Abrahamia 1 (1 & 2): pages 100-116 (2015)
An International Journal of Plant Sciencs

Similarity Assessment among Accessions of *Geophila repens* L. Using Morphological Markers

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ABSTRACT

Geophila repens L. of the family Rubiaceae is a prostrate herb having medicinal properties, found in the forest areas of Western Ghats, Eastern Ghats, Assam and Andaman islands. This plant is used in the traditional medicinal practices as a drug to combat severe jaundice and other lever ailments. *Geophila* is a totally under explored herb with respect to its active compounds and its action. Further the plant is not widely distributed and is seen only in the forest undercover only in specific patches where there is less sunlight and high humidity. The genetic relationship among 11 accessions of *Geophila repens* were analysed using 46 qualitative and 32 quantitative morphological characters. Statistical analysis of the quantitative characters showed maximum similarity among the accessions. *Geophila repens* collected from different regions in Kerala and from Andaman islands, were in closely formed groups which clearly indicated minimum geographic differentiation of the accessions.

Key words : Dendrogram, *Geophila repens,* proximity matrix, qualitative characters, quantitative characters

INTRODUCTION

Assessment of genetic diversity within a plant population has important consequences in conservation of genetic resources (Sarikamis *et al.,* 2010). Measurement and characterization of genetic diversity had always been a primary concern in population and evolutionary genetic studies (Cheema *et al.,* 2010). Various numerical taxonomic techniques have been successfully used to classify variation patterns at both intra-and inter-specific levels (Ariyo, 1991; Virk *et al.,*

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2000, Campbell *et al.,* 2009). The genetic diversity between plant genotypes can be estimated either by determining their level of polymorphism for genetic markers or by analysis of morphological traits.

Morphological characterization is a conventional technique used for evaluating diversity among plant populations. Morphological markers have been used to Identify varietal genotype and genetic purity based on the assessment of phenotypic characteristics (Bayorbor *et al.,* 2010). Morphological characters constitute basic information for plant systematics. Prior to the development of molecular markers, genetic characterization was mainly carried out using morphological characters (Patterson and Weathercup, 1984: Mignouna *et al.,* 1996). Morphological characters like growth habit, leaf morphology and floral characters have been used to define the taxonomic status. Polygenic morphological traits also serve as genetic markers for various plant germplasm management and taxonomy.

Traditional diagnostic keys for naming taxa based on morphological studies have long played a fundamental role with regard to practical biological identification. Morphological variation not only reflects the genetic make-up (genotype) of an organism, but also of the influence of particular environment on gene expression or the phenotype (De Vienne *et al.,* 2003).

Geophila repens (Fig. 1.), not uniformly distributed and is seen only in specific niches in the undercovers of moist deciduous forests, about 2000-5000ft above the sea level (Beddome, 1996). This plant is used in traditional medicinal practices by the tribals of Thiruvananthapuram as a guarded drug to combat severe jaundice and other liver ailments. Though with great medicinal potential, *G. repens* is a totally under explored herb with respect to its active compounds and its activity. Being restricted to specific pockets in the forest areas of Western Ghats, Assam and Andaman islands, the accessibility to this plant is quite difficult. Further, there is a threat to its existence due to scarcity in distribution, over exploitation and dwindling nature of forest areas due to rapid urbanization.

Fig.1. *Geophila repens*

MATERIALS AND METHODS

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 (Table 1). Morphological analysis of 11 accessions of the plant was carried out using 78 morphological characters- 46 qualitative and 32 quantitative characters (Tables 2 and 3), selected according to the plant characteristics. For each character 10 samples were analysed from each accession. The qualitative foliar characters were studied following the classification of leaf characters by Hickey (1973). The colour of the lamina was determined by comparing with the Colour Chart by Wilson (1938, 1941). The data were recorded and tabulated for further analysis. The data were subjected to statistical analysis using the software package NTSYS Pc 2.0 (Rohlf, 2002). Statistical parameters such as variability, analysis of variance, heritability and genetic gain were calculated. The morphological characters analysed from the 11 accessions were pooled together, standardized and subjected to Hierarchical Cluster Analysis (Johnson and Wichern, 2001) employing the Average Linkage Study using NTSYS. A Proximity Matrix was prepared by calculating the Squared Euclidean Distances between accessions and a dendrogram was constructed illustrating the closeness of the relationship between the accessions.

RESULTS

Morphological data concerning 46 qualitative characters and 32 quantitative characters were collected from 11 accessions of *G. repens.* The qualitative morphological characters were uniformly expressed in all the members studied and hence were not subjected to statistical analysis. Of the 32 quantitative characters studied, statistical analysis was restricted to 26 morphological characters which showed variation among eleven accessions. The remaining six characters such as number of stipules, number of bracts, number of calyx lobes, number of corolla lobes, number of stamens and number of stigma remained constant in all the accessions collected.

Table 1. Accessions of *G. repens* collected from different localities

Sl. No.	Location	Collection site	Acc. Code	Acc. No.
1	Palode	TBGRI gene bank	Tg	KUBH 5588
2	Kulathoopuzha	Dalikarikkakam	DK	KUBH 5590
3	Anchal	Kadamankode	Kd	KUBH 5592
4	Kollam	Mukkada	Md	KUBH 3157
5	Kottayam	Uzhavoor	Ur	KUBH 5595
6	Perumbavoor	Iringol	Il	KUBH 5596

7	Ernakulam	Thattekkad	Td	KUBH 3024
8	Thrissur	Peechi	P	KUBH 5598
9	Palakkad	Dhoni forest	Dh	KUBH 5599
10	Kannur	Mattanoor	Mr	KUBH 5600
11	Andaman islands	Dhania khadi	An	KUBH 2801

Table 2. List of qualitative characters selected for morphological analysis of *G. repens*

Sl. No.	Characters selected	Character Code	Sl. No.	Characters selected	Character Code
1	Distribution	Dn	24	Calyx surface	Clsr
2	Habit	Ht	25	Calyx longevity	CII
3	Stem surface	Ssf	26	Calyx colour	Cle
4	Presence of stipule	Stp	27	Corolla shape	Cos
5	Type of stipule	Stt	28	Corolla colour	Coc
6	Shape of stipule	Stsp	29	Corolla dorsal surface	Cod
7	Nature of petiole	Npt	30	Corolla ventral surface	Cov
8	Petiole surface	Pts	31	Uniformity in stamen length	Slu
9	Leaf colour	Lc	32	Anther colour	Anc
10	Leaf shape	Ls	33	Anther shape	Ans
11	Leaf texture	Lt	34	Filament colour	Flc
12	Leaf surface	Lsr	35	Filament nature	Fln
13	Leaf margin	Lm	36	Heterostyly	Ht
14	Leaf apex	La	37	Style colour	Syc
15	Leaf base	Lb	38	Style surface	Sys
16	Equality of leaf base	Lbe	39	Stigma colour	Sgc
17	Venation	Vn	40	Stigma type	Sgt
18	Inflorescence type	Int	41	Fruit shape	Fts
19	Peduncle surface	Pns	42	Fruit colour	Ftc
20	Pedicel surface	Pds	43	Fruit surface	Fts
21	Bract (present/absent)	Bt	44	Seed type	Sdt
22	Shape of bract	Bts	45	Seed colour	Sdc
23	Shape of calyx	Cls	46	Seed shape	Sds

The plant is pantropical in distribution (Verdcourt, 1989) and is seen throughout the Western Ghats. The plant is a creeping herb with pubescent stem. Leaves are reniform, simple, opposite, petiolar and stipulate. Petiole is appressed and pubescent, stipules are connate and inter petiolar, characteristic to the family. The leaves are spinach green in colour, chartaceous and glabrous with entire margin and campylodromous venation.

The leaf apex is variable, sometimes rounded, acute retuse or mucronate and leaf base is cordate and symmetrical. Inflorescence is a simple cyme, 1-3 flowered. flowers actinomorphic and pentamerous. Peduncle and pedicel pubescent, bract lanceolate and pubescent, green obconic. Calyx is pubescent and persistent. Corolla hypocrateriform and offwhite in colour with pubescent ventral surface and glabrous dorsal surface. Anthers 5 in number of uniform length, dorsifixed and sagitate. Both anthers and filament white in colour. Style homostylous with white bifid stigma. Fruit capsicum red in colour, globose and glossy. Seed straw coloured and pyrene and widely ovate in lateral view.

Table 3. List of quantitative characters selected for morphological analysis of *G. repens*

Sl. No.	Characters selected	Character Code	Sl. No.	Characters selected	Character Code
1	Internode length	Il	17	Length of calyx lobe	CLI
2	Petiole length	Pl	18	Length of corolla	Col
3	Lamina length	Ll	19	Number of corolla lobes	Con
4	Lamina breadth	Lb	20	Length of corolla lobes	Coll
5	Lamina area	La	21	Breadth of corolla lobes	Cob
6	Lamina perimeter	Lp	22	Length of corolla tube	Ctl
7	Lateral vein pair number	Lv	23	Number of stamens	Sn
8	Number of stipules	St	24	Length of anther	Al
9	Length of stipules	Ls	25	Length of filament	Fl
10	Number of flowers/ inflorescence	Fl	26	Height of ovary	Oh
11	Length of peduncle	Pd	27	Length of style	Stl
12	Number of bracts	Bt	28	Number of stigma	Stn
13	Length of bracts	BI	29	Fruit diameter	Fd
14	Pedicel length	PLl	30	Fruit height	Fh
15	Calyx length	Cl	31	Seed length	Sdl
16	Number of calyx lobe	Cn	32	Seed diameter	Sd

Analysis of Statistical Parameters

The mean values of quantitative characters studied from 11 accessions of *G. repens* were calculated. The overall mean, range, critical difference, standard deviation, standard error and variance for each character were analysed. The mean values and the range of character expression did not show much variation among the accessions studied. The genotypic co-efficient of variation (GCV) and phenotypic co-efficient of variation (PCV) were analysed and the details are given in Table 4. Values of GCV and PCV obtained in the present study are less than 30% revealing a low degree of variability among the accessions with regard to the characters studied. Analysis of variance does not reveal significant variation at 5% level of significance for all the quantitative characters suggesting there is not much variation among the accessions in this study. The mean values for the foliar characters showed a higher value.

Heritability for each of the quantitative characters was calculated to determine whether the variability obtained for the quantitative characters are heritable (Table 5). Higher values above 60% showed that the genotypic variance and phenotypic variance are very close, suggesting that the phenotypic variability is mainly due to the genotypic effect and not because of the environmental influence. Values below 60% reveal the influence of the environment on the phenotype. In the present study only five characters namely petiole length, pedicel length, lamina breadth, lamina length and lamina perimeter showed percentage of heritability value above 60. All these showed values just above 60, the highest being 79.77% for petiole length. The lowest value obtained was below 10%, for length of bracts and length of corolla lobes (6.33 and 1.36% respectively).

Table 4. Genotypic and phenotypic co-efficients of variation mean in twenty six quantitative morphological characters of the accessions of *G. repens*

Sl. No.	Character	Genotypic co-efficient of variation(GCV)	Phenotypic co-efficient of variation(PCV)
1	Il	8.26	16.84
2	Pl	41.37	46.32
3	Ll	13.89	17.53
4	Lb	17.25	21.34
5	La	25.11	33.06
6	Lp	13.49	17.11
7	Lv	8.01	13.07
8	Ls	18.73	26.37

Sl. No.	Character	Genotypic co-efficient of variation(GCV)	Phenotypic co-efficient of variation(PCV)
9	Fl	14.93	34.89
10	Pdl	7.66	13.76
11	Bl	3.99	15.86
12	PLl	22.19	27.03
13	CI	5.99	13.08
14	CLl	16.49	25.75
15	Col	4.53	9.07
16	Coll	1.99	17.08
17	Cob	7.97	18.05
18	Ctl	5.63	8.94
19	Al	12.46	24.93
20	FI	10.59	23.48
21	Oh	14.96	42.33
22	Stl	12.98	18.1 I
23	Fd	14.38	20.27
24	Fh	7.46	18.39
25	Sdl	4.46	12.25
26	Sd	6.69	16.13

The study revealed that the variation obtained is not heritable in majority of the characters and the changes if any are due to environmental influence. The genotypic and phenotypic correlations between pairs of quantitative characters were analysed. High correlations above 0.26 and 0.32 suggested high genetic association between the two characters at 5% and I % levels of significance. In the present study all the leaf characters were found to be highly correlated genotypically and phenotypically at both 5% and I % levels. But other characters, no significant correlations were observed and much of the values obtained were negative. The floral characters did not show any correlation with one another and were negatively correlated with all other characters.

Proximity Matrix

The quantitative morphological characters analysed from the 11 accessions were subjected to Hierarchical Cluster Analysis. A proximity matrix was obtained and a dendrogram was generated based on the data obtained. The dendrogram was grouped into two main branches, A and B, which is further divided into four clusters (Fig. 2). Cluster I consisted of five accessions. Cluster II contained two accessions and Cluster III consisted of three accessions. Cluster IV included only a single accession. Cluster I contained accessions 4, 8, 9, 10 and II. The similarity matrix obtained also supported the above result (Table 6).

Table 5. Heritability for quantitative morphological characters of accessions of *G. repens*

Sl No.	Characters	Heritability (%)
1	Petiole length	79.77
2	Pedicel length	67.37
3	Lamina breadth	65.31
4	Lamina length	62.85
5	Lamina perimeter	62.23
6	Lamina area	57.72
7	Length of style	51.37
8	Length of stipules	50.44
9	Fruit diameter	50.33
10	Length of calyx lobe	41.02
11	Length of corolla tube	39.62
12	Lateral vein pair number	37.56
13	Length of peduncle	30.99
14	Length of corolla	25.00
15	Length of anther	24.98
16	Intenode length	24.03
17	Calyx length	20.94
18	Length of filament	20.34
19	Breadth of corolla lobes	19.52
20	Number of flowers/inflorescence	18.30
21	Seed diameter	17.17
22	Fruit height	16.47
23	Seed length	13.27
24	Height of ovary	12.50
25	Length of bracts	6.33
26	Length of corolla lobes	1.36

An 1.00

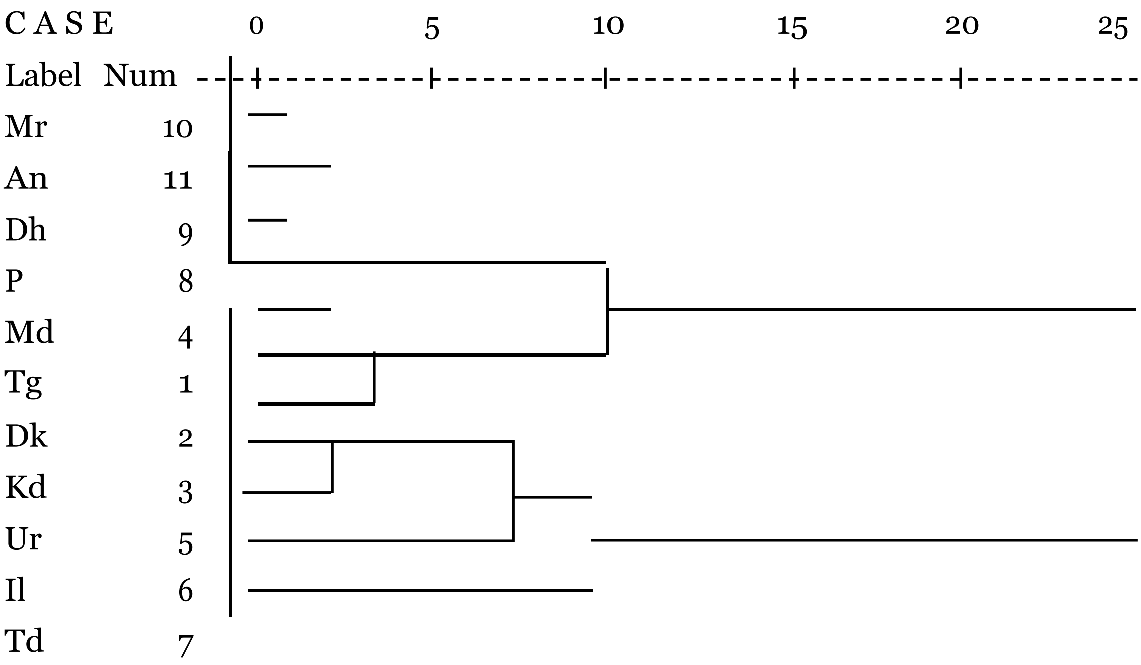

Fig. 2. Dendrogram based on the morphological data among accessions of *G. repens*

Table 6. Proximity matrix obtained from morphological characters using Dice's coefficient in eleven accessions of *G. repens*

Accession	Accession										
	Tg	Dk	Kd	Md	Ur	II	Td	P	Dh	Mr	An
Tg	1.00	0.99	0.94	0.98	0.95	0.90	0.93	0.98	0.98	0.99	0.99
Dk		1.00	0.94	0.99	0.95	0.90	0.94	0.98	0.98	0.99	0.99
Kd			1.00	0.98	1.00	0.99	0.99	0.97	0.98	0.97	0.97
Md				1.00	0.99	0.95	0.98	1.00	1.00	1.00	1.00
Ur					1.00	0.99	0.99	0.98	0.98	0.98	0.98
II						1.00	0.98	0.95	0.95	0.94	0.94
Td							1.00	0.98	0.98	0.97	0.97
P								1.00	1.00	1.00	1.00
Dh									1.00	1.00	1.00
Mr										1.00	1.00

The proximity matrix obtained had the highest similarity value of 1.00 (100% similarity) among accessions 9 (Dh), 10 (Mr) and 11 (An), which are placed close to each other in the dendrogram as a sub clade in Cluster I. A bootstrap value (96%) was observed at the node of the two accessions. Accession 4 (Md) and accession 8 (P) grouped together as a sub clade, also exhibited a similarity value of 1.00 (cent per cent correlation). The grouping of these accessions exhibited a high level of confidence with the bootstrap value of 98%.

In cluster II, accessions I (Tg) and 2 (Dk) which were from the nearby geographic localities were grouped together with a similarity value of 0.99. The grouping of these accessions was supported by a higher bootstrap value of 91 %. Cluster III included accessions 3 (Kd), 5 (Ur) and 6 (Il), where accessions 3 and 5 were in close association and accession 6 lies separately. The association of accessions 3 and 5 revealed complete similarity (proximity value of 1.00) which was supported by a high level of confidence (93% bootstrap value). Cluster IV contained one accession Td (7) which stands as an outlier of branch B. The clustering of Td as an outlier is substantiated by an absolute level of confidence (100% bootstrap value). In general the matrix obtained from the morphological data showed high similarity with highest similarity value of 1.00 (i.e. 100% similarity) among all the accessions included in cluster I and accessions 3 (Kd) and 5 (Ur) contained in cluster Ill. The least similarity value recorded was 0.90 between accession 6 (Il) and accessions 1 (Tg) and 2 (Dk).

Principle Component Analysis

The two dimensional ordination plot generated by PCA confirmed the results of cluster analysis with minor difference (Fig. 3). The eigen values of the principal components (PCs) ranged from 9.562 to 1.291. The PCs accounted for about 92.81% of the cumulative variance (Table 7). The percentage of variance ranges from 35.42%

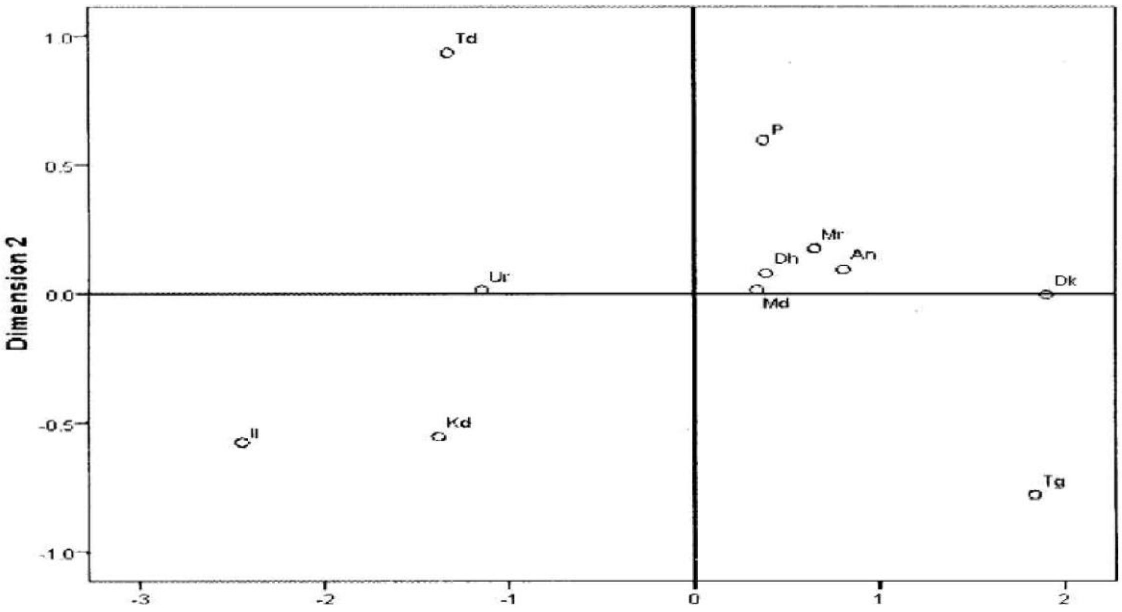

Fig. 3. Scatter plot showing relationship among accessions of *G. repens* based on morphological data

to 4.78%. In the scatter plot the distribution of the accessions is almost similar to the distribution in the dendrogram, though there are minor variations. Accessions 1 and 2, though grouped together in the dendrogram, lie at a distance in the scatter plot but in the same quadrant. But accessions 3 and 5 which showed a similarity value of 1.00 lies separate from each other in two quadrants in the scatter plot and so are accessions 4 and 8.

Table 7. Eigen value, percentage of variability and accumulated variability for morphological analysis

Component	Initial Eigen values		
	Total	% of Variance	Cumulative %
1	9.562	35.416	35.416
2	4.906	18.171	53.587
3	4.134	15.312	68.899
4	3.763	13.938	82.837
5	1.403	5.195	88.032
6	1.291	4.782	92.813

Discussion

Morphological traits provide a simple way of measuring genetic diversity while studying genotype performance under normal growing conditions, but are influenced by environmental factors (Tuinstra *et al.*, 1996; Beuningen and Busch, 1997; Abdi *et al.*, 2002; Fufa *et al.*, 2005). They are used to evaluate distinctness, uniformity and stability and also to establish the description of genotype (Peterson *et al.*, 1994). Studies on germplasm characterization have been carried out frequently using characterization of plant morphological attributes for alfalfa (Rumbaugh *et al.*, 1988), wheat (Pecetti *et al.*, 1992), white clover (Rosso and Pagano, 2001), white lupin (Rubio *et al.*,2004), apricot (Ruiz and Egea, 2008), fenugreek (Mc Cormick *et al.*, 2009), water melon (Szamosi *et al.*, 2009), sesame (Morris, 2009), safflower (Elfadl *et al.*, 2010), vineyard peach (Nikolic *et al.*, 2010) and Sorghum (Gerrano *et al.*, 2014).

In the present study morphological characters were used for the characterization of the accessions of *G. repens.* The qualitative characters ana lysed does not show any kind of variation among the eleven accessions chosen for the study. This clearly indicated that the accessions are morphologically similar and variations were only in the quantitative aspect of the characters. Among the quantitative characters analysed, six of the characters were similar with no variation. The characters that remained constant in all the members were number of stipules, number of bracts, number of calyx lobes, number of corolla lobes, number of stamens and number of stigma. In statistical analysis GCV and PCV showed very low values indicating little variation among the accessions. In this analysis, only one or two values regarding foliar characters were above 30%, which showed that variation is more in those characters only. Analysis of variance and heritability analysis does not reveal any significant variation among the accessions.

The genotypic and phenotypic correlation between the accessions were also analysed for the characters among accessions. The genotypic correlation coefficient reflected the inherent association between two characters either due to pleiotropic effect of genes or linkage while the phenotypic correlations refer to the observable correlation between two plant characters. The latter gives a measure of modified genotypic correlation coefficient by the environment. Here also the foliar characters showed higher phenotypic and genotypic correlation. Rest of the accessions revealed lower values of correlation. Much of the characters do not reveal any correlation between genotypic and phenotypic expressions. This shows that the variations revealed are not heritable and is greatly influenced by the environmental conditions. The variations between the genotypic correlation coefficient and phenotypic correlation coefficient of the characters analysed were high in all the cases, suggesting that environmental effects had greater influence on variability.

Different statistical procedures, ranging from simple univariate to the more complex multivariate techniques, have been used in the analysis of characterization data in the germplasm collection. Although statistics such as means, ranges and variances are helpful in providing information on the diversity of accessions in germplasm collections, they do not enable the simultaneous comparison of the accessions and the plant attributes (Harch *et al.*, 1995). Pattern analysis techniques such as clustering and ordination, have been used extensively to study the diversity among accessions for various plant species (Jahufer *et al.*, 1997; DeLacy *et al.*, 2000; Rosso and Pagano, 2001). In the present study clustering of accessions were done based on the similarity matrix obtained using Dice coefficient.

The morphological analysis based on the dendrogram and subsequent distance matrix revealed greater similarity among the accessions. Similarity among accessions was cent per cent (100%) among a number of accessions. The lowest similarity value between accessions were also as high as 0.90 as given by accession 6 (Il) with accessions 1 (Tg) and 2 (Dk). The classification obtained in the dendrogram was substantiated by a higher bootstrap value. All this combined with other statistical analysis revealed the similarity among the accessions collected from regions across the Western Ghats and from the Andaman islands.

Although, the morphological dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among accessions, diverse results could be obtained in morphological grouping, when experiments were repeated owing to variations in environmental conditions such as soil types, and soil fertility levels (Steel, 1972); light temperature and moisture regime (Summerfield and Huxley, I 973; Morakinyo and Ajibade,1998). The clustering pattern obtained in the dendrogram should be further confirmed using the supporting evidence from the PCA scatter plot.

Cluster analysis decreases the number of individual variable units by classifying such variation into groups which are translated into a dendrogram using the coefficient of similarity (Sneath and Sokal, 1973; Tatineni *et al.*, 1996). Multivariate analysis of principal components and cluster analysis based on morphological traits was performed to determine the level of variability and the relationship among the accessions.

In the present study, PCA substantiated the result obtained in cluster analysis with minor exceptions. The distribution of the accessions is almost similar to the distribution in the dendrogram. In the scatter plot, accessions which gave 100% similarity value were seen grouped in one quadrant of which accessions 9, 10 and 11 lie very closely as depicted by the dendrogram and similarity values. But accession 8 which showed cent percent similarity with accession 4 in the matrix was placed at a distance among the group in the scatter plot. Accessions 1 and 2, though grouped together in the dendrogram, lie at a distance in the scatter plot but in the same quadrant. Accessions 3 and 5 which showed a similarity value of 1.00 also lies separate from each other in two quadrants in the scatter plot.

The difference in scatter plot and the dendrogram may be due to the difference in the axis that is not visible in the 2D scatter plot, but is expressed in the dendrogram. Another possibility for variation may be due to the algorithemic difference between cluster analysis and PCA. But the overall patterning goes well with the cluster analysis, showing similarity among the accessions. However, the morphological studies conformed the relationship between eleven accessions of *G. repens.*

Accessions of *G. repens* collected from different regions in Kerala and from Andaman islands, were in closely formed groups which clearly indicates that the geographic differentiation of the accessions is not extensive. Reduced variability may be the reason for scanty distribution and 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.

Acknowledgement

The authors thank Head of the Department of Botany, University of Kerala for the facilities provided.

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