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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF RAPD AND ISSR IN CHARACTERISATION OF GEOPHILA REPENS L.

***V.R. Sumitha[1], A.Gangaprasad[2] and G.M. Nair[3].**

1. Department of Botany, Mahatma Gandhi College, T.V.P.M. Kerala. Pin-695 004.
2. Department of Botany, University of Kerala, Kariavattom Campus, T.V.P.M. Kerala. Pin- 695581.
3. Inter-University Centre for Genomics and Gene Technology, Department of Biotechnology, University of Kerala, Kariavattom Campus, T.V.P.M. Kerala. Pin- 695581.

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****Corresponding Author***

V.R. Sumitha.

Abstract

Geophila repens, of the family Rubiaceae, is used as a hepatoprotectant in the folk medical practices in Thiruvananthapuram district, Kerala,India. This plant is also reported to possess several other medicinal properties. Being restricted to specific pockets in under covers of forest areas, the accessibility to this plant is quite difficult. Further, due to scarcity in distribution there is a threat to its existence due to over exploitation, urbanization and industrialization. In order to formulate effective conservation strategies for frequently exploited plants, assessment of its genetic diversity and population structure is urgent. Genetic diversity among accessions of a plant can be analysed using various marker techniques. Molecular characterization of the accessions of *G. repens* was done using RAPD and ISSR markers. Random amplified polymorphic DNA (RAPD) markers and ISSR markers are widely applicable because they are rapid, inexpensive, simple to perform, do not require prior knowledge of DNA sequence and require very little starting DNA template. A total of 11 accessions of *G. repens* were collected from various localities, out of which 10 accessions were from Kerala and one from Andaman islands. RAPD analysis was carried out using a set of 20 decamer primers. ISSR analysis was also carried out in *G. repens*. The analysis was carried out using a set of 12 primers. The effectiveness of the molecular markers used for the characterization of *Geophila* was analysed at various levels like polymorphism, Polymorphism information content (PIC) and resolving power (Rp) and primer index values in the case of DNA based markers. This reveals the efficiency of ISSR marker system compared to RAPD.

Introduction:-

The assessment of genetic diversity and DNA fingerprinting of germplasm are of paramount importance in medicinal plant research for the maintenance, utilization and further acquisition of the germplasm resources. It is has important consequences in practical applications like conservation of genetic resources (Sarikamis *et al*., 2010) and in population and evolutionary genetic studies (Cheema *et al*., 2010). DNA-based assays have revolutionized and modernized our ability to characterize genetic variation. Random amplified polymorphic DNA (RAPD) markers and ISSR markers are two molecular typing approaches that have been used to detect variation among plants. These methods are widely applicable as they are rapid, inexpensive, simple to perform, do not require prior knowledge of DNA sequence and require very little starting DNA template (Esselman *et al*., 1999).

Geophila repens L. is a small slender prostrate herb with rooting at nodes and having medicinal properties in the family Rubiaceae. It is used in the treatment of diarrhoea, sore, earache, cough and intestinal ailments (Phytochemical database, 2005) and in traditional medicinal practices of the tribals of Thiruvananthapuram, Kerala, India, as a guarded drug to combat severe jaundice and other liver ailments. *G. repens*, not uniformly distributed, is seen only in specific niches in the undercover of moist deciduous forests, about 2000-5000ft above the sea level (Beddome, 1996). Being restricted to specific pockets in Western Ghats and Eastern Ghats forest areas and in the forest under covers in Assam and Andaman islands, the accessibility to this plant is quite difficult. Further, due to scarcity in distribution there is a threat to its existence owing to over exploitation and dwindling nature of forest due to rapid urbanization and industrialization. PCR based markers have been used to characterize a wide range of plant species, however no such reports are available with *Geophila repens*.

Materials and methods:-

A total of 11 accessions of *Geophila repens* (Fig.1.) were collected from various localities, out of which 10 accessions were from Kerala and one from Andaman islands (Table 1.) DNA based molecular markers i.e., RAPD and ISSRwere used for haracterisation of the acessions.

For DNA isolation, young and fresh leaves were taken from the collected plants. RAPD analysis was carried out using a set of 20 decamer primers (Vision Scientific, India), and ISSR analysis was carried out using a set of 12 primers (Finnzymes, Bangalore). The data obtained were analysed satistically so as to determine the relationship among accessions. All the analysis were expedited using the software package NTYSYS Pc 2.0 (Rohlf, 2002).

Fig.1: Habit of G. Repens.

Table 1: Accessions of *Geophila repens* collected from different localities.

No.	Location	Collection site	Acc. Code	Acc. No.
1	Palode	TBGRI gene bank	Tg	KUBH 5588
2	Kulathoopuzha	Dalikarikkakam	DK	KUBH5590
3	Anchal	Kadamankode	Kd	KUBH5592
4	Kollam	Mukkada	Md	3157
5	Kottayam	Uzhavoor	Ur	KUBH5595
6	Perumbavoor	Iringol	Il	KUBH5596
7	Ernakulam	Thattekkad	Td	3024
8	Trichur	Peechi	P	KUBH5598
9	Palakkad	Dhoni forest	Dh	KUBH5599
10	Kannur	Mattanoor	Mr	KUBH5600
11	Andaman islands	Dhania khadi	An	2801

The data obtained were analysed by considering each accession as an operational taxonomic unit (OTU). The1/0 matrix was prepared for all the scored bands pertaining to corresponding marker systems for further analysis of data. The total number of bands, number of polymorphic bands and average number of bands per primer were calculated. The binary data obtained from the marker systems were analysed individually using Dice's coefficient (Dice, 1945) to determine pair wise comparison to estimate the genetic relationship among the accessions of *G. repens*. Using the distance matrix, a sequential, agglomerative, hierarchical and nested (SAHN) cluster analysis was preformed with Unweighted Pair Group Method Arithmetic average (UPGMA) algorithm.

Estimates of the differences between the dendrograms based on RAPD and ISSR markers were assessed by computing the cophenetic values and constructing cophenetic matrix for each primer set. These cophenetic matrices were compared using Mantel's test for matrix correspondence (Mantel, 1967; Liedloff, 1999) using the package 'ade4' in R software. Eigenvalues and eigenvectors were calculated by the Eigen programme using a correlation matrix as input and 2D plot were used to generate the two-dimensional PCA plot from the software. Multivariate relationships among OTUs were estimated through principal component analysis (PCA) and parallel analysis of distances. The information content for the marker systems, were calculated for each locus and marker using Polymorphism Information Content (PIC) (Lynch and Walsh, 1998). Resolving power (Rp) was calculated to reveal polymorphism and distinguish between genotypes.

Results:-

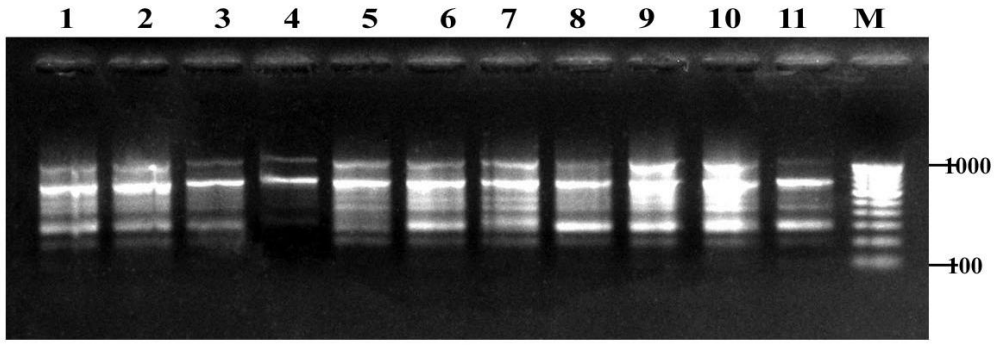
RAPD analysis:-

The 20 RAPD primers gave reliable amplification profile for obtaining the genetic relationships among 11 accessions of *Geophila repens* (Fig. 2.). The banding profiles generated by the primers yielded a total of 163 amplification products of which 98 bands were polymorphic and the remaining 65 were monomorphic. The percentage of polymorphism exhibited by the accessions is 60.12%. Polymorphism revealed by each primer ranged from 2-9 with an average number of 8.4 bands per primer.

The distance matrix generated in RAPD on the basis of Dice coefficient revealed high level of genetic similarity among the accessions though there is no 100% similarity (Table 2.). The pair wise genetic similarity ranged from 0.78 to 0.95. The similarity matrix was subjected to UPGMA clustering and a dendrogram was obtained (Fig. 3). The dendrogram analysis indicates that the 11 accessions are grouped together into two major clusters. Highest value for genetic similarity (0.95) was estimated between accessions 6 (Il) and accession 9 (Dh) and between accessions 8 (P) and 10 (Mr). These accessions were seen clustered together in the dendrogram, in two subclusters respectively. Lowest estimated value (0.78) is between accession 1 (Tg) and accession 4 (Md).

Associations among the accessions were also revealed by a two dimensional PCA based on the same set of matrix. Overall, the grouping pattern from PCA corresponded well with the clustering pattern of the dendrogram (Fig. 4.). The percentage variability accounted by the first PC was 52.13%, 10.05% for the second principle component and 8.001 for the third PC. The three components together showed a cumulative variance of 70% and the Eigen values ranged from 5.73 to 0.88. (Table 3.).

Fig. 3.22-3.24. RAPD patterns generated from the genomic DNA of 11 accessions of *Geophila repens*. The lanes contain accessions in the order same as that in Table 3.1. 'M' is the marker ladder



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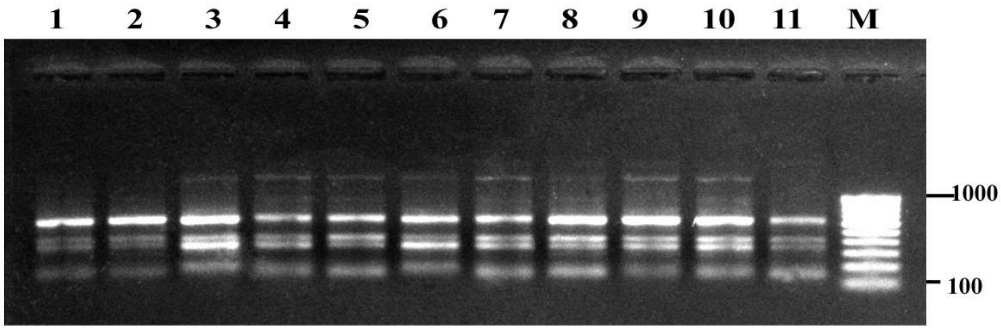

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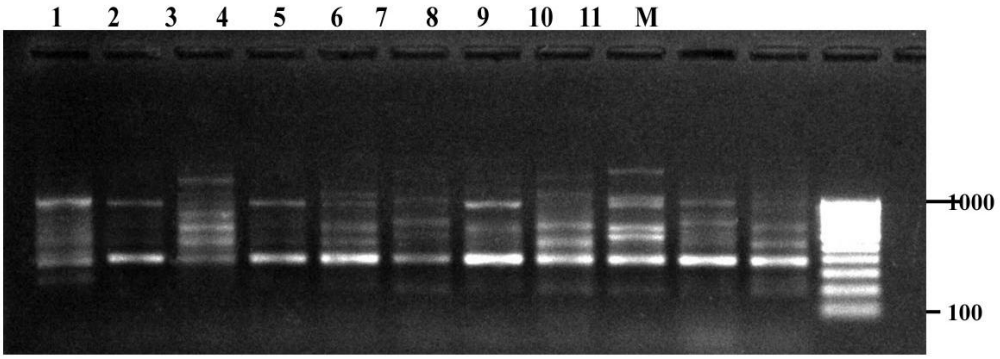


S150 (44.

Table 2: Similarity matrix obtained from the RAPD data of eleven accessions of *Geophila repens* using Dice's coefficient.

Accession	Accession										
	Tg	Dk	Kd	Md	Ur	Il	Td	P	Dh	Mr	An
Tg	1.00	0.86	0.83	0.78	0.80	0.83	0.81	0.81	0.81	0.81	0.79
Dk		1.00	0.83	0.83	0.85	0.88	0.86	0.84	0.86	0.84	0.84
Kd			1.00	0.85	0.87	0.89	0.87	0.86	0.88	0.87	0.84
Md				1.00	0.91	0.87	0.91	0.85	0.87	0.87	0.85
Ur					1.00	0.90	0.94	0.91	0.92	0.91	0.86
Il						1.00	0.94	0.93	0.95	0.92	0.91
Td							1.00	0.94	0.94	0.94	0.91
P								1.00	0.94	0.95	0.89
Dh									1.00	0.94	0.90
Mr										1.00	0.90
An											1.00

Table 3: Eigen value, percentage of variability and accumulated variability for RAPD analysis.

PCos	Eigen value	Percentage Variability	Cumulative percentage
1	5.734	52.129	52.129
2	1.105	10.045	62.173
3	0.88	8.001	70.175

Fig 3. Dendrogram based on the RAPD matrix in accessions of *Geophila repens*

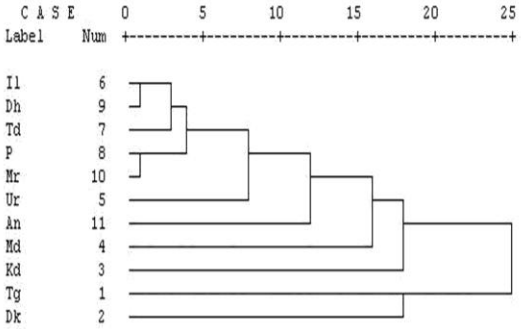
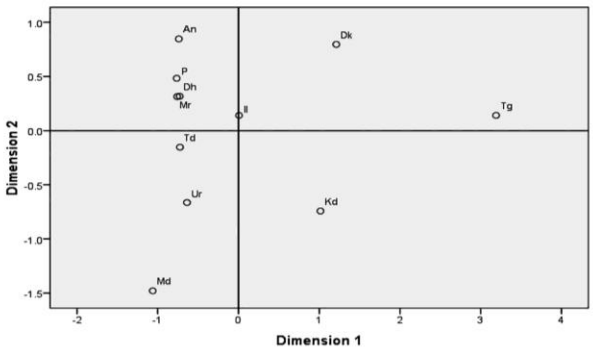


Fig 4. Principal component analysis showing the relationships among *Geophila repens* based on RAPD data



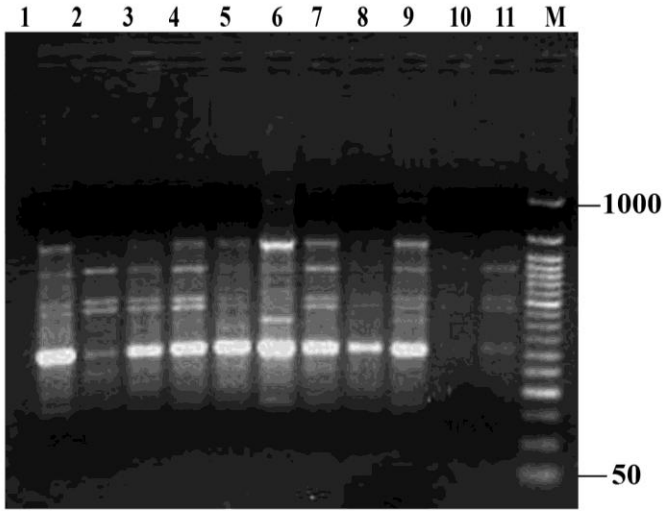
ISSR analysis:-

ISSR analysis was carried out on 11 OTUs using 172 amplicons from 12 primers (Fig. 5.). The polymorphic fragments produced per primer ranged from 7-19 with an average of 14.33 bands per primer. Out of the total 172 amplicons produced by the primers 156 were polymorphic and 16 monomorphic revealing a polymorphism of 90.69 %.

Statistical analysis revealed high level of genetic similarity among the accessions (Table 4). The pair wise genetic similarity ranged from 0.521 to 0. 778. Greater similarity was estimated between accession 6 (Il) and accession 7 (Td), which had highest value 0.778. Lowest value for genetic similarity (0. 521) was estimated between accessions 1 (Tg) and accession 10 (Mr). A tree diagram of the accessions was obtained when the similarity values were subjected to UPGMA clustering where the 11 accessions were grouped into two clusters (Fig. 6.).

Principle component analysis was carried out to confirm the result obtained by the cluster analysis regarding the association among the accessions (Fig. 7.). The positioning of accessions in the scatter plot was almost similar to that of cluster analysis, with minor differences in the spatial arrangement of the accessions. The percentage variability accounted by the PC ranged from 29.47% to 9.93%. The two components together showed a cumulative variance of 53.53% and the Eigen values ranged from 3.24 to 9.93. (Table 5).

Fig 5. Representative figures showing amplification products revealed by ISSR primers. The lanes contains accessions of *G. repens* in the order same as that in table 1. 'M' is the marker ladder.



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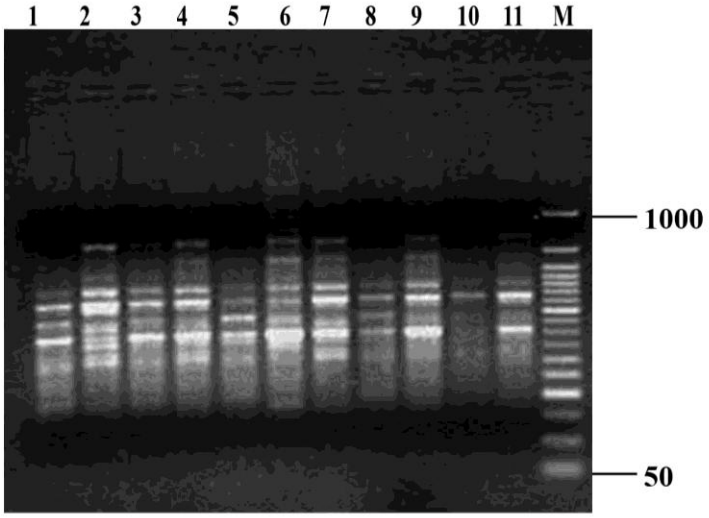

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Table 4: Similarity matrix of ISSR data using Dice coefficient in eleven accessions of *Geophila repens*.

Accession	Accession										
	Tg	Dk	Kd	Md	Ur	Il	Td	P	Dh	Mr	An
Tg	1.000	.735	.689	.735	.686	.652	.658	.606	.623	.521	.593
Dk		1.000	.772	.739	.677	.661	.667	.667	.680	.636	.631
Kd			1.000	.759	.700	.637	.660	.639	.654	.575	.615
Md				1.000	.757	.767	.760	.683	.667	.615	.660
Ur					1.000	.705	.723	.730	.700	.603	.663
Il						1.000	.778	.679	.742	.624	.676
Td							1.000	.696	.734	.608	.683
P								1.000	.732	.635	.674
Dh									1.000	.641	.687
Mr										1.000	.725
An											1.000

Table 5: Eigen value, percentage of variability and accumulated variability for ISSR analysis.

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	3.242	29.473	29.473
2	1.555	14.135	43.608
3	1.092	9.925	53.533

Fig 6. Dendrogram based on the ISSR data in accessions of *Geophila repens*

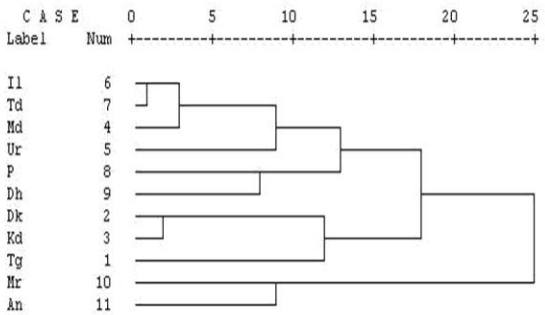
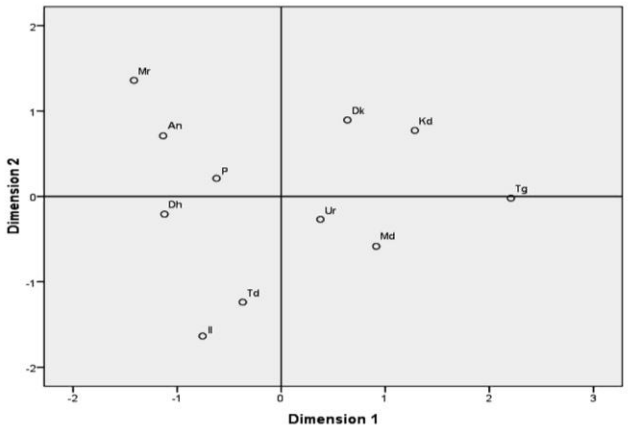


Fig 7. Principal component analysis obtained using ISSR data showing the relationships among accessions of *Geophila repens*



Effectiveness of different marker systems in characterization of accessions of *G. repens*:-

The effectiveness of the molecular markers used for the characterization of *G.repens* was analysed at various levels like polymorphism, Polymorphism information content (PIC) and resolving power (Rp) and primer index values. The polymorphism level between the markers was calculated as the ratio of the number of bands present to the total number of bands. The markers showed a lesser polymorphism of 60.12% for RAPD and 90.69 % for ISSR markers. The percentage polymorphism exhibited by ISSR markers were very high compared to the RAPD markers. The average number of polymorphic bands per primer was also high for ISSR analysis with an average of 14.33 bands per primer against 8.4 bands per primer for RAPD. This reveals the efficiency of ISSR marker system compared to RAPD.

The Polymorphism Information Content (PIC) value is often used to measure the informativeness of a genetic marker for linkage studies. The RAPD and ISSR markers showed a range of PIC scores among the primers used (Table 6). The PIC score for RAPD ranged from 0 to 0.843. Maximum PIC score was observed for the primer S 127, which gave the minimum number of amplicons. In the case of ISSR markers the PIC score ranged from 0.426 to 0.769. Maximum PIC score was observed in primer 843 which showed 100% polymorphism.

Table 6: Polymorphism information content (PIC) revealed by DNA based marker systems.

	RAPD		**ISSR**	
	Primer code	PIC	Primer code	PIC
	S113	0.3684	809	0.5500
	S105	0.0000	811	0.6216
	S104	0.3278	816	0.4263
	S119	0.3492	818	0.5589
	S162	0.0902	823	0.6560
	S150(68.8)	0.2107	834	0.5914
	S150(50.5)	0.5289	835	0.7331
	S164	0.2452	836	0.6057
	S182	0.2184	840	0.5275
	S166	0.5179	843	0.7698
	S187	0.4472	845	0.5382
	S183	0.2269	847	0.4744
	S143	0.3339		
	S142	0.3099		
	S127	0.8430		
	S150(44.3)	0.6416		
	S193	0.0967		
	S191	0.3447		
	S190	0.1839		
	S196	0.0630		

Resolving power (Rp) was analysed to measure the value of the primer used in DNA marker analysis. In the case of RAPD primers, maximum Rp value was recorded for the primer S162 (10.46) and the least value of 1.18 for the primer S 150 (50.5) (Table 7). In ISSRs the Rp values of the primer is slightly different from that of RAPD. The Rp values of the ISSR primers ranged from 3 (843) to 13.82 (847). The average Rp value of RAPD primers is 6.32 and that of ISSR primers is 8.42. Primer index values differed considerably in the case of RAPD and ISSR systems. The primer index value for RAPD (RPI) ranged between 0.298 and 3.21 with a mean value of 1.41. For ISSR the primer index value (SPI) ranged from 2.08 to 7.5 with an average of 4.97.

Table 7: Resolving power and (Rp) and Primer index of RAPD and ISSR primers in *Geophila repens*.

RAPD			ISSR		
Primer code	Rp	RPI	Primer code	Rp	SPI
S113	5.182	2.579	809	5.091	4.95
S105	7	0	811	7.727	8.702
S104	6.727	2.95	816	8.272	5.116
S119	6.364	2.793	818	10	8.942
S162	10.455	0.992	823	4.091	5.248
S150(68.8)	5.182	1.265	834	11.091	10.665
S150(50.5)	4.545	4.231	835	9.091	14.661
S164	5.091	1.471	836	8	8.479
S182	6.182	1.529	840	11.546	9.496
S166	5	4.661	843	3	5.388
S187	6	4.025	845	9.364	8.612
S183	8.364	2.496	847	13.818	9.488
S143	7.455	3.339			
S142	6.546	2.479			
S127	1.182	2.529			
S150(44.3)	5.456	7.058			
S193	9.182	0.967			
S191	5.546	2.413			
S190	7.091	1.471			
S196	7.727	0.504			

Discussion:-

Characterization of 11 accessions of *G. repens* was carried out by examining the data obtained from DNA based markers like RAPD and ISSR to assess the level of genetic similarity among the accessions. Genetic characterization of natural resources is an essential step for a better understanding of genetic resources for the implementation of in situ and ex situ conservation activities (NBPGR, 2000). Molecular data can be of great value to conservationists and it is possible to investigate directly many issues of concern such as gene pool fragmentation and genetic erosion (Falk and Holsinger, 1991). Molecular techniques are powerful tools to reveal polymorphisms at the DNA level and for characterization as RAPD and ISSR primers can resolve the genetic diversity more precisely. DNA fingerprinting of medicinal plants is a necessity for generating a molecular database and since morphology of plants are dependent on interactions between genes and environment, it makes all the more imperative to use molecular markers to catalogue *Geophila repens* accessions based on the geographical variations.

The random amplified polymorphic DNA technology (Williams *et al*., 1990) is the most appropriate and convenient technique in genotype fingerprinting (Kumar *et al*., 2006). Being multilocus (Karp *et al*., 1997) it is the simplest and fastest detection technology for diversity analysis. In RAPD analysis accessions of *G. repens* were found to be highly similar revealing a polymorphism of about 60% only. In this analysis only two primers gave full polymorphism and a single primer was found to be monomorphic. Similarity matrix obtained using Dice coefficient gave pair wise genetic similarity values ranging from 0.95 to 0.78. Dendrogram obtained using these similarity values, showed greater similarity between accession 6 and accession 9 and also between accessions 8 and 10 as depicted by the matrix. The similarity values obtained with the RAPD data were higher, which was also evident from the gel. Almost similar pattern of bands were obtained among the accessions in the gel when RAPD was done using 20 arbitrary primers. The result is on par with the RAPD analysis of *Typhonium* sp. by Rout (2006) and in *Ixora* sp. (Rajaseger *et al*., 1997).

The clustering of accessions in the dendrogram based on the similarity matrix is supported by a PCA scatter plot. Results obtained from the principal component analysis correspond well with the grouping of accessions based on cluster analysis with the exception of minute rearrangements. A higher similarity would mean more genetic relatedness among the accessions within the group. Three principal components obtained accounted for 70% of the total RAPD variation (52.13%, 10.05% and 8.001, respectively). Though these components explained higher cumulative variation, the results of the PCA were generally consistent with those obtained through the clustering analyses.

RAPD technique is advantageous as it can yield a large number of loci and may provide a more representative sample of the genome than proteins and allozymes do. RAPD markers are even more abundant because numerous random sequences can be used for primer construction. However, the RAPD technique has also some limitations. The dominant allelic expression will bias the estimates of genetic diversity and population genetic structure (Lynch and Milligan, 1994; Isabel *et al*., 1993; Szmidt *et al*., 1996). But detailed analyses of qualitative and quantitative phenotypic variation are still necessary in plant systematic studies (Hörandl, 2002). DNA markers, especially those based on microsatellites, are useful in assessing a large number of accessions, quickly and reliably. Inter-simple sequence repeat–PCR (ISSR–PCR) is a simple, cost-efficient, robust, multilocus marker method which is extremely useful in determining genetic variability (Reddy *et al*., 2002).

Similarity assessment using ISSR:-

ISSR- PCR is a microsatellite based multilocus marker technique, which is simple and useful for estimating genetic diversity in several crop plants. The technique is similar to RAPD but is advantageous showing higher level of polymorphism, reproducibility and cost effectiveness which is advantageous when differentiating closely related cultivars, because variable regions in the genome are targeted (Hantula *et al*., 1996; Goula˜o and Oliveira, 2001, Chowdhury *et al*., 2002). In addition, ISSR markers are designed to anneal to a microsatellite sequence and are longer than RAPD primers, allowing higher annealing temperatures to be used. DNA markers, especially those based on microsatellites, are useful in assessing a large number of accessions, quickly and reliably (Gupta and Varshney, 2000).

ISSR analysis was carried out using 12 primers to characterize the accessions of *Geophila*. The primers revealed a polymorphism of almost 91% and about 4 primers gave 100% polymorphism. Higher level of polymorphism (94.3 %) using ISSR markers was reported in seven populations of *Pongamia pinnata*, from three different eco-geographical regions of Orissa (Sahoo *et al*., 2009). Similarity values among accessions ranged between 52-78% and the accessions were grouped based on the similarity matrix. The grouping of accessions in the cluster is supported by a high bootstrap value. The association among the accessions as revealed by the dendrogram is substantiated by the scatter plot obtained by PCA analysis. The placement of accessions in the scatter plot is similar to that in the dendrogram with a minute exception. There were only minor differences in the spatial arrangement of the accessions in the scatter plot. Accessions 8 and 9 grouped together in cluster A was seen located near accessions 10 and 11, were grouped in cluster B. The similarity value also supports the scatter plot. The principal components accounted for 53.53% (29.47% to 9.93%) of the total variation accounted by ISSR. The results obtained in PCA were generally consistent with those obtained in the cluster analyses.

ISSR markers are preferred more than RAPD for the assessment of genetic characterization as they amplify large number of DNA fragments per reaction, representing multiple loci across the genome (Kaushik *et al*., 2003). In the present study higher polymorphism was observed using ISSR marker system compared to RAPD. The difference may be due to difference in the methods used to characterize the DNA. The difference could be explained in terms of functional constraints since some of the RAPD bands are concerned with functional loci (Penner, 1996). Further, in RAPD, polymorphism occurs mainly due to point mutation or insertion- deletion mechanisms, whereas slippage accounts for the polymorphism in ISSR (Milbourne *et al*., 1997).

Comparison between the markers:-

Both RAPD and ISSR were compared using polymorphism information content (PIC), resolving power (Rp) and primer index (PI). PIC scores for a primer represent the gene diversity of a specific locus and can be used to evaluate markers so that the most appropriate marker can be selected for genetic mapping and phylogenetic analysis (Anderson *et al*., 1993; Powell *et al*., 1996). The probability of obtaining greater polymorphism using a primer is high when the PIC value for that particular primer is high (Rana and Bhatt, 2004). In the present study RAPD markers showed a mean PIC value of 0.317 and ISSR had mean PIC value of 0.588. Lower PIC value was obtained for RAPD markers denoting lesser variability among the accessions. This was also evident from the banding pattern.

Resolving power (Rp) and primer index (PI) were calculated for RAPD and ISSR primers to analyze the effectiveness of each marker in detecting polymorphism. These two techniques measure the ability of the primers to distinguish between the genotypes by measuring the polymorphism obtained. The resolving power and primer index were comparatively higher for ISSR (Rp= 8.42 SPI= 4.97) than RAPD (Rp= 6.31, RPI= 1.41) highlighting the importance of ISSR markers in the identification and characterization of species. The efficiency of ISSR

markers in the detection of polymorphism is well established (Liu and Wendel, 2001; Bolibok *et al*., 2005). The polymorphism in banding pattern was also higher for ISSR compared to RAPD. The grouping of accessions was also different among the markers. All these techniques reveal variable results which reveal information about distinct and different regions of the genome.

However considerable variation was noted in the band polymorphism and clustering pattern of accessions in the dendrogram obtained from the individual data analysis of the marker systems. The two DNA based markers gave varying clustering patterns and no shared clustering was detected. It has been shown that different markers might reveal different class of variation and the difference obtained may be attributed to the different character and properties of RAPD and ISSR loci (Fernandez *et al*., 2002; Hou *et al*., 2005). The differences found among the dendrogram generated by RAPDs and ISSRs could be partially explained by the different number polymorphim of loci obtained, reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships as observed by Loarce *et al*. (1996) in barley. Another explanation could be the low reproducibility of RAPDs (Karp *et al*., 1997). The similarity values obtained in ISSR analysis (0.52 to 0. 78) were low compared to that of RAPD (0.95 to 0.78). The ability of the ISSR markers to detect greater polymorphism was reported by many authors (Fernandez *et al*., 2002; Hou *et al*., 2005; Bhuyan *et al*., 2007). In all the dendrograms, accessions were found clustered distinctly. The genetic similarity of these genotypes is probably associated with their similarity in the genomic and amplified region. Similar results of distinct dendrogram pattern for RAPD and ISSR markers were reported in Artemisia annua (Kumar *et al*., 2011). The ISSR markers target regions between microsatellite loci distributed across the genome, while the RAPD markers scan the entire genome and, hence, genome-wide genetic variation could be detected by the use of DNA-based marker systems (Pamidiamarri *et al*., 2009).

At higher similarity levels, clusters sub group into small clusters. Accessions collected from same regions as well as different regions in Kerala and the one collected from Andaman islands, were in closely formed groups which clearly indicates that the geographic differentiation of the accessions of *Geophila repens* is not extensive. The accessions, named after their respective specific locations of collection, were gathered from the different parts of the state of Kerala, a probable reason why they have very little variability. Clustering of cultivars from same or nearby region was also reported by Shashidhara *et al*. (2003). Steiner and Santos (2001) noted that geographic distance among the collection site is not associated with plant genetic distance but ecologic similarity is related to genetic similarity. The accession collected from Andaman islands also showed greater similarity to accessions collected from different areas of Kerala which may be due to similar environmental conditions.

Genetic diversity is critical for adaptation to environmental changes and for long term survival of a species. Lower level of variation is effected when the plant population undergoes increased selfing, thereby leading to homozygosity (Ellstrand and Elam, 1993). Small population size increases the level of inbreeding and genetic drift, thereby reducing genetic variability. So in species with small range and reduced number of individuals, low levels of variability are expected (Barret and Kohn, 1991). There is a prevalent view that rare species are genetically not very diverse. Habitat fragmentation has several genetic consequences, such as erosion of genetic variability and increased inter-population divergence (Young *et al*., 1996), depending on several factors such as size of the remnant subpopulations, distance and connectivity (Saunders *et al*., 1991). Models of the effect of habitat fragmentation on population genetic structure indicate that increased isolation of populations will decrease gene flow and increase genetic differentiation among populations (Fore and Guttman, 1992; Husband and Barrett, 1996). Founder effects in newly established populations, accompanied by limited gene flow between populations, may further reduce the genetic variation within a population, thus constraining the adaptive flexibility of the population and potentially contributing to reduction in fitness (Williamson and Werth, 1999). In the present study reduced variability is observed among the accessions of *Geophila repens*. The plant is not widely distributed and is seen only in the forest undercover where there is less sunlight and more humidity. Also in the forest areas they are seen only as specific patches where the above conditions are there. Fragmentation of continuous habitat into smaller and more isolated patches can potentially alter the spatial distribution of genetic diversity (Gilpin and Soule, 1986; Lande, 1988; Barrett and Kohn, 1991; Fenster and Dudash, 1994). Reduced variability may be the reason for scanty distribution of this species.

Conclusion:-

In the present study, RAPD and ISSR markers were used to analyse the genetic relatedness among 11 accessions of *G. repens*. Both the marker systems revealed higher level of similarity among the accessions of *G. repens*, when the banding patterns obtained were analysed statistically. This lack of variability may be the reason for the lack of adaptability of the species to thrive even in slightly varied environmental conditions. Hence effective measures have to be taken for the conservation and multiplication of this medicinal plant as habitat destruction may create threat to the existence of this valuable species.

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