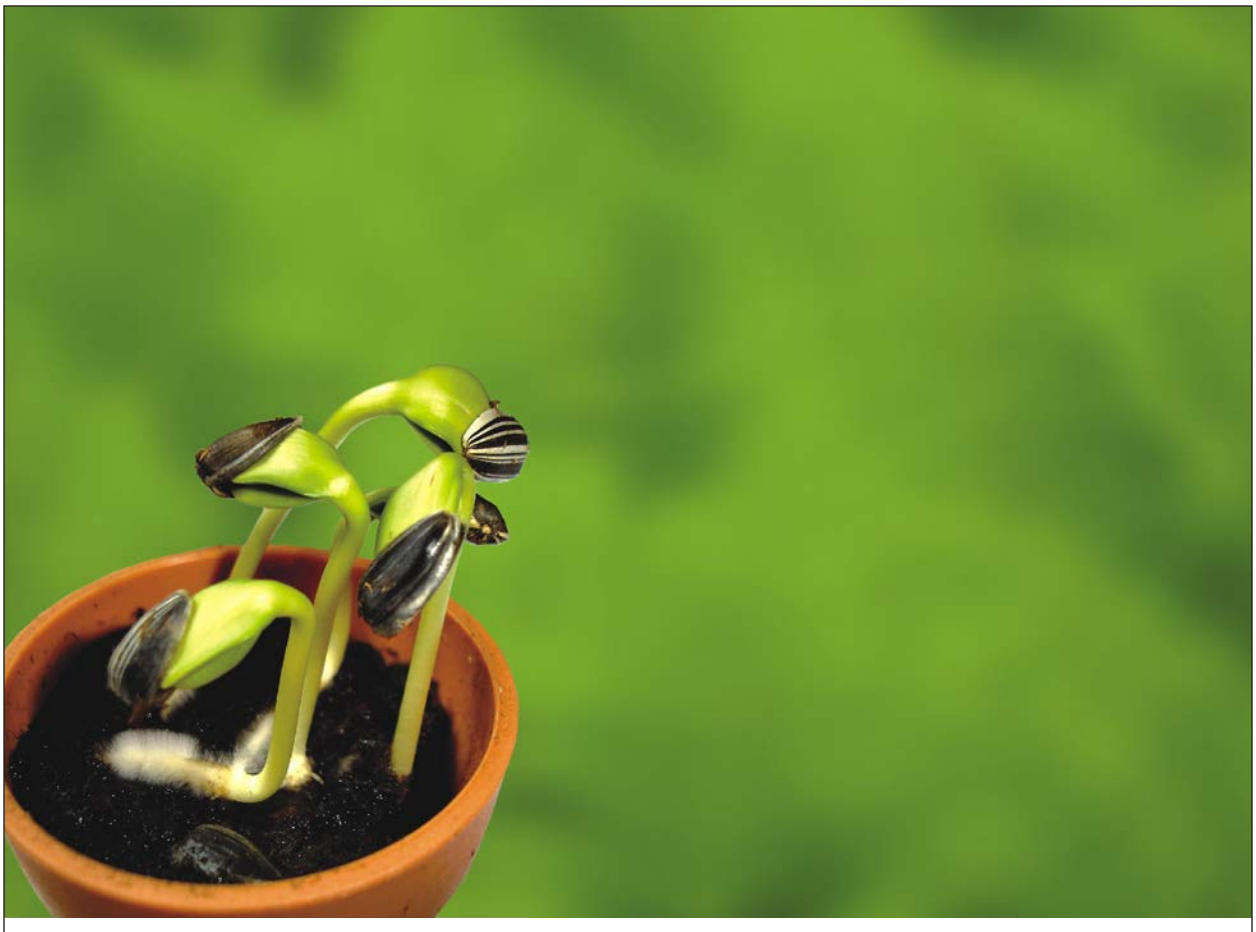

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Studies on Morphological and Phytochemical Variability of Different Populations of *Tribulus terrestris*

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Abstract: An attempt was made to analyze the variability and correlation of characters among five accessions of *Tribulus terrestris* collected from different geographical and agroclimatic regions of South India. All the five accessions showed different levels of morphological and phytochemical variability. Leaf area showed the maximum morphological variability. Phytochemical analysis by using HPLC revealed differential levels of chemical affinities. Among the six morphometric characters studied, all the characters except internodal length showed significant positive correlation towards each other. Results from this study can be exploited for commercial and breeding purposes of this important medicinal plant.

Key words: Puncture vine, variability, HPLC, *Tribulus terrestris*, phytochemical analysis

INTRODUCTION

Tribulus terrestris Linn. (Zygophyllaceae) commonly known as puncture vine is an annual or perennial prostrate herb with many slender, spreading branches up to 90 cm in length, commonly found throughout India, up to an altitude of 5,400 m. Whole plant is used in medicine. It is useful in strangury, dysuria, renal and vesical calculi, anorexia, dyspepsia, helminthiasis, cough, asthma, cardiopathy, skin diseases and rheumatic arthritis (Sivarajan and Balachandran, 1994; Warrier *et al*., 1996).

Some previous researchers have conducted some studies on the diversity of *Tribulus* populations (Scott and Morrison, 1996; Louveaux *et al*., 1998). Mas and Verdu (2001) has studied the relationship between mean length of the main stem and taproot width and total, vegetative and reproductive biomass. Phytochemical analysis was attempted by Ganzera *et al*. (2001) for the determination of steroidal saponins in this plant. The present study is an effort to analyze the variability of five *Tribulus terrestris* populations collected from different geographical and agroclimatic regions of two southern states of India at morphological and phytochemical levels and to find out the interrelationship of characters by correlation analysis.

MATERIALS AND METHODS

Morphological Variability

The study on morphological variability has been carried out on the basis of morphological differences between the five accessions collected from different sources of South India as follows:

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Table 1: Morphological variations in quantitative characters of *Tribulus terrestris*

Characters	Mean	SE	Range	SD	CV
Leaf length (cm)	3.58	0.23	2.6-4.8	0.74	20.67
Leaf breadth (cm)	2.59	0.22	1.57-3.74	0.71	27.41
LL/LB	1.41	0.04	1.25-1.7	0.13	9.21
Leaf area (cm^2)	6.90	0.99	2.99-12.80	3.14	45.50
Leaflet No.	12.0	0.0	-	0.0	0.0
Petiole length (cm)	0.88	0.06	0.54-1.1	0.19	21.59
Stabilized internodal length (cm)	4.83	0.13	4.3-5.6	0.42	8.69
First branch length (cm)	26.9	2.04	18.0-37.0	6.47	24.05

Malappuram (Dt.), Kerala (Acc. No. TT01); Coimbatore (Dt.), Tamil Nadu (TT02); Palakkad (Dt.), Kerala (TT03); Erode (Dt.), Tamil Nadu (TT04); Thrissur (Dt.), Kerala (TT05). Twelve replications per accession were observed. Morphological variability among the accessions was analyzed using different morphological characters (Table 1) with the help of mean, range, Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV) (Khan, 2000).

Phytochemical Variability

The five accessions described above were subjected to analysis of phytochemical variability using HPLC method. Leaves with some shoot parts were used as samples for phytochemical analysis. The samples were dried in shade, powdered and 10 g each of the dried samples were reflux condensed using methanol (3×100 mL) for 8 h at 50°C. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator below 50°C. Ten milligram each of the concentrated extracts were dissolved in 10 mL methanol and used for HPLC analysis.

Shimadzu HPLC system consisting of LC-10ATVP pump, a rheodyne injector, SPD M10AVP photodiode array detector and CLASS-VP 6.12 SP5 integration software was used for the analysis. The stationary phase was Phenomenex Luna C 18 (250×4.6 mm) column with 5 µ particle size and a guard column. The mobile phase was passed through 0.45 µ PVDF filter, degassed and used. The column was equilibrated with the mobile phase for one hour and then pumped with a back pressure of 200 kg cm^{-2}. The injection volume was 20 µL and the chromatograms were run for 20-40 min under the mobile phase of MeOH: GAA (7:3).

The chromatographic patterns of the various accessions were compared and the Paired Affinity Indices (PAI) were computed. The PAI between A and B is calculated by,

$$PAI = [(\frac{\text{No. peaks similar to A and B}}{\text{No. similar peaks + No. dissimilar peaks in A and B}})] \times 100$$

where A and B stand for any two accessions (Ravindran *et al*., 1992). PAI is a measure of chemical affinity between any two accessions.

Correlation of Characters

Correlation analysis is used to analyze such relationships between characters. Correlation of the characters in the case of the different species studied has been worked out using correlation coefficient and its significance using t-test (Rangaswamy, 1995).

RESULTS AND DISCUSSION

Morphological Variability

Study of variability of quantitative morphological characters based on eight parameters revealed maximum variability in the case of leaf area (Table 1). Morphological variability of characters shows

the genotypic differences between the different accessions of the species studied. It can be used as an index to analyze the genotypic differences present within the species and such differences indicate the genetic diversity of the species that has accumulated in the course of evolution of the species in different populations. Such diversities, since they have originated in different habitats and have contributed significantly towards the adaptability and divergence of the species, can be considered as valuable sources of genes and genotypes for the selection of superior accessions leading to their use in conservation, propagation and commercial exploitation. Similar studies have been carried out in medicinal plants (Misra *et al*., 1998; Sangwan *et al*., 2004), rice (Shoba, 1993), tea (Ramasubramanian, 2005), coffee (Nikhila *et al*., 2002; Raghu *et al*., 2003), cardamom (Radhakrishnan *et al*., 2005), Coriander (Srivastava *et al*., 2000), *Casearia sylvestris* (Silva *et al*., 2006) by earlier workers and such studies have helped in the identification of superior genotypes of the corresponding plants.

Phytochemical Variability

The number of peaks appeared in the HPLC chromatogram (Fig. 1) in each accessions were arranged pattern wise to study the Paired Affinity Index (PAI) (Table 2). Almost identical Rt values were checked by spectrum analysis and classified on its basis. Among the accessions used for the study, the accession from Malappuram (Dt.), Kerala, (TT 01) showed the highest chemical affinity (70.58%) with the accession from Coimbatore (Dt.), Tamil Nadu (TT 02). Phytochemical characters are the indicators of the chemical constituents of plants and the study of phytochemical variation is very important in medicinal plants since medicinal property of any plant is the result of the action of such chemicals. Analysis of the phytochemical variability between accessions in the case of medicinal plants will help to identify them in terms of their differences in phytochemical constitution. More over, phytochemical differences are good indicators of their genotypic distances. Studies on phytochemical variability have been attempted by earlier workers in different crops like cinnamon (Ravindran *et al*., 1992), pepper (Ravindran and Nirmal Babu, 1994), tea (Ramasubramanian, 2005), etc.

Correlation of Characters

Biological characters are controlled by genes which may be oligogenic or polygenic in nature. Characters, which show continuous distribution, are polygenic in nature. Most of the plant characters related to growth, yield and productivity belong to this category. The agronomic characters of medicinal plants are no exemptions. Such characters show different levels of interrelationships between them and such relationships can be identified by correlation analysis.

Six morphometric characters viz., leaf length, leaf breadth, petiole length, leaf area, internodal length and first branch length were studied for correlation analysis in this species (Table 3). All the characters except internodal length showed significant positive correlation towards each other. Correlation of characters shows their interrelation ship. Characters with significant positive correlation usually show similar trends of variation providing an opportunity for their selection jointly. This is very much important in breeding programmes by selection since the bulk of characters to be used for selection get reduced considerably. Similar approaches have been used by earlier workers in different crops like tea (Ramasubramanian, 2005), Dahlia (Misra *et al*., 1990), Taramira (Sodani *et al*., 1990).

Table 2: Paired affinity index (PAI) of the different accessions of *Tribulus terrestris*

Accessions	TT 01	TT 02	TT 03	TT 04	TT 05
TT 01	100				
TT 02	70.58	100			
TT 03	21.73	20.0	100		
TT 04	20.0	20.0	29.03	100	
TT 05	18.18	17.39	18.51	17.39	100

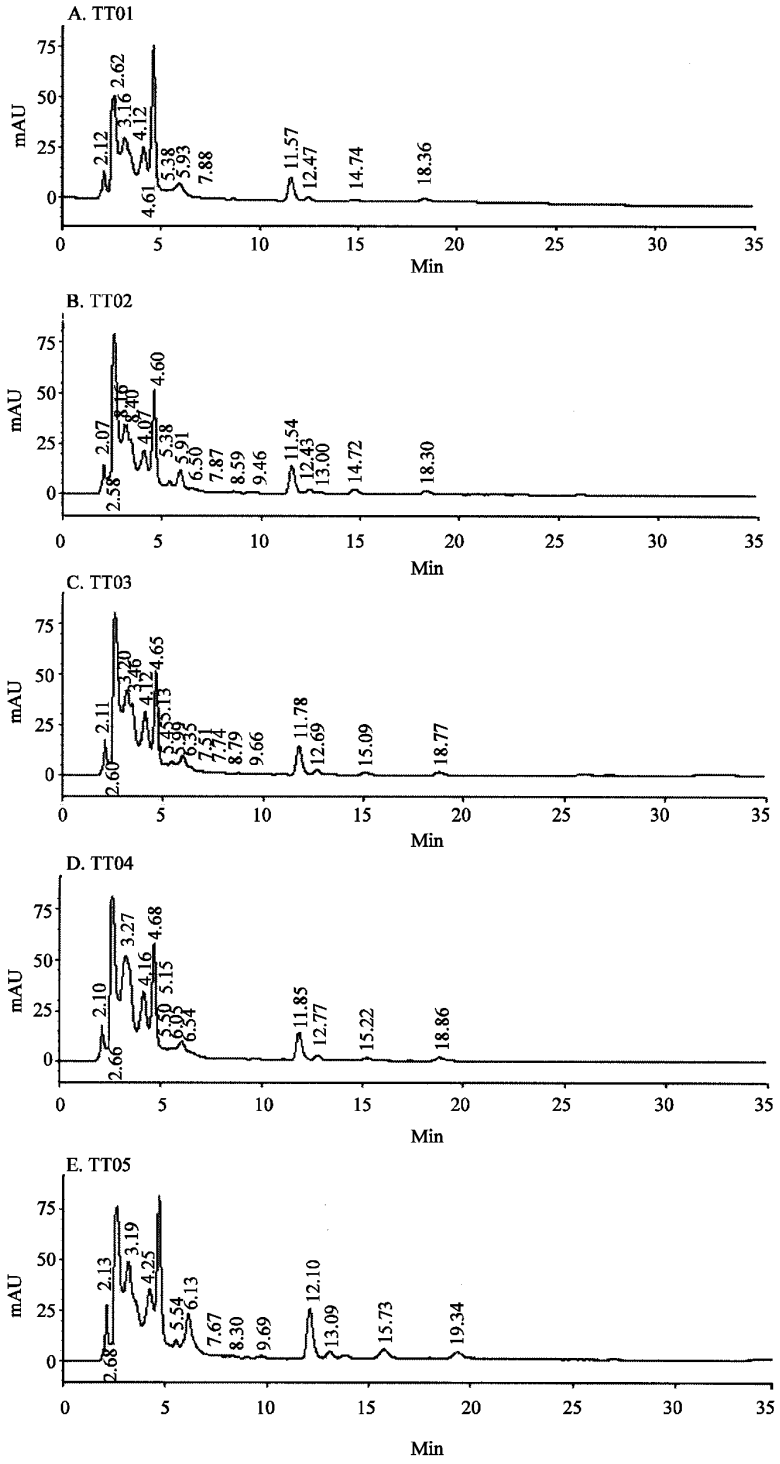

Fig. 1: HPLC chromatograms of *Tribulus terrestris*

Table 3: Correlation of quantitative morphological characters of *Tribulus terrestris*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area	Stabilized internodal length
Leaf breadth	0.98*				
Petiole length	0.76*	0.80*			
Leaf area	0.99*	0.99*	0.78*		
Stabilized internodal length	-0.33	-0.35	-0.72	-0.29	
First branch length	0.67*	0.73*	0.78*	0.73*	-0.17

*Significant at 5% level

In conclusion, among the characters studied for morphological variability leaf area showed the maximum variability. Morphological variability of characters indicates genotypic differences between the different accessions of the species studied. Such differences can be considered as valuable sources of gene differences that can be exploited in propagation and breeding programmes. Phytochemical analysis with the help of HPLC in the case of the above five accessions revealed differential levels of chemical affinities, very often associated with population distances and differences. Such variation can also be exploited both for commercial and plant breeding purposes.

REFERENCES

Ganzera, M., E. Bedir and I.A. Khan, 2001. Determination of steroidal saponins in *Tribulus terrestris* by reversed phase high performance liquid chromatography and evaporative light scattering detection. J. Pharma. Sci., 90: 1752-1758.

Khan, M.A., 2000. Plant Breeding. Biotech Books, Delhi, India, pp: 25-50.

Louveaux, A., M. Jay, O.T.M. Hadi and G. Roux, 1998. Variability in flavonoid compounds of four *Tribulus* species: Does it play a role in their identification by desert locust *Schistocerca gregaria*?. J. Chem. Ecol., 24: 1465-1481.

Mas, T.M. and M.A. Verdu, 2001. Relationships between plant size and biomass in *Tribulus terrestris*. Actas Congreso, Sociedad Espanola de Malherbologia, Leon Spain, 20, 21y 22 de-noviembre de, pp: 251-254.

Misra, R.L., H.L. Saini, D. Dhyani, T.S. Verma and P.C. Thakur, 1990. Genetic variability in dahlia (*Dahlia variabilis*). Indian J. Genet., 50: 51-55.

Misra, H.D., J.R. Sharma and S. Sharma, 1998. Genetic variability and path analysis in Ashwagandha (*Withania somnifera*). JMAPS., 20: 753-756.

Nikhila, K.R., A.G.S. Reddy, V.B. Sureshkumar and K.V. Mohanan, 2002. Consequences of sib mating in C×R (*Coffea congensis*×*Coffea canephora*) coffee. Proceedings of PLACROSYM XV, pp: 83-87.

Radhakrishnan, V.V., K.V. Mohanan and P.M. Priya, 2005. Comparative performance of certain promising genotypes of Cardamom. J. Non Timber For. Prod., 12: 38-41.

Raghu, A.V., K.V. Mohanan, A.G.S. Reddy and V.B. Sureshkumar, 2003. Variability in a sib mated progeny of CxR (*Coffea congensis*×*Coffea canephora*) Coffee. Indian J. Agric. Res., 37: 110-114.

Ramasubramanian, B., 2005. Studies on variability, genetic divergence and crop improvement in Tea (*Camellia assamica* (Masters) Wight). Ph.D Thesis, University of Calicut, Kerala, India.

Rangaswamy, R., 1995. A Text Book of Agricultural Statistics. New Age International Publishers Ltd., New Delhi, India.

Ravindran, S., N.R. Krishnaswamy, K.S. Manilal and P.N. Ravindran, 1992. Chemotaxonomy of *Cinnamomum* Schaef. occurring in Western Ghats. J. Indian Bot. Soc., 71: 37-41.

Ravindran, P.N. and K. Nirmal Babu, 1994. Chemotaxonomy of south Indian Piper. JMAPS., 3: 6-13.

Sangwan, R.S., N.D. Chaurasiya, L.N. Misra, P. Lal, G.C. Uniyal, R. Sharma, N.S. Sangwan, K.A. Suri, G.N. Qazi and R. Tuli, 2004. Phytochemical variability in commercial herbal products and preparations of *Withania somnifera* (Ashwagandha). Curr. Sci. 8693, pp: 461-465.

Scott, J.K. and S.M. Morrison, 1996. Variation in populations of *Tribulus terrestris* (Zygophyllaceae) 1. Burr morphology by using Burr morphology. Aust. J. Bot., 44: 175-190.

Shoba, B., 1993. Studies on mutagenesis in early upland rice. Ph.D Thesis, University of Calicut, Kerala, India, pp: 318.

Silva, M.A.S., L.C. Ming, A.M.S. Pereira, B.W. Bertoni, A.P. Batistini and P.S. Pereira, 2006. Phytochemical and genetic variability of *Casearia sylvestris* Sw. From Sao Paulo State Atlantic Forest and Cerrado populations. Rev. Bras. Plant Med., Botucatu, 80: 159-166.

Sivarajan, V.V. and I. Balachandran, 1994. Ayurvedic Drugs and Their Plant Sources, Oxford and IBH Publishing Co. Pvt. Ltd., 66 Janpath, New Delhi.

Sodani, S.N., E.V.D. Sasthry and M.R. Nehra, 1990. Divergence analysis in Taramira (*Eruca sativa* Mill.). Indian J. Genet., 50: 9-12.

Srivastava, S.B., L. Kamaluddin, S.M. Tripathi and J.P. Srivastava, 2000. Genetic Divergence in Coriander (*Coriandrum sativum* L.). In: Spices and aromatic plants-Challenges and opportunities in the new century. Ramana, K.V., S.J. Eapen, K. Nirmal Babu, K.S. Krishnamurthy and A. Kumar (Eds.), Indian Society for Spices, Calicut, Kerala, India.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty, 1996. Indian Medicinal Plants-a compendium of 500 species. Orient Longman Pvt. Ltd. Chennai, India, 5: 311-315.