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Assessment of Genetic Diversity in Medicinal Climber of *Tinospora cordifolia* (Willd.) Miers (Menispermaceae) from Gujarat, India

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Abstract: Genetic diversity of 25 accessions of the medicinal climber *Tinospora cordifolia* was measured by isozymes from Gujarat. The germplasm was reared in a field plot under identical conditions and mature stems were used for the presented study. Analysis by using battery of ten isozymes revealed the presence of 16 gene loci and 33 alleles in 25 accessions. The percentage of polymorphic loci (P) was 45.0% and mean observed number of alleles per locus (A) was 1.57. The average observed heterozygosity (Ho) and expected heterozygosity (He) were 0.443 and 0.270, respectively shows high levels of genetic variation among different accessions. The UPGMA dendrogram clearly depict the spectra of genetic diversity among various accessions. The clustering of accession TC-1 (Kheda) and TC-2 (Songhad) appeared at the top of the dendrogram which are genetically rich. These accessions should be conserved for future breeding programme.

Key words: Genetic diversity, conservation, medicinal plant, *Tinospora cordifolia*

INTRODUCTION

The questions of population structure and conservation biology have been addressed the genetic diversity of plant species using isozyme analysis by Hamrick *et al*. (1979) and Crawford (2000). Knowledge on the amount and distribution of genetic variability within a species is vital to plant breeders because it is important consideration when selecting germplasm to be included in a breeding programme. It is also helpful to geneticists to it's manage plant genetic resources and provides information for designing sampling protocols (Bretting and Widrlechner, 1995). Attempts have been made to study genetic diversity in medicinal plant species (Grubbs *et al*., 2004; Siva and Krishnamurthy, 2005). Further research on medicinal plant species is required to develop the conservation strategies and crop improvement programmes.

Tinospora cordifolia (Willd.) Miers. (Galo) plant is known, as heart leaved moonseed plant in English and Guduchi in Sanskrit. It is a large, glabrous, deciduous, climbing shrub belonging to the family Menispermaceae. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m. Stems are useful in diabetes, fever, flatulence, hypertension, jaundice and leucorrhoea. Roots are useful in gynecological disorders, spleen troubles (Kirtikar and Basu, 1975; Zhao *et al*., 1991). The dried stem and bark are used as tonic, antiperiodic and aphrodisiacs. The starch of roots and stems are nutritious and are used to cure diarrhoea (Bhattacharjee, 2004). A variety of constituents such as alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides have been isolated from *T. cordifolia* plant and their structures were elucidated. Leaves of this plant are rich in proteins (11.2%) and are also fairly rich in calcium and phosphorus (Khosa and Prasad, 1971; Zhao *et al*., 1991).

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The present study was undertaken to find out the genetic diversity among the different accessions of *T. cordifolia* collected from various parts of Gujarat using isozyme analysis.

MATERIALS AND METHODS

Plant Material

Twenty five germplasm accessions of *T. cordifolia* were collected from different localities of South Gujarat, North Gujarat, Central Gujarat, Saurashtra and Kachchh Region of Gujarat State (from arid zone to moist deciduous habitats) during the year 2003-04. The geographical distribution of the collected sample materials was shown in Table 1. The stem cuttings were grown and maintained in the uniform agroclimatic conditions at Sardar Patel University Botanical Garden, Vallabh Vidyanagar, Gujarat. The stem cuttings were used for the analysis of genetic diversity among the accessions.

Preparation of Enzyme Extract

Seven samples per accession were used to prepare crude enzyme extracts. One gram of plant materials were washed with distilled water and macerated individually in extraction buffer [1 mL of 0.25 M phosphate buffer (pH 7.5) containing 5% Sucrose, 5% PVP (polyvinyl pyrrolidone), 14 mM mercaptoethanol]. The homogenate was centrifuged at 15,000 rpm for 30 min at 4°C in a Beckman Coulter Avanti J-25, USA. The supernatant was collected and stored under refrigeration.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was carried out following the standard procedure (Laemmli, 1970). Seven samples were loaded into vertical discontinuous native PAGE (Genei, Bangalore), 10×10 cm glass plate with 1mm thick polyacrylamide gels. Electrophoresis was conducted for 3 h at 4°C with an applied voltage of 100 Vm^{-1}. Fifteen enzymatic systems were tried to identify the best gel and buffer systems, in samples. Ten enzyme systems were selected using tris-glycine

Table 1: Geographic distribution of *Tinospora cordifolia* accessions used for genetic diversity

Accession No.	Village	District
TC-1	Kheda	Kheda
TC-2	Vasad	Anand
TC-3	Akhaj	Mehsana
TC-4	Magarvada	Banaskantha
TC-5	Vansada	Navsari
TC-6	Sukhpur	Surendranagar
TC-7	Vertej	Bhavnagar
TC-8	Lathi	Amreli
TC-9	Badhergadh	Kachchh
TC-10	Junagadh	Junagadh
TC-11	Bileshwar	Rajkot
TC-12	Kutiyana	Porbandar
TC-13	Lalpur	Jamnagar
TC-14	Kewala	Ahmedabad
TC-15	Dathivada	Banaskantha
TC-16	Patan	Patan
TC-17	Dehagam	Gandhinagar
TC-18	Edar	Sabarkantha
TC-19	Pavaghad	Panchmahal
TC-20	Chottaudepur	Chottaudepur
TC-21	Kabervad	Bharuch
TC-22	Rajpipala	Narmada
TC-23	Dharampur	Valsad
TC-24	Ahwa	Dangs
TC-25	Songhad	Surat

electrophoresis buffer system (Laemmli, 1970). Gels were stained with a staining solution specific for each enzyme i.e., Alcohol dehydrogenase (ADH) E.C.1.1.1.1 (Tanksely, 1979), Glutamate dehydrogenase (GDH) E.C.1.4.1.2 (Scheid *et al*., 1980), diaphorase (DIA) E.C.1.6.99 (Wendel and Weeden, 1989), Aspartate Amino Transferase (AAT) E.C.2.6.1.1 (Decker and Rao, 1963), Catalase (CAT) E.C.1.11.1.6 (Thorup *et al*., 1961), Glucose-6-phosphate dehydrogenase (G-6-PDH) E.C.1.1.1.49 (Brewer and Sing, 1970), peroxides (PEX)E.C.1.11.1.7 (Guikema and Sherman, 1980), amylase (AMY) E.C.3.2.1.1 (Frydenberg and Nielsen,1966), Ribulose Biophosphate Carboxylase (RBC) E.C.4.1.1.39 (Weeden, 1984), superoxide dismutase (SOD) E.C.1.15.1.1 (Beauchamp and Fridovich, 1971) without any modification. The most anodally migrating locus was designated with numbers and allelic variation at a locus was coded alphabetically. The genetic interpretations of banding pattern of isozymes were done as per the Wendel and Weeden (1989) interpretation of plant isozymes.

Data Analysis

The POPGENE (version 1.31) computer programme developed by Yeh and Boyle (1997) was used to analyse allelic data. Population genetic parameters were calculated for each species as a whole and on population basis such as; allelic frequency (A), percentage of polymorphic loci (P), observed heterozygosity (Ho), expected heterozygosity (He), observed homozygosity (O), expected homozygosity (E), Shannon's information index (I), genetic distance, genetic identity (I), dendrogram was prepared using UPGMA (Unweighted pair group method with arithmetic averaging) among populations was clustered on the basis of their genetic distance estimates. In addition, the programme PHYLIP (Phylogeny inference package, version 3.5) was used to obtain the genetic distances by resampling the data and clustered into a dendrogram (Felsenstein, 1993).

RESULTS

Allelic Frequency

In the present study, 10 enzyme systems DIA, G6P, GDH, ADH, SOD, AAT, RBC, PEX, AMY and CAT (Fig. 2A-C, 3A-C, 4A-D) were selected to find out the genetic diversity in 25 accessions of *Tinospora cordifolia*. The enzyme systems provided 16 loci and 33 alleles in 25 accessions of *T. cordifolia*. GDH-1, CAT-1, CAT-2 and CAT-3 were found to be invariant and monomorphic in nature (Fig. 1). CAT-1 and CAT-2 were detected in all the accessions in monomorphic form (Fig. 1). The GDH-1 was found to be monomorphic in 18 out of 25 accessions, where as CAT-3 was observed as monomorphic in 5 out of 25 accessions. The DIA-1and DIA-2,

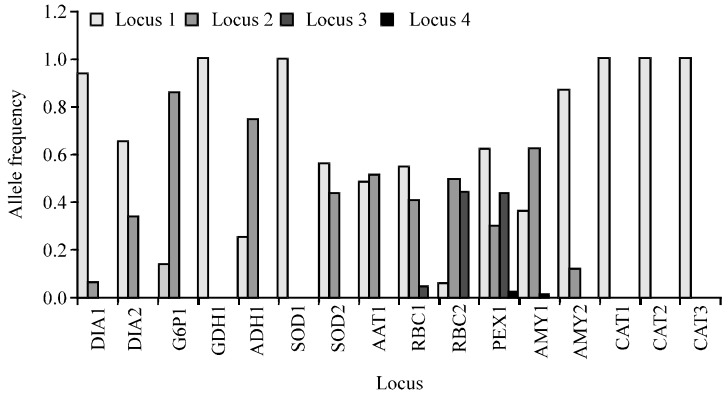

Fig. 1: Allele frequency at 16 loci in 25 accessions of *T. cordifolia*

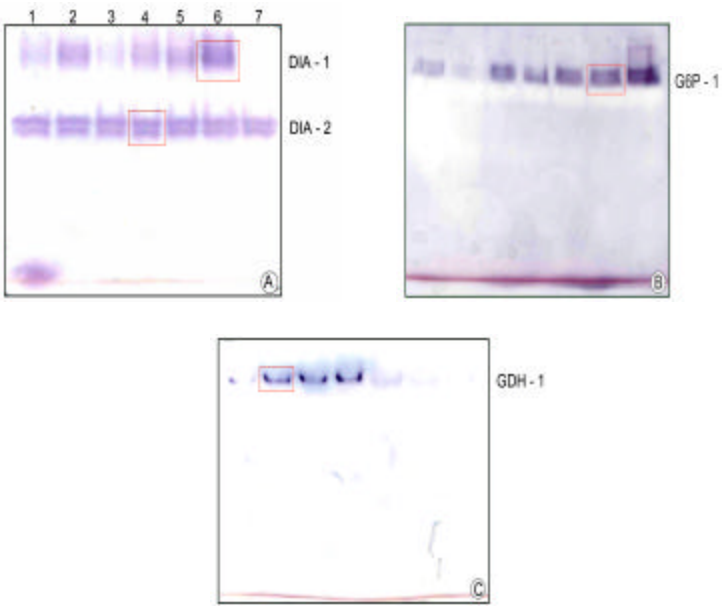

Fig. 2: Isozyme profile of different alleles and locus of *Tinospora cordifolia* (Willd.) Miers and Th. (A) DIA-1, DIA-2, (B) G6P-1 and (C) GDH-1. Lane 1 to 7: Accessions individual sample

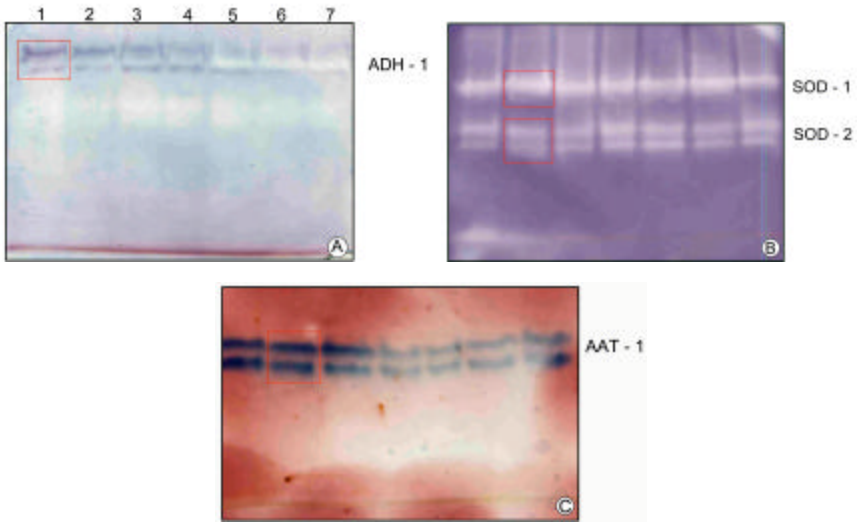

Fig. 3: Isozyme profile of different alleles and locus of *Tinospora cordifolia* (Willd.) Miers (A) ADH-1, (B) SOD-1, SOD-2 and (C) AAT-1. Lane 1 to 7: Accessions individual sample

G6P-1, ADH-1, SOD-1, SOD-2, AAT-1 and AMY-2 were invariant in all accessions and were dimorphic in nature. RBC-1, RBC-2, PEX-1 and AMY-1 were detected in all the accessions as polymorphic alleles (Fig. 1).

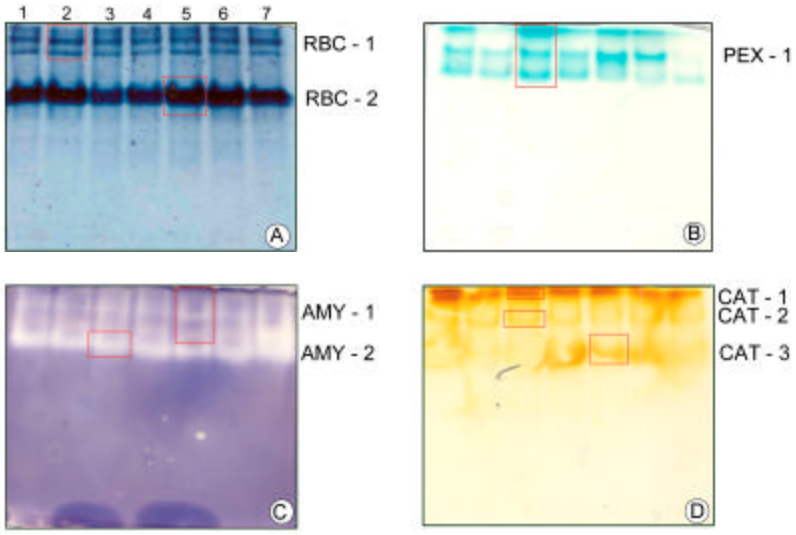

Fig. 4: Isozyme profile of different alleles and locus of *Tinospora cordifolia* (Willd.) Miers (A) RBC-1, 2, (B) PEX-1, (C) AMY-1, 2 and (D) CAT-1, 2, 3). Lane 1 to 7: Accessions individual sample

Polymorphic Loci

Total 12 polymorphic loci found in all the accessions with an over all percentage of polymorphic loci at 75%. The percentage of polymorphic loci (P) (0.99 criterion) varied from 31.25 to 62.50% (overall loci) in all the 25 accessions. The lowest percentage of polymorphic loci was observed in accessions TC-15, TC-16 and TC-25 (31.25%) and highest percentage of polymorphic loci was observed in accession TC-8 (62.50%). The mean percentage of polymorphic loci (overall loci) was observed as 45.00±7.86% (Table 2).

Allelic Number

Mean observed number of alleles per locus was lowest in accession TC-25 (1.33) and highest in accession TC-8 (1.85). The mean value of observed number of alleles per locus was found to 1.57±0.123. PEX-1 was found to possess the highest observed number of alleles (4.000) for all loci where as the lowest number of alleles were found in GDH-1, CAT-1, CAT-2 and CAT-3 loci. The mean of observed number of alleles for all loci was 2.062±0.853 (Table 2).

Effective Number of Alleles

The most effective number of alleles was found in RBC-2 (2.249) for all loci in 25 accessions of *T. cordifolia*. The mean value of effective number of alleles was recorded as 1.533±0.483 (Table 2).

Shannon's Information Index (I)

Out of 25 accessions studied, the Shannon's information index (I) was found highest in accession TC-8 (0.520) and lowest in accession TC-25 (0.194). The mean value of Shannon's information index for all loci was 0.357±0.070. The highest Shannon's information index (I) for genetic variation was located in PEX-1 (0.891) locus and the lowest in GDH-1, CAT-1, Cat-2 and CAT-3 (0.000) loci. The mean value of genetic variation for all loci was 0.436±0.348 (Table 2).

Table 2: Descriptive statistics for overall loci of 25 accessions of *T. cordifolia*

Accession No.	P	A	I	Ho	He	O	E
TC-1	37.50	1.50	0.305	0.327	0.228	0.672	0.228
TC-2	37.50	1.50	0.322	0.428	0.248	0.571	0.248
TC-3	43.75	1.63	0.381	0.401	0.294	0.598	0.294
TC-4	50.00	1.75	0.390	0.436	0.283	0.563	0.283
TC-5	43.75	1.57	0.322	0.367	0.238	0.632	0.238
TC-6	50.00	1.57	0.380	0.485	0.292	0.514	0.292
TC-7	56.25	1.84	0.482	0.552	0.353	0.417	0.353
TC-8	62.50	1.85	0.520	0.511	0.384	0.488	0.384
TC-9	43.75	1.50	0.345	0.449	0.268	0.551	0.268
TC-10	50.00	1.61	0.413	0.571	0.318	0.428	0.318
TC-11	43.75	1.61	0.353	0.428	0.262	0.571	0.262
TC-12	50.00	1.69	0.426	0.538	0.320	0.461	0.320
TC-13	50.00	1.57	0.386	0.520	0.298	0.479	0.298
TC-14	43.75	1.53	0.365	0.505	0.282	0.494	0.282
TC-15	31.25	1.42	0.261	0.345	0.196	0.654	0.196
TC-16	31.25	1.40	0.251	0.284	0.187	0.715	0.187
TC-17	43.75	1.61	0.323	0.377	0.234	0.622	0.234
TC-18	43.75	1.61	0.349	0.421	0.259	0.578	0.259
TC-19	50.00	1.61	0.401	0.538	0.307	0.461	0.307
TC-20	50.00	1.64	0.404	0.520	0.302	0.479	0.302
TC-21	56.25	1.64	0.411	0.561	0.319	0.438	0.319
TC-22	43.75	1.46	0.311	0.428	0.240	0.571	0.240
TC-23	37.50	1.50	0.308	0.404	0.234	0.595	0.234
TC-24	43.75	1.50	0.339	0.459	0.261	0.540	0.261
TC-25	31.25	1.33	0.194	0.234	0.146	0.765	0.146
Mean	45.00	1.57	0.357	0.443	0.270	0.554	0.270
SD	07.86	0.123	0.070	0.088	0.052	0.090	0.052

P: Percentage of polymorphic loci (0.99 criterian), A: Mean observed number of alleles per locus, I: Shannon's information index, Ho: Observed heterozygocity, He: Expected heterozygocity, O: Observed homozygosity, E: Expected homozygosity

Table 3: Summary of heterozygosity for all loci in 25 accessions of *T. cordifolia*

Locus	Sample size	Obs Hom	Obs Het	Exp Het*	Nei**	Ave-Het
DIA-1	326	0.871	0.128	0.125	0.120	0.069
DIA-2	348	0.459	0.540	0.453	0.451	0.281
G6P-1	350	0.754	0.245	0.241	0.240	0.172
GDH-1	178	1.000	0.000	0.000	0.000	0.000
ADH-1	90	0.844	0.155	0.384	0.380	0.037
SOD-1	348	0.994	0.005	0.005	0.005	0.005
SOD-2	330	0.139	0.860	0.493	0.491	0.465
AAT-1	326	0.024	0.975	0.501	0.499	0.497
RBC-1	304	0.118	0.881	0.537	0.535	0.491
RBC-2	336	0.029	0.970	0.557	0.555	0.499
PEX-1	336	0.392	0.607	0.516	0.514	0.427
AMY-1	348	0.310	0.689	0.481	0.479	0.372
AMY-2	54	0.740	0.259	0.229	0.225	0.020
CAT-1	278	1.000	0.000	0.000	0.000	0.000
CAT-2	290	1.000	0.000	0.000	0.000	0.000
CAT-3	42	1.000	0.000	0.000	0.000	0.000
Mean	260	0.605	0.390	0.282	0.281	0.208
SD		0.384	0.384	0.229	0.229	0.215

*Expected homozygosity and heterozygosity were computed using levene in 1949. **Nei (1973) expected heteroztgosity

Heterozygosity

The lowest observed heterozygosity (Ho) was recorded in accession TC-25 (0.234) and highest in accession TC-10 (0.571) with a mean value of 0.443±0.088 (Table 2). The lowest expected heterozygosity (He) was noticed in accession TC-25 (0.146) and highest in accession TC-8 (0.384) with mean value of 0.270±0.052. The lowest observed heterozygosity for genetic variation was recorded in DIA-1 (0.128) loci and highest in AAT-1 (0.975) loci.

The over all mean value of observed heterozygosity (Ho) was 0.390±0.384 (Table 3). The over all highest expected heterozygosity was seen in loci RBC-2 (0.557) and RBC-1 (0.537) with mean

value of 0.282±0.229. The over all highest observed heterozygosity was observed in RBC-2 (0.499) and AAT-1 (0.497) loci and the mean value at 0.390±0.384. Nei's (1973) expected heterozygosity was shown in the Table 3, which revealed that the highest expected heterozygosity in RBC-2 (0.555) loci with the mean value of 0.281±0.229 (Table 3).

Homozygosity

The minimum observed homozygosity in accession TC-7 and maximum observed homozygosity in accession TC-25 was found with the mean value of 0.554±0.090. The observed homozygosity was perceived highest (1.000) in the GDH-1, CAT-1, CAT-2 and CAT-3 loci with the mean value of 0.605±0.384 (Table 3). The lowest expected homozygosity (E) was seen in accession TC-25 and highest in accessions TC-8 with the mean value of 0.270±0.052 (Table 2).

Dendrogram

The UPGMA (Unweighted pair group method using arithmetic average) dendrogram clearly depicted various spectra for genetic diversity among 25 accessions of *Tinospora cordifolia* using the genetic distance estimation (Modified from NEIGHBOR procedure of PHYLIP version 3.5). The dendrogram based on UPGMA clustering revealed that the accession TC-1 was placed at first position and accession TC-16 was placed at the end. The dendrogram contained mainly 2 major clusters. Cluster No. 1 contained 5 accessions (TC-2, 3, 14, 15, 16) where as cluster No. 2 included 20 accessions. Dendrogram based on Nei's (1978), suggests genetic distance was maximum between the accessions TC-21 and TC-23 and minimum between the accessions TC-6 and TC-5 (Fig. 5, Table 4).

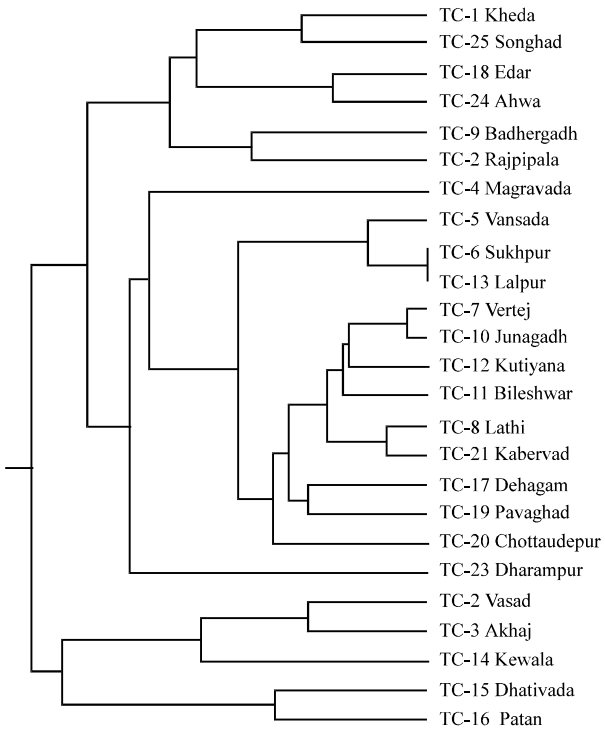

Fig. 5: Dendrogram based UPGMA clustering of 25 accessions of *T. cordifolia* using the genetic distance estimates (Modified from NEIGHBOR procedure of PHYLIP Versions 3.5)

Table 4: Genetic relationship among 25 accessions of *T. cordifolia from* different localities. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal)

Acce No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	-	0.894	0.836	0.892	0.756	0.854	0.879	0.859	0.915	0.914	0.945	0.88	0.862	0.831	0.785	0.819	0.847	0.906	0.870	0.872	0.897	0.874	0.869	0.868	0.931
2	0.829	-	0.928	0.883	0.741	0.845	0.850	0.829	0.815	0.899	0.936	0.866	0.856	0.916	0.884	0.812	0.850	0.904	0.850	0.855	0.890	0.789	0.852	0.857	0.850
3	0.178	0.073	-	0.798	0.678	0.784	0.757	0.754	0.719	0.812	0.866	0.785	0.794	0.85	0.811	0.731	0.765	0.818	0.777	0.790	0.815	0.724	0.751	0.774	0.803
4	0.113	0.124	0.225	-	0.76	0.855	0.863	0.835	0.814	0.900	0.938	0.822	0.857	0.810	0.773	0.812	0.844	0.795	0.856	0.859	0.889	0.790	0.854	0.755	0.849
5	0.279	0.299	0.387	0.273	-	0.966	0.849	0.895	0.779	0.860	0.804	0.888	0.951	0.831	0.734	0.779	0.885	0.746	0.861	0.844	0.903	0.803	0.721	0.727	0.726
6	0.157	0.167	0.242	0.156	0.038	-	0.888	0.940	0.830	0.915	0.905	0.923	0.994	0.902	0.832	0.869	0.899	0.808	0.905	0.888	0.956	0.835	0.822	0.770	0.819
7	0.128	0.162	0.277	0.147	0.163	0.118	-	0.941	0.906	0.972	0.926	0.932	0.888	0.825	0.734	0.798	0.913	0.821	0.903	0.903	0.926	0.815	0.906	0.800	0.815
8	0.151	0.187	0.281	0.18	0.110	0.061	0.603	-	0.845	0.940	0.907	0.91	0.948	0.87	0.763	0.820	0.880	0.785	0.879	0.887	0.956	0.824	0.845	0.773	0.787
9	0.088	0.203	0.329	0.205	0.249	0.186	0.097	0.167	-	0.917	0.875	0.867	0.840	0.775	0.755	0.796	0.898	0.922	0.885	0.848	0.876	0.906	0.897	0.872	0.859
10	0.089	0.106	0.207	0.105	0.149	0.088	0.028	0.061	0.086	-	0.966	0.960	0.926	0.857	0.780	0.831	0.957	0.865	0.940	0.936	0.967	0.949	0.916	0.840	0.848
11	0.056	0.065	0.142	0.063	0.217	0.099	0.075	0.096	0.132	0.033	-	0.944	0.913	0.864	0.819	0.863	0.896	0.861	0.935	0.920	0.947	0.840	0.925	0.823	0.888
12	0.127	0.143	0.241	0.125	0.117	0.079	0.069	0.094	0.142	0.040	0.057	-	0.919	0.828	0.775	0.832	0.935	0.839	0.936	0.922	0.942	0.851	0.865	0.814	0.837
13	0.148	0.155	0.229	0.153	0.050	0.006	0.117	0.053	0.173	0.076	0.090	0.063	-	0.912	0.842	0.872	0.910	0.816	0.905	0.895	0.966	0.839	0.831	0.785	0.812
14	0.184	0.086	0.162	0.210	0.185	0.103	0.191	0.138	0.254	0.154	0.146	0.188	0.091	-	0.899	0.835	0.829	0.854	0.809	0.824	0.897	0.746	0.782	0.818	0.777
15	0.241	0.123	0.209	0.257	0.308	0.183	0.308	0.270	0.280	0.247	0.199	0.253	0.171	0.105	-	0.920	0.777	0.833	0.778	0.791	0.824	0.735	0.778	0.810	0.798
16	0.199	0.207	0.313	0.207	0.248	0.140	0.224	0.198	0.227	0.185	0.146	0.183	0.136	0.18	0.082	-	0.802	0.781	0.848	0.878	0.860	0.761	0.833	0.811	0.855
17	0.165	0.161	0.267	0.168	0.121	0.105	0.090	0.127	0.107	0.043	0.109	0.066	0.094	0.187	0.251	0.219	-	0.867	0.930	0.910	0.944	0.865	0.841	0.826	0.811
18	0.098	0.100	0.200	0.228	0.292	0.212	0.196	0.241	0.080	0.144	0.149	0.175	0.203	0.156	0.182	0.246	0.142	-	0.863	0.822	0.851	0.882	0.820	0.943	0.864
19	0.138	0.162	0.252	0.155	0.149	0.099	0.101	0.128	0.121	0.061	0.066	0.065	0.099	0.211	0.250	0.163	0.071	0.146	-	0.909	0.924	0.874	0.881	0.814	0.832
20	0.136	0.155	0.235	0.151	0.169	0.117	0.101	0.119	0.164	0.065	0.082	0.080	0.111	0.192	0.233	0.129	0.093	0.195	0.095	-	0.920	0.827	0.844	0.892	0.902
21	0.108	0.116	0.204	0.116	0.101	0.044	0.076	0.041	0.131	0.033	0.054	0.058	0.034	0.108	0.192	0.149	0.057	0.16	0.078	0.083	-	0.891	0.871	0.822	0.840
22	0.134	0.236	0.322	0.234	0.218	0.179	0.204	0.192	0.097	0.162	0.173	0.161	0.175	0.292	0.307	0.272	0.144	0.124	0.134	0.189	0.115	-	0.801	0.828	0.833
23	0.140	0.159	0.285	0.157	0.327	0.195	0.098	0.167	0.107	0.087	0.076	0.144	0.184	0.244	0.249	0.182	0.172	0.198	0.126	0.168	0.137	0.221	-	0.771	0.811
24	0.140	0.153	0.255	0.280	0.318	0.252	0.222	0.256	0.135	0.173	0.194	0.205	0.241	0.200	0.209	0.209	0.191	0.058	0.205	0.113	0.195	0.188	0.259	-	0.897
25	0.071	0.162	0.219	0.162	0.319	0.199	0.203	0.239	0.151	0.164	0.118	0.176	0.207	0.251	0.224	0.156	0.208	0.145	0.183	0.102	0.173	0.182	0.208	0.107	-

DISCUSSION

In plants, isozyme data allows quantification of the similarity or difference between populations, groups of populations and species (Gottlieb, 1981). Populations and species can be characterized on the basis of the differences in allelic frequencies. *Tinospora cordifolia* is most important ingredient in ayurvedic preparations and extensively used now-a-days in India. Considerable efforts are being made to evaluate the genetic resources of plants and its close relatives, within species and tribes (Macqueen, 1993; Pottinger, 1996).

The genetic diversity of *T. cordifolia* had higher level of allozyme variation within population and less variation among the populations than the species with other combinations of traits (Hamrick *et al*., 1979; Gottlieb, 1981; Loveless and Hamrick, 1984).

Tinospora cordifolia is a wide spread species through out Gujarat. At the species and population level, geographical range was the best predictor for the level of allozyme variation (Hamrick *et al*., 1979). Endemic species have the lowest genetic diversity where as regionally distributed and wind spread species had geographically a history of large and continuous population which was less susceptible to loss of genetic variation due to genetic drift (Qiu *et al*., 2005; Contle *et al*., 1998). The wide spread species had an average genetic diversity within populations than more geographically restricted species. *Tinospora cordifolia* is more wide spread species and has greater genetic diversity within population where heterozygosity obtained was (0.208±0.215). The second factor affecting the level of variation within the population is variability (breeding system and seed dispersal) of the species. Wind pollinated species had more genetic variation within population. Since, *T. cordifolia* is the cross pollinated species probably it might have higher genetic variation within population.

In *T. cordifolia* higher genetic diversity within population was observed in comparison with the genetic diversity within population ranged approximately from 0.35-0.0 for *Echium agineum* (Burdon and Brown, 1986), *Dicea abies* (Ludkvist and Rudin, 1977) and *Pinnus longaeva* (Hiebert and Hamrick, 1983).

A dendrogram showing the phylogenetic relationship among 25 accessions of *T. cordifolia* based on the data of genetic distance obtained by native gel electrophoresis revealed that the size of the population was widely spread. The UPGMA (Unweighted pair group method with arithmetic mean) dendrogram clear by depicted spectra of genetic diversity among various accessions. Accession sample collected from Kheda region (Central Gujarat) and Songhad (South Gujarat) region were clustered at the top of the dendrogram.

Attempts have been made to understand population structure for a number of medicinal plant species to establish commercial level propagation for useful secondary metabolites using isozyme markers (Scott and Morrison, 1996; Uma Shanker and Ganeshaiah, 1997; Siva, 2003; Siva and Krishnamurthy, 2005). Geographical conditions also affect the active constituents of medicinal plants and hence their activate profiles. Estimation of genetic diversity is an important aspect in designing the crop improvement programme as well as management of germplasm and also help in making strategies for conservation.

CONCLUSION

Based on the data obtained from this study on *T. cordifolia* suggested that the accessions collected from Kheda (Central Gujarat) and Songhad (South Gujarat) region be conserved in field gene bank. This will felicitate that the genetic diversity could be maintained in field gene bank and new genetically rich material may be enriched through the collections from other locations for future use and conservation.

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