has been widely popular in tribes as well as in modern pharmaceutical practices. During the survey visit and discussion with the local population of Jammu (Jammu and Kashmir) it was found that they use Giloy to cure malaria. Some people are also using it to cure skin problems although use is limited. The whole plant is used to cure various ailments by general tribes and local people throughout the country. Choudhury *et al*. (2012) had also reported that Vanrui (Chorei) Cheragi community in Karimganj district (Assam) utilized the extract to cure skin infections, stomach problems, and diabetes and malaria treatment. So, there is a great similarity in the use of *Tinospora cordifolia* among the natives of hilly regions. Stem extract of the plant is used to cure headache, migraine, skin diseases and to cure cough by the people of Kachchh Arid Ecosystem, Gujarat, India (Joshi *et al*. 2013). Similar usages of this medicinal therapist were found among the local population of Udaipur region. Amrita formulations are used for treatment of various ailments after mixing with boiled cow milk or with cold water by the Soren clan of Santals of Rajshahi district (Bangladesh) (Rahmatullah *et al*. 2012). Tribal community of Jammu (J&K) and Bigwada (Rajasthan) uses decoction of stem, administered
orally for the treatment of fever on the other hand the inhabitants of Badala (U.P.) take the juice of stem orally with honey for the treatment of swasa (Asthma) (Anonymous, 1999). Interaction with tribals of Rajasthan living in the periphery of Udaipur city and in Dahanu forest division of Maharashtra, tribal races, viz. Agaris, Bhils, Dhodias, Dublas, Khakaris, Rimoshis, Thakurs, Vardaris, Vagharis and Varlis use decoction with cold or hot water (about 3-4) in morning in an empty stomach as a tonic in general debility. The Muslims tribals of Rajouri (Jammu) comprising Gujjars and Bakerwals use the plant in treatment of bone fracture (Jee et al. 1984) while paste or juice of Amrita (T. cordifolia) leaves is usefully applied locally in case of Daha (burning sensation). Most common use of Tinospora cordifolia among the people of most of the regions is its use in the treatment diarrhea, fever and Jaundic. The results of the present investigation are in agreement to findings of Shah et al. 1983 among the tribals of Mumbai and its adjoining areas and the fishermen along the sea coast for the treatment of chronic diarrhea, fever, jaundice, and dysentery.

5.2 BIOCHEMICAL CHARACTERIZATION OF BERBERINE

5.2.1 Quantification of Berberine Content Using HPLC

High Performance Thin Layer Chromatographic (HPLC) analysis was employed for characterization of collected germplasm on the basis of Berberine content. This is the first report of screening of 60 accessions of T. cordifolia for identification of high berberine yielding lines. Literature survey reveals that till date no effort has been made on this front. In pursuance of this, Stem samples of 60 accessions of T. cordifolia were collected (wild as well as cultivated) from eleven states Jammu and Kashmir, Punjab, Himachal Pradesh, Rajasthan, Haryana, Gujarat, Uttar Pradesh, Orissa, Arunachal Pradesh, Tamilnadu, Kerala and one from NCR (NBPG). HPLC being one of the most sensitive, selective, precise and robust technique for biochemical characterization used for screening of collected accessions. Crude extract isolated (Figure 3.3) through the extraction method (Figure 3.4). HPLC chromatogram of berberine standard was identified at 346 nm (Figure 3.5). A good linear relationship existed between peak areas and Beberine content in reference compound as shown in Figure 3.6.

Developed protocol represented the efficiency of extraction, as peaks of berberine were sharp at 16.0 min. in different collected accessions (Figure 4.1-Figure 4.6). The calibration curve demonstrated linearity in the range of 5–20 µg ml⁻¹ for berberine (Table 4.2). Linear least-square regression analysis of the negative values of % Bias in the recovery Characterization of Tinospora cordifolia (Willd.) Miers germplasm
Discussion

Characterization of *Tinospora cordifolia* (Willd.) Miers germplasm of standard samples, verified the accuracy of the analytical method (Table 4.3). For total 9 determinations for berberine the values of mean % recovery and mean relative standard deviation (% RSD) were 99.45 and 0.71, respectively (Table 4.4). No significant variation was observed in intra-day and inter-day analysis of berberine at three standard concentrations (5, 10 and 20 µg g⁻¹). The value of mean recovery as well as mean and individual RSD is less than 2.0% verified the repeatability and precision of the developed method. LOD and LOQ values for berberine were 2.38 µg ml⁻¹ and 7.07 µg ml⁻¹ respectively (Table 4.5). Among different eco-geographical locations highest concentration of Berberine was found in Jammu & Kashmir as compared to other states. Among different geographical locations highest concentration of Berberine (0.132 µg mg⁻¹) was found in the samples collected from Indian Institute of Integrative Medicine TC-2 (IIIMJ-2), (Jammu, Jammu & Kashmir). Least concentration of Berberine 0.014 µg mg⁻¹ found in wild accessions TC-20 (HRAB) collected from Ambala, Haryana. Table 4.6 represents the concentration of Berberine in all 60 accessions gathered from entire country and Figure 4.7 represents graphical presentation of berberine concentration.

Berberine concentration varied from 0.132 to 0.096 µg mg⁻¹ in accessions collected from Jammu and Kashmir, whereas, a lower amount of Berberine was obtained in the accession from Himachal Pradesh 0.018 µg mg⁻¹ in (TC-15, HP-1) and 0.041 µg mg⁻¹ in (TC-16) HP-3. In accession from Punjab, concentration varied from .029 µg mg⁻¹ (TC-5, PBPA-2) to .067 µg mg⁻¹ (TC-7, PBAT). 0.096 µg mg⁻¹ Berberine was obtained from two accessions RJMM-1 (Motimagari, Udaipur, Rajasthan) and IIIMJ3 (Jammu & Kashmir). In Arid region accessions of Rajasthan RJBD-1 (Baghdara, Udaipur, Rajasthan) has highest Berberine concentration 0.10 µg mg⁻¹, while RJSJG-1 (Sajangarh, Udaipur, Rajasthan) has lowest concentration (0.019 µg mg⁻¹). In case of Haryana state highest concentration (0.044 µg mg⁻¹), was obtained in the genotype IC-550221 (HAU, Hisar), whereas, HRAB (Ambala, Haryana) showed lowest concentration (0.014 µg mg⁻¹). Among different cultivated accessions conserved at NBPGR, New Delhi the concentration of Berberine varied from 0.016 µg mg⁻¹ (TC-38, IC-281967) to 0.125 µg mg⁻¹ (TC-42, IC-417329). Coastal region accessions gathered from Tamilnadu Agriculture University showed a narrow disparity in Berberine concentration ranging from 0.019 µg mg⁻¹ (TNAU-10) to 0.077 µg mg⁻¹ (TNAU-4).

Few reports are available regarding quantitative estimation of Berberine in stem samples of *T. cordifolia* using HPLC methods. Jatrorrhizine is a protoberberine type alkaloid
Discussion

from *Tinospora* was evaluated through HPTLC based method using ethyl acetate-isopropanol (10%) and aqueous ammonia 6:10:6 (v/v) as mobile phase. Limits of detection and quantification were found to be 20 and 40 ng per band respectively (Mallavadhani *et al.* 2009). Attempts were made to compare the methanolic extracts of stem portions of *T. cordifolia* and *T. sinensis* with respect to berberine content by Srinivasan *et al.* 2008. TLC and HPLC comparison of both the species revealed significant variation in the chemical constitution of two species as the concentration of berberine in the stem of *T. cordifolia* was found to be 0.3192% while only 0.0967% in *T. sinensis*. Similarly, HPLC-UV-DAD based separation and quantitation of some important markers (20 β-hydroxyecdysone, tinosporaside, cordioside, and columbin) in three species of *Tinospora viz.* *T. cordifolia, T. malablica, and T. crispa* in 70% ethanolic extract has revealed that out of the three species highest amount of the marker compounds were present in *T. cordifolia* (especially from accessions collected from higher altitudes of the Jammu province (North India) as compared to two other species are in agreement with present investigation. (Ahmed *et al.* 2006a). In present investigation the concentration of Berberine was found 0.031 µg mg⁻¹ in two accessions TC-18 (HRMZ-1, Minizoo Pipli, Kurukshetra, Haryana) and TC-48 (ORGM, Ganjam, Orissa) which is in agreement with Patil *et al.* 2009, thirty one Tinospora samples (both wild and cultivated) exhibited higher Berberine concentration, while 29 accessions showed exactly similar range of Berberine to our study, further confirms the accuracy and comparability of the present study. Contrary to its higher content of berberine was found in red, yellow and green fruit (1.30 µg mg⁻¹, 1.10 µg mg⁻¹, 1.0 µg mg⁻¹, respectively) after evaluation of Berberine and lycopene profiling during the ontogeny of *Tinospora cordifolia* fruit (Khan *et al.* 2011). Study revealed that inflorescences had the highest alkaloid content as compared to mature fruit pulp. HPLC analysis of Berberine in other plant species including *B. croatica* and *B. vulgaris* revealed a higher Berberine concentration in root extracts (1.217% and 1.120% ) than in leaf or twig extracts (1.424% and 0.805%) in both plant species (Kosalec *et al.* 2009). Berberine content in root and fruit of *Berberis lyceum royle* was obtained 4.5% and 2.9%, respectively. Whereas, 0.3% Berberine content was obtained in the roots of *Justicia adhatoda* L., although absent in leaves (Gulfraz *et al.* 2004).

A wide variation was observed in the amount of Berberine concentration among all collected accessions. The possible reason may that the production of secondary metabolites is affected by various eco-geographical (different rainfall and temperature) regions and growth (developmental stage) variations. Similarly, Sharma *et al.* (2013) also reported that
Variations may be due to differences in the species, stem size, collection time, season and maturity of the plant. Influence of various other parameters (seasonal variation, geographical location, average rainfall, planting strength, genotype of plant, time of sowing, harvesting period and extracting solvents) on the concentration of bioactive agents have also been observed in other medicinal plants like *Lepidium sativum* L. (Nayak *et al.* 2009, 2012), *Plantago ovata* Forsk (Mann and Vyas, 1996) and *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Saxena *et al.* 2000). Similarly, our study also found that considerably genotypes collected from high altitude are having high berberine content. However, All genotypes did not follow the same pattern as others factors are also responsible for the variation, it needs to be investigated further. This extended and validated method developed in the present investigation allows simultaneous estimation of Berberine alkaloids and can be used for routine quality control of crude drugs and polyherbal formulations.

5.3 MOLECULAR CHARACTERIZATION OF *TINOSPORA CORDIFOLIA* USING MOLECULAR MARKERS

5.3.1 Molecular Characterization in *T. cordifolia* Accessions Through RAPD

*Tinospora cordifolia* is found throughout the great parts of India but now it is listed amongst threatened species in many areas in the country due to over harvesting and destruction of habitat. Population growth, urbanization and the unrestricted collection for pharmaceutical purpose from the wild is resulting in an overexploitation of natural resources. Keeping this in view, a feasible conservation strategy is needed for preserving the declining genetic resources of this species.

In present investigation an attempt has been made to explore the genetic diversity based on RAPD in 60 accessions of *T. cordifolia* collected from different eco-geographical regions of the entire country (Table 3.1). Genomic DNA was isolated from leaves of four months old seedlings using modified CTAB method (Figure 3.7). Subsequently quality and purity of isolated DNA was improved via addition of Proteinase K and RNase A in a step wise manner (Figure 3.8).

Out of 50 reandom primers only forty four primers produced amplicons. Figure 4.9 to 4.20 represents banding profile and polymorphism generated using RAPD primers of OPA18, OPC-16, OPC-15, OPD-05, OPJ-08, OPJ-11, OPL-05, OPAB-05, OPAE-01, OPAE-05, OPAP-9 and OPAP16, respectively. A clear and conspicuous band in electrogram shows the reproducibility of RAPD primers. Forty four primers which showed amplification and
Discussion

produced scorable bands of which 64.81% were polymorphic and 35.18% were monomorphic (Table 4.7). The number of bands produced per primer ranged from 6 in OPAE-06 to 18 in OPL-07. OPAO-01 showed highest percentage of polymorphism among the primers i.e. 91.67% while OPJ-05 showed lowest percentage was i.e. 27.27%.

Genetic differentiation and gene flow and other parameters all genotypes grouped into 4 groups and subjected to Popgene analysis (Table 4.8). The overall value of Genetic differentiation (Gst), and Gene flow (Nm) comes to be 0.191 and 2.114 based on RAPD (Table 4.9). First time association of RAPD marker with that of berberine content studied using AMOVA in *Tinospora cordifolia*. Degree of freedom among the groups and between the cultivars are 3 and 56, respectively based on analysis of molecular variance (AMOVA). Variance component varied as 16.417 and 55.709 respectively (Table 4.10). Table 4.11 represents PIC, MI, DI values of 44 RAPD primers out of 50. PIC, DI, MI values ranged from 0.07-0.30, 0.81-0.92 and 7.86-17.96, respectively.

In UPGMA based cluster analysis all 60 accessions grouped into four clusters with number of accessions 8, 10, 27 and 15, respectively as per Figure 4.21. Cluster 1st was further divided into 2 sub clusters with 2 (TC-18 and TC-27) and 6 accessions, respectively. TC-18 (HRMZ-2) and TC-27 (IC-550229) both belong to the same eco-geographical region of Haryana with similarity coefficient 0.72. Rana *et al*. 2012 also reported the similarity coefficient varying from 0.33 to 0.79 which is in conformity with our result. Two and three dimensional plot of principal component analysis in RAPD analysis of 60 *T. cordifolia* accessions also revealed almost similar grouping pattern (Figure 4.22 and 4.23). Jaccard’s pairwise similarity matrix revealed by RAPD markers for 60 accessions of *Tinospora cordifolia* varied from 0.58 to 0.92 (Figure 4.24).

5.3.2 Molecular Characterization in *T. cordifolia* Accessions Through ISSR

In present investigation the genetic diversity based on ISSR in 60 accessions of *T. cordifolia* collected from different eco-geographical regions of India (Table 3.1) carried out. Thirty six ISSR primers were found suitable from a total of fifty primers employed in the study among the 60 *Tinospora* accessions. Among ISSR primers, ISSR-12, 13, 14, 16, 27, 35, 39, 42, 44, 47, 48 and 50, representative electrogram are presented as Figure 4.25 to 4.36, respectively. All the primers were polymorphic with variable percentage of polymorphism 14.29% to 100%. (Table 4.12).

Table 4.13 reveals the value of Shannon’s information index 0.448, 0.431, 0.341 and 0.358 in Himalayan region, Gangetic region, Coastal region and Arid region, respectively.
A high value of Shannon’s information index is indicative of a diverse community. Among the four eco-geographical regions Himalayan region genotypes are more diverse and equally distributed. The value of Heterogenity was highest in Himalayan region (0.306), whereas lowest in coastal and arid region (0.229). Table-4.14 shows the overall value of Genetic differentiation (Gst) and Gene flow (Nm), which are 0.180 and 2.279. Analysis of molecular variance (AMOVA) depicted by Table-4.15, showed Berberine content % variance component with respect to ISSR marker which is 9.059 among group and 35.634 between the group. Table 4.16 summarises the Polymorphic Information content, Diversity Index, Resolvling power and Marker Index values revealed through ISSR.

Figure 4.40 represents the dendrogram generated using ISSR marker with Jaccard’s similarity coefficient which clearly segregates all 60 accessions of *Tinospora cordifolia*. Jaccard’s similarity coefficient ranged from 0.59 to 0.86 with an average similarity of 0.75 (Table 4.20). Four clusters with accessions 7, 13, 27 and 13 accessions respectively formed in the dendrogram. The cluster I contained 2 sub clusters with TC-27 (IC-550229) and 6 accessions. II\textsuperscript{nd} Major Cluster contains 13 accessions; Major cluster III\textsuperscript{rd} enclosed 27 accessions separated into two sub–clusters. First of the subcluster is having all accessions belonging to Punjab region except TC-6 as well nine accessions from Haryana region. II\textsuperscript{nd} sub cluster is having 14 accessions belonging to Haryana and New Delhi region. In this subcluster maximum similarity was observed in accessions TC-36 and TC-37 i.e. 84 %. IV\textsuperscript{th} cluster contained 13 accessions out of which genotypes from Tamilnadu region from one subcluster while remaining from another subcluster. Maximum 86% similarity observed in TC52 and TC56 accessions.

Figure 4.38 represents two dimensional PCA analysis of ISSR primers in which four major groups were shaped with 7 accessions in I\textsuperscript{st} cluster (TC-27, TC-49, TC-3, TC-48, TC-16, TC-15, TC-1); 15 accessions in II\textsuperscript{nd} cluster and thirteen in III\textsuperscript{rd} while 25 accessions in fourth as shown in. In Figure 4.39, all the accessions were grouped in three dimensional principal component analyses. Figure 4.40 represents Jaccard’s pairwise similarity matrix revealed by ISSR markers.

### 5.3.3 Molecular Characterization in *T. cordifolia* Accessions Through Combined data of RAPD and ISSR

The value of Nei’s gene diversity was reported to be 0.224, 0.228, 0.278, 0.308 for Arid region, Coastal region, Gangetic and Himalayan regions, respectively on the basis of The POPGENE analysis. The percentage of polymorphism varied from 59.35% to 91.43%.
Discussion

Characterization of *Tinospora cordifolia* (Willd.) Miers germplasm (highest in Gangetic region, while lowest in coastal) (Table-4.17). Table 4.18 shows the overall value of Shannon’s Information index 0.491 and Nei’s gene diversity comes to be 0.327. The overall value of Genetic differentiation (Gst), and Gene flow (Nm) comes to be and 0.187 and 2.176, respectively (Table-4.18). Table-4.19 depicts that degree of freedom, among the groups and between cultivars as 3 and 56 respectively, based on analysis of molecular variance (AMOVA) based on RAPD and ISSR while Variance component varied as 25.903 and 91.328 respectively.

In Combined RAPD and ISSR dendrogram, TC-40 (IC-281972) with TC -41 (IC-417328) was the closet pair of accessions with 92 % similarity (Figure 4.41), whereas in the ISSR, TC-52 (TNAU-1) with TC-56 (TNAU-5) was the closet pair with 86%, respectively (Figure 4.37). These results showed that a high degree of genetic similarity exists among the accessions used in the present study for diversity analysis. Study reveals that due to vegetatively propagation genetic recombination and reshuffling of genes phenomenon is generally precluded; a major agent of genetic variation. Low dispersal of seeds, clonal reproduction and population colonization history and pattern of population structure are other possible reasons which resolve its low level of genetic diversity. Results obtained in the present study can be utilized for designing conservation strategies for this valuable and endangered species. These results were in accordance with the previous studies applied in different plant species (Goulao *et al.* 2001, Mattioni *et al.* 2002).

Ahmed *et al.* (2006b) found low genetic diversity among different cultivars of *Tinospora cordifolia* where six primers generated polymorphic bands out of 38 random primers and three restriction enzymes exhibit polymorphism among 11 restriction enzymes. A very low degree of genetic variation was obtained in twenty samples of *T. cordifolia* collected from different regions of India using RAPD markers. Only four decamer primers (OPA-16, OPC-7, OPC-13 and OPG-5) out of 120 only showed reproducible polymorphic banding pattern (Shinde and Dhalwal, 2010).The genetic similarity matrix generated from pooling of RAPD and ISSR data reanging from 0.33 to 0.79 with an average of 0.56. Similarly, in our investigation 0.59 to 0.88 genetic similarity was obtained with an average of 0.59. Among 37 accessions three accessions found to be most divergent with 49% similarity (*Rana et al.* 2012). Similarly in our study two accessions (TC-18 and TC-27) found to be most divergent with 70 % similarity.

The present investigation deals with biochemical and molecular characterization of *Tinospora cordifolia* accessions collected from diverse locations of India. HPLC based...
biochemical analysis revealed that two locations viz: TC-18 (HRMZ-1, Minizoo Pipli, Kurukhetra, Haryana) and TC-48 (ORGM, Ganjam, Orissa) found to be having Berberine content i.e. 0.031 µg mg\(^{-1}\) similar to what Patil et al. 2009 reported. Also, Ahmed et al. 2006 found that high altitude are having high berberine content same was found in our study. TC-2(IIMJ-2) from Jammu, Jammu & Kashmir contained highest concentration of Berberine i.e. 0.132 µg mg\(^{-1}\) while least concentration of Berberine 0.014 µg mg\(^{-1}\) found in HRAB collected from Ambala. Fifty RAPD and fifty ISSR primers were employed in genetic diversity study of 60 accessions of *T. cordifolia*. Using modified CTAB method genomic DNA was isolated and subjected to PCR analysis at standardized conditions with 45 cycles for RAPD and 35 cycles for ISSR. Visualization of gels using UV light and band scoring on the basis of 0 and 1 done. Popgene analysis based on RAPD revealed the overall value of Genetic deifferentiation (Gst), Gene flow (Nm), comes to be 0.191, 2.114 respectively. Analysis of molecular variance (AMOVA) based on RAPD, ISSR and RAPD + ISSR gave variance component among groups as 16.417, 9.509 and 25.903. Level of polymorphism based on RAPD, ISSR and RAPD+ISSR varied as 64.81%, 64.81%, 63.98% respectively.

UPGMA dendrogram of all the three trees obtained from RAPD (Figure 4.23), ISSR (Figure 4.39) and combined data of RAPD+ISSR (Figure 4.43) were comparable. Also, Two and three dimensional plot of principal component analysis using RAPD analysis, ISSR analysis and RAPD+ISSR analysis were comparable to their same domain. Low genetic diversity among cultivars of *Tinospora cordifolia* observed by Ahmed et al. (2006b) as well as by (Shinde and Dhalwal, 2010). Rana et al. 2012 obtained the genetic similarity matrix generated from pooling of RAPD and ISSR data ranging from 0.33 to 0.79 with an average of 0.56 which is in similarity with our study.

The present study revealed the development of an efficient DNA isolation and purification protocol with optimization of RAPD and ISSR conditions. The genus *Tinospora* has not been taken any considerable priority in terms of genetic diversity, population structures as well as other qualitative and quantative studies related to their genetic improvement and conservation. Utilization of large number of accessions (60 accessions covering the major Indian states) and increased resolution may provide effective marker system to access the genetic diversity. To the best of knowledge, no work has been published on the molecular characterization of *Tinospora* germplasm from such diverse locations throughout the entire country. These finding can also serve as guidelines to preserve the genetic resources of this valuable medicinal plant.