ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 12, pp.144-149, December, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVOLUTIONARY RELATIONSHIP AND DIVERGENCE AMONG SIX CULTIVARS OF *Trichosanthes anguina* L. BASED ON SDS-PAGE AND RAPD ANALYSIS

[1]Bhuvaneshwari, N. C., [1] *Ravindhran, R., [2]Santhosh Kumar, A., [2]Antony Raj, G. and [2]Vincent, S.

[1]Department of Plant Biology and Biotechnology, Loyola College, Chennai- 600 034, Tamil Nadu, India
[2]Division of Environmental Biotechnology, Loyola College, Chennai – 600 034, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 24th September, 2012
Received in revised form
08th October, 2012
Accepted 18th November, 2012
Published online 18th December, 2012

Key words:
Trichosanthes anguina,
RAPD,
Genetic diversity,
SDS-PAGE,
Dendrogram analysis.

ABSTRACT

A set of 6 cultivars of snake gourd (*Trichosanthes anguina*) collection was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Random amplified polymorphic DNA analysis (RAPD) analysis. By using SDS-PAGE, total soluble leaf protein was fractionated into 11 bands, which showed heterogeneity among different varieties. Thiruvallur cultivar (TVL) exhibited maximum number of bands (11) and Nagapattinum cultivar (NAP) exhibited least number of 6 bands. Polymerase chain reaction (PCR) with 4 arbitrary decamer oligonucleotide primers, applied to the 6 cultivars, produced a total of 46 different marker bands of which 63.4 per cent were polymorphic. The size range of the amplified DNAs was mostly between 106 bp and 6132 bp. Thus, with the selected primers sufficient polymorphism could be detected to allow identification of individual cultivars. Visual examination of electrophoresis gels and analysis of banding patterns confirmed that many of the snake gourds under cultivation with similar morphological characters are genetically quite different. A dendrogram displaying the relative genetic similarities between the cultivars showed a range of 56 to 89 per cent similarity. RAPD analysis offered a rapid and reliable method for the estimation of variability between different cultivars which could be utilized by the breeders for further improvement of the snake gourd genotypes.

INTRODUCTION

Trichosanthes anguina L., the snake gourd (chichinda) also known as Chinese cucumber occupies an important place among vegetables in India. It is believed to have originated in India. It is cultivated wide from India to Australia. The plants are commonly cultivated in south India for their snake-like greyish white spongy fruits. It is an annual plant and belongs to the gourd family Cucurbitaceae. It is of exceedingly rapid growth and of climbing habit. Snake gourd is a very nutritious vegetable. An analysis of this vegetable shows it to consist of moisture 94.6%, protein 0.5%, fat 0.3%, fibre 0.8% and carbohydrate 3.3% per 100 grams of edible portion. Cultivar development is an important part of the plant breeding and the identification of these varieties by different parameters plays an important role in breeding industry. However, with the increase in the number of cultivars of each crop, it is difficult to distinguish the cultivars on the basis of morphological characters alone. This has led to the development of the new stable parameters such as use of their genetic material (nucleic acids and proteins) as a tool for varietal identification. In crop improvement programme, genetic diversity as been considered as an important factor which is also essential pre-requisite for hybridization programme for obtaining progenies with important desirable characters like disease resistance, earliness, quality or even performance of a particular character (Chowdhury *et al.*, 1975). In current scenario, the DNA marker has become the marker of choice for the study of crop genetic diversity. A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily be monitored. The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis f or designing strategies to exploit for applied purposes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. RAPD is a PCR-based technology. RAPDs have a very high genomic abundance and are randomly distributed throughout the genome.

Besides that, RAPD markers also show levels of polymorphism similar to iso-enzyme markers. Knowledge of genetic diversity among existing cultivars of any crop is essential for long-term success in breeding programme and to maximize the exploitation of the germplasm resources (Belaj *et al.*, 2002). According to Ahmed *et al.*, (2000), there has been a great scope for genetic improvement of snake gourd as there is a wide range of variability exists in Asia. Genetically diverse and geographically isolated lines may generate a wide range of variation when brought together. Thereby, information on its genetic architecture of snake gourd is essential. Under such circumstances, this study was aimed to assess the genetic diversity among cultivars of snake gourd from six different farmer's fields in Tamil Nadu.

Corresponding author: raviloyola1998@gmail.com

MATERIALS AND METHODS

Seed collection

Six cultivars of *Trichosanthes anguina* were collected from the farmer's fields of Tamil Nadu (Nagapattinam, Adhudhurai, Tanjavur, Vellore, Thiruvallur and Ranipet) and maintained at the Loyola College garden.

SDS-PAGE analysis

About 100 mg of fresh leaf samples were collected from 6 cultivars of snake gourd plants. The samples were homogenized using 0.5 ml of 1M Tris-HCl buffer (pH 7) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. To 0.5 ml of supernatant, equal volumes of TCA: acetone solution (1:1 w/v) was added. The tubes were incubated at -20 °C overnight. The protein precipitate was collected by centrifugation at 10,000 rpm for 10 minutes at 4 °C. The pellet was washed using 80% acetone. The contents were centrifuged for 10,000 rpm for 10 minutes at 4°C. The pellet was finally dissolved in 100 µl of 1M Tris-HCl buffer (pH 7). The SDS plates were assembled and a 12% separating gel with a 4% stacking gel was cast. The samples and the standard marker (116-14.4 KDa) were loaded in their corresponding wells and the SDS PAGE was run at 50 V for stacking and 100 V for separating gel. After the run was complete, the separating gel was then immersed in Coomassie Brilliant Blue (CBB) staining solution and left overnight. The gel was then de-stained using methanol and glacial acetic acid solution (2:3 v/v). The developed gel was stored in 7% glacial acetic acid. The bands were visualized under white illuminator and documented using gel documentation instrument.

Genomic DNA isolation and RAPD analysis

Total genomic DNA was extracted from different cultivars of snake gourd as described by Kuta *et al*., (2005). The isolated DNA was suspended in 30 µl of TE buffer. The concentration of DNA was determined by ultraviolet–visible spectrophotometer (Systronics, India). Intactness of genomic DNA was checked on 0.8 % agarose gel. Random amplified polymorphic DNA analysis was carried out in a DNA thermal cycler (Mastercycler gradient, Eppendorf, Germany). Ten decamer oligonucleotides from Operon technologies were used in this study. The PCR was carried out in a volume of 10 µl reaction mixture consisting of 5 µl Ampliqon *Taq* DNA Polymerase master mix RED (2.0X – Ampliqon, Denmark), 0.8 µl template DNA (10 ng), 0.6 µL random primer (10 ng), and 3.6 µL sterile DNase-free water. The PCR was performed with an initial denaturing step for 5 min at 94ºC, 35 cycles consisting each of a denaturing step of 1 min at 94ºC, primer annealing step of 1 min at 37ºC and primer extension step of 2 min at 72ºC. Final extension of 7 min at 72ºC was given for polishing the ends of PCR products. PCR products were electrophoresed on 1.8 % (w/v) agarose gel. The amplified bands were photographed with UV gel documentation system. A 100 bp ladder (100bp – 1031 bp) and Lamda DNA/*Hin*d III (300 – 23130 bp) were used as molecular weight standards.

Data analysis

The RAPDistance Package Software Version 1.04 (Armstrong *et al.*, 1994) and Numerical taxonomy and Multivariate Analysis System (NTSYS-pc) were used in this study. The molecular weights of bands were estimated based on the standard bands from Gene Ruler DNA ladder marker. The presence of band was scored from the photograph. Only clear and reproducible bands were given consideration. These bands were considered as polymorphic when they were absent in some sample in frequency greater than 1 % (Jorde *et al.*, 1995) and change in band intensity were not considered as polymorphism. Clear bands were scored as present (1) or absent (0) at particular position or distance migrated on the gel. The data matrix of 1's and 0's were prepared from the scorable bands and was entered into the data analysis package (Armstrong *et al.*, 1994). The indexes of similarity were calculated across all possible pair wise comparisons of individual within and among population (Nei and Li 1979).

Calculations were done based on the formula,

$$SI = 2NXY/ (NX+NY)$$

Where, NXY is the number of RAPD bands shared in common between individuals X and Y, NX and NY are the total number of bands scored in X and Y respectively.

The similarity index was used to calculate the genetic distance values and to construct the dendrogram. The dendrogram provides a visual representation of the differences in the varieties of Snake gourd. The dendrograms were constructed using the Unweighted Pair-Group Method of Arithmetic (UPGMA) employing Sequential, Agglomerative, Hierarchical and Nested Clustering (SAHN) from NTSYS-pc program (Rohlf, 1994).

RESULTS

Establishment of plants

The six cultivars collected from different places (Table 1) were successfully grown in the Loyola College garden. After a period of one month, they were photographed (Fig 1) and used for SDS PAGE and RAPD analysis.

Table 1. Collection of different seed cultivars of *Trichosanthes anguina* in Tamil Nadu

Cultivars	Place of collection
NAP	Nagapattinum
ADT	Adhudhurai
TJN	Tanjavur
VEL	Vellore
TVL	Thiruvallur
RPT	Ranipet

SDS-PAGE analysis

The proteins that were separated were distinguished and grouped based on the standard marker A wide variation was observed in the pattern of protein bands of the cultivars studied. By using SDS-PAGE, total soluble leaf protein was fractionated into 11 bands. Thiruvallur cultivar (TVL) exhibited maximum number of bands (11) followed by Ranipet cultivar (RPT) and Adudhurai cultivar (ADT) with 10 bands each (Fig 2). Nagapattinum cultivar (NAP) exhibited least number of 6 bands (Fig 2). Entire protein banding profile was divided into 4 regions (A to D) based on its decreasing molecular weight when compared with standard protein marker (M). The molecular weight of leaf protein ranged between 114-4 KDa among snake gourd cultivars (Table 2). In region A (82-

Table 2. Protein molecular weight profile of six cultivars of snake gourd

Region	Position of band	Molecular weight of proteins (KDa)					
		*NAP	ADT	TJN	TVL	RPT	M
A	1st	111	114	114	114	115	116
A	2nd	96	94	94	94	94	
A	3rd	-	82	-	81	-	
B	4th	-	72	-	71	72	
B	5th	63	62	62	62	62	
B	6th	-	57	57	57	56	
C	7th	47	46	46	46	45	45
C	8th	-	38	38	36	36	35
C	9th	-	-	-	27	26	
D	10th	12	12	12	13	13	
D	11th	5	6	6	5	5	

* NAG- Nagapattinum cultivar, ADT- Adudhurai cultivar, TJN- Tanjavur cultivar, VEL- Vellore cultivar, TVL- Thiruvallur cultivar, RPT- Ranipet cultivar.

Table 3. Description of the operon random primers used in the present study for screening six cultivars of snake gourd

Primers	Sequences	% of G+C	Melting/ annealing temp (Tm)	No. of basepairs
OPE4	3'GTGACATGCC5'	60	72ºC	10
OPE6	3'AAGACCCCTC5'	60	72ºC	10
OPE8	3'TCACCACGGT5'	60	72ºC	10
OPE9	3'CTTCACCCGA5'	60	72ºC	10

Table 4. Details of amplified fragments obtained in snake gourd and percentage of polymorphism

Primers	Size of fragments (bp)	Total number of fragments	No. of polymorphic fragments	No. of monomorphic fragments	Polymorphism (%)
OPE4	106-2060	10	6	4	60
OPE6	192-6132	18	11	7	61.1
OPE8	164-1841	10	7	3	70
OPE9	572-2227	8	5	3	62.5
Total		46	29	17	63.4

Table 5. Similarity matrix using Jaccard's coefficient for six snake gourd cultivars based on the 46 bands obtained with 4 RAPD primers

Sample	NAG	ADT	TJN	VEL	TVL	RPT
NAG						
ADT	0.782609					
TJN	0.858696	0.782609				
VEL	0.891304	0.782609	0.858696			
TVL	0.565217	0.565217	0.565217	0.565217		
RPT	0.695652	0.695652	0.695652	0.695652	0.565217	

Range: 0.5652- 0.8913, Average: 0.7826. NAG- Nagapattinum cultivar, ADT-Adhidhurai cultivar, TJN- Tanjavur cultivar, VEL- Vellore cultivar, TVL- Thiruvallur cultivar, RPT- Ranipet cultivar.

114 KDa) all the six cultivars showed the presence of highest molecular weight protein (114 and 94 KDa). But 82KDa protein was distinctly observed only in Adudhurai cultivar (ADT) and Thiruvallur cultivar (TVL). In region B (56-72 KDa), all the six cultivars of snake gourd showed the presence of medium molecular weight protein (62 KDa), whereas 72 KDa, 57 KDa protein was completely absent in Nagapattinum cultivar (NAP) (Table 3). In region C (27-47 KDa), 46 KDa protein was observed in all six cultivars of snake gourd. Nagapattinum cultivar (NAP), Adudhurai cultivar (ADT), Tanjavur cultivar (TJN) showed absence of 27 KDa protein. In between 27-46 KDa proteins, 38 KDa of protein was also registered in all five cultivars except in Nagapattinum cultivar (NAP). In region D (4-13 KDa), all the six cultivars of snake gourd registered the presence of 12 KDa, 4-6 KDa protein (Table 3).

RAPD analysis

RAPD analysis was performed using 10 decamer primers of which only 4 primers (Table 3) showed distinct banding patterns. Totally 46 fragments were generated by the four primers (Fig 3). The size of fragments generated by all the 4 primers ranged from 106 bp to 6132 bp. The total numbers of monomorphic fragments were 17. The total numbers of polymorphic fragments were 29. The primers OPE4 generated 6 fragments, OPE6 generated 11 fragments, OPE8 generated 7 fragments and OPE9 generated 5 fragments. The percentage of polymorphism generated for all the primers was 63.4% (Table 4). The similarity index for six cultivars of snake gourd was analyzed using Jaccard's coefficient. The similarity index for all six cultivars of snake gourd within Tamil Nadu ranged from 0.565217-0.891304 (Table 5). Higher similarity indices suggest that the individuals in the population have closer genetic relation among them. Nagapattinum cultivar (NAP) and Vellore cultivar (VEL) showed highest similarity index (0.891304). The dendrogram analysis for six cultivars of snake gourd was done using NTSYS-PC software. Genetic distance of six cultivars of snake gourd ranged from 0.57 to 0.89 (Fig 4). The dendrogram produced by snake gourd varieties showed 5 clusters. The first cluster consisted of varieties Nagapattinum cultivar (NAP), Vellore cultivar (VEL). The second cluster consisted of Tanjavur cultivar (TJN). These 2 groups were linked together at about 0.85 genetic distance level. The third cluster consisted of Adudhurai cultivar (ADT). The group was

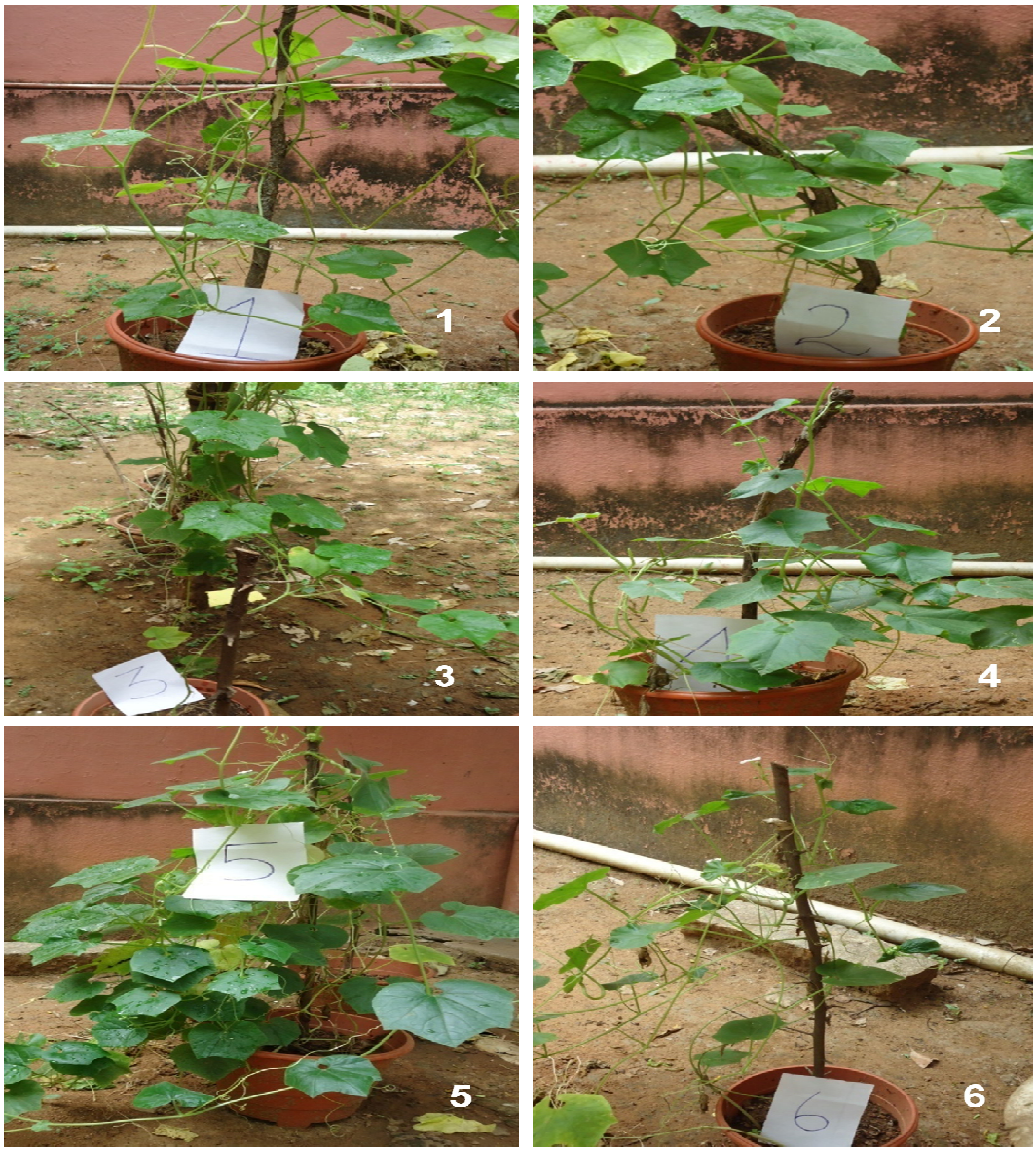

Fig. 1. Six different cultivars of snake gourd. 1: Nagapattium cultivar, 2: Adhudhurai cultivar, 3: Tanjavur cultivar, 4: Vellore cultivar, 5: Thiruvallur cultivar, 6: Ranipet cultivar.

linked together to first and second clusters at about 0.78 genetic distance level. The first three clusters were joined to the fourth cluster comprising of Ranipet cultivar (RPT) at about 0.69 genetic distance level. Last cluster consisted of Thiruvallur cultivar (TVL) linked to other clusters by 0.56 genetic distance level.

DISCUSSION

SDS-PAGE Analysis

Gel electrophoresis has shown that many isoenzymes and polymorphic proteins are widely distributed in plants (Cherry and Ory, 1972) and that protein polymorphism signals the existence of allelism (Goodenough, 1978). The results from SDS-PAGE analysis of snake gourd protein indicate differential banding pattern for different snake gourd cultivars but the overall degree of variation is relatively low, similar result was reported in the previous study for 13 varieties of wheat (Shuaib *et al.*, 2007). The diversity in high molecular weight protein subunits could be as a result of gene silencing in some cultivars encoding these proteins

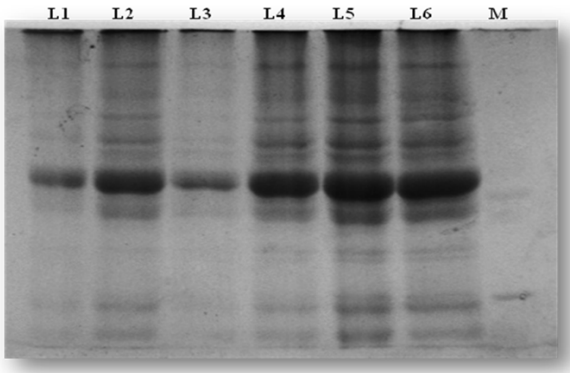

Fig. 2. 12% SDS-PAGE gel showing protein profile of six cultivars of snake gourd. Lanes: L1-Nagapattinum cultivar, L2-Adudhurai cultivar, L3-Tanjavur cultivar, L4-Vellore cultivar, L5-Thiruvallur cultivar, L6-Ranipet cultivar and M-116-14.4 KDa marker.

(Lawrence and shepherd, 1980). In our study, there is a difference in the density of common major bands, which suggests heterogeneity among different cultivars. These regions were proven to be distinct since all six cultivars

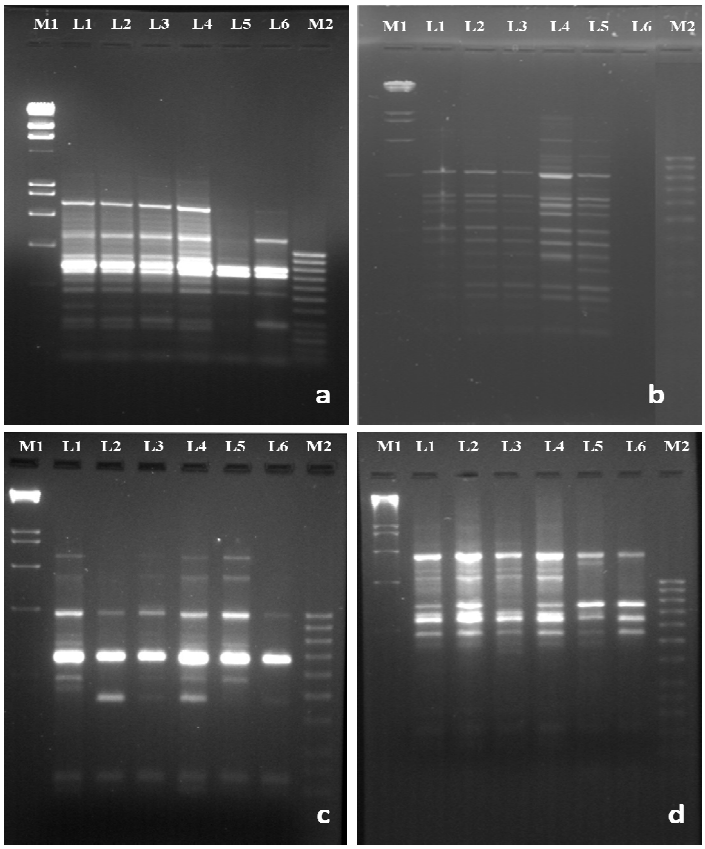

Fig. 3. Agarose gel electrophoresis of RAPD products of 6 genomic isolates of snake gourd. (a) OPE4; (b) OPE6; (c) OPE8; (d) OPE9. (Lanes: M1- Lamda DNA/ *Hin*d III plus marker (300-23130 bp), L1-Nagapattinum cultivar, L2–Adudhurai cultivar, L3-Tanjavur cultivar, L4-Vellore cultivar, L5-Thiruvallur cultivar, L6-Ranipet cultivar and M2-100bp marker (100bp- 1031bp).

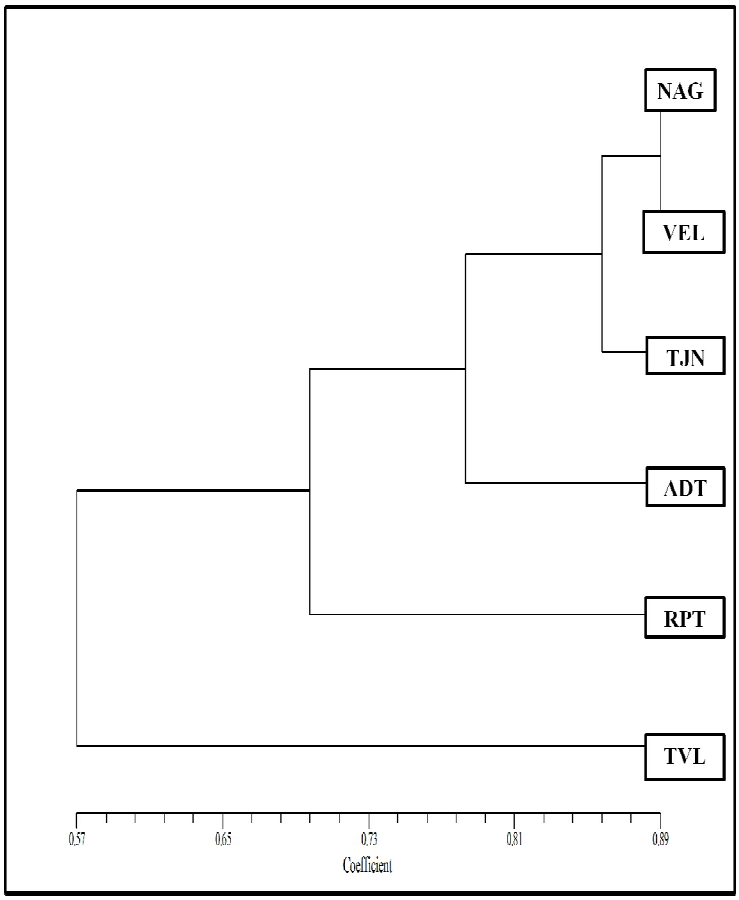

Fig. 4. NTSYS-PC Dendrogram showing the genetic diversity among 6 cultivars of snake gourd of Tamil Nadu. NAG- Nagapattinum cultivar, ADT- Adudhurai cultivar, TJN- Tanjavur cultivar, VEL- Vellore cultivar, TVL- Thiruvallur cultivar, RPT- Ranipet cultivar.

registered presence of all lower molecular weight proteins. By the comparative analysis, each cultivar had its unique profile which was different from other cultivars. From the results based on molecular weight it is evident that 82 KDa, 72 KDa, 27 KDa of proteins were unique among the cultivars, hence these protein types could be useful to identify the cultivars of snake gourd. SDS-PAGE technique has proven to be a useful tool in supporting classical taxonomy studies (Thang *et al*., 2003). Protein content variations among different cultivars can be used in identification of the species or cultivars and also to get information on purity of genetic resources.

RAPD Analysis

RAPD technique has been used in genetic study of snake gourd cultivars. The results showed different primers generated different fragment numbers and length of DNA amplification products. The results on similarity index of six cultivars of snake gourd suggested that the individuals in the Thiruvallur population (TVL) have farther genetic relation due to low similarity index and Nagapattinum (NAP) and Vellore cultivar (VEL) have closer genetic relations as their similarity index value was high. Based on the dendrogram analysis, Nagapattinum cultivar (NAP) and Vellore cultivar (VEL) were most similar cultivars and Thiruvallur cultivar (TVL) stood diverse among all other cultivars.

Conclusion

SDS-PAGE protein profile was able to generate only a moderate level of polymorphism. Snake gourd cultivars possess considerable variation in their genetic base. Genetic makeup of a crop species is not affected by environment and only expression profiles can be the targets of environment proved in present study as the snake gourd cultivars were collected from different geographical zones grouped together on the basis of RAPD banding profiles. The cultivars with wide genetic distance can be used as parents to exploit heterosis in future snake gourd breeding programs. The rare or unique alleles observed can further be employed for marker assisted selection programs. The primers which proved more information can be converted to sequence tagged sites (STS) and sequence characterized amplified regions (SCAR) for amplification of specific alleles which could be further utilized in snake gourd genome analysis. Also the information gained from clustering behavior of accessions can be useful to design strategies for their management in the gene-bank.

REFERENCES

Ahmed, M.S., Rasul, M.G., Bashar, M.K and Mian, A.S.M. 2000. Variability and heterosis in snake gourd (*Trichosanthes anguina* L.). Bangladesh. J. P1. Breed. Genet. 13(1): 27-32.

Armstrong, J.S., Gibbs, A.J., Peackall, R and Weiller, G. 1994. The RAPDistance package, Australian National University, Camberra, Australia.

Belaj, A., Satovic, Z., Rallo, L. and Trujillo. 2002. Genetic diversity and relationship in olive (*Oleaeuropea* L.) germplasm collection as determined by RAPD. Theor. Appi. Genet. 105(4):638-644.

Cherry, J.P. and Ory, R.T. 1972. Electrophoresis of six selected enzymes of peanut cultivars. Phytochem. 12: 283 -289.

Choudhury, B.D., Bhat, P.N. and Singh, V.P. 1975. Genetic diversity in cluster beans. Indian. J. Agric. Sci. 45: 530-535.

Goodenough ,U. 1978. Genetics. Holt, Rinehart and Winston, Inc. (second edition). Great Britain. pp. 748.

Jorde, L.B. 1995. Population specific genetic markers and diseases in biology and biotechnology: A comprehensive desk reference. Meyesr, RA (Ed.) New York: VCH publisher. pp. 724-728.

Kuta, D.D., Kwon-ndung, E., Dachi, S., Bakare, O. and Ogunkanmi, L.A. 2005. Optimization of protocols for DNA extraction and RAPD analysis in West African fonio (*Digitaria exilis* and *Digitaria iburua*) germplasm characterization. Afr. J. Biotechnol. 4(12): 1368- 1371.

Lawrence, G.J. and Shepherd, K.W. 1980. Variation in glutenin protein sSubunits of wheat. Aust. J. Biol. Sci. 33: 221-233.

Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of National Academy of Science USA. 7: 5269 5273.

Rohlf, F.J. 1994. NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter software. New York.

Shuaib, M., Zeb, A., Ali, Z., Ali, W., Ahmad, T. and Khan I. 2007. Characterization of wheat varieties by seed storage-protein electrophoresis. Afr. J. Biotechnol. 6(5): 497-500.

Thanh, V.C., Tran, N.N., Yutaka, H and Nguen, V.T. 2003. Antenna protein diversity of prawns (*Macro brachium*) in the Mckong Delta. Biopshere conservation. 5: 11-17.

****