December 2009 Volume 3 Issue 4

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(4), 2009 [197-200]

Studies on morphometric variations and distribution pattern of proteins in wild cucurbit (*Cucumis trigonus*)

A.M.Mali, N.S.Chavan*

Department of Botany, Shivaji University, Kolhapur- 416004, (INDIA)

E-mail : niranjanac_2006@yahoo.com; arunammali@yahoo.in

Received: 9th September, 2009 ; Accepted: 19th September, 2009

ABSTRACT

Mature fruits and dried seeds of wild member of Cucurbitaceae were analysed with SDS-PAGE electrophoresis for their protein polymorphism and morphometric variations with view to observe how far the protein polymorphism data would help to assess the relatedness as well as differentiation among single species. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Cucumis trigonus;
Morphometric variations;
Environmental interaction;
Genetic diversity;
Jaccard's similarity coefficient;
Protein profile;
SDS-PAGE.

INTRODUCTION

The use of the biochemical components of plants as an aid to their systematic is now familiar concept. The Cucurbitaceae members are famous for medicinal and ethnic uses. The numbers of wild species are also notable. The cucurbit shows phenotypic plasticity and variation in morphology[17], embryology[7,15,16] and palynology[8,15,17]. Biochemically the family has also been screened with respect to their non protein amino acids and bitter principles[13]. Reliable, quick and accurate methods of germplasm characterization and cultivar identification are essential for their effective utilization in breeding programme. Seed proteins are widely used in plant genetic studies[4]. Considerable knowledge has been added to the chemosystematics with the use of SDS gel electrophoresis.

Cucumis trigonus Roxb. is an important potential cucurbit from the Cucurbitaceae (Vernacula: Kachri, Mekki, Balli). It is perennial climbing herb. It is available in various parts of India, and it is a highly acceptable wild vegetable across south India. Fruits are edible and famous among the agricultural community. It is also valued for its medicinal properties due to cucurbitacin B and D. The herb is recommended as antidote for snake poison, purgative, astringent, analgesic, anti-inflammatory, anabolic activity[11] and diuretic[21] useful in bilious disorder[3]. It is also valued for its medicinal properties due to steroidal and triterpenic compounds[19]. Kachri fruit, contains high protease activity[1] and has been used as a meat tenderizer in the Indian subcontinent[12].

There is a morphological variation in fruits and seeds. Fruit of *Cucumis trigonus* are diverse in skin colour pattern, size and shape, ranging from small and round to large and variably shaped. Greater the level of plasticity, the more the spatial pattern of phenotypes within and among population may be dependent on the environmental pattern and not on the organization of phenotype[8]. To accomplish these pur-

FULL PAPER

poses, morphological traits have been analysed. It is also probable that a comparative study of seed proteins of the morphological different fruit samples of same species will be useful guide to chemical taxonomy and ecological diversity. Keeping this view the present work was under taken to evaluate the morphological differentiation and genetic diversity among single species of *C. trigonus*.

MATERIALS AND METHODS

Five mature fruit samples of *Cucumis trigonus, w*hich shows morphometric variations, were collected from Bijapur district of Karnataka. Morphometric variation based on the morphological traits like fruit and seed (skin) colour pattern, circumference: length ratio etc.[9,10].depicted in TABLE 1. The measurements of the quantative traits were made with a milimetric ruler and thread. All the measurements were made by the same person. Each sample consider as Type:1, Type:2, Type:3, Type:4, Type:5 respectively. To observe the variation of protein, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure outlined by Sadashivam and Manickam[14] employing 15% of polyacrylamide gel containing 10% of SDS.

Homogenization and protein extract

Dried seeds were homogenized with 5ml extraction buffer (0.62 M Tris-Cl pH 6.8 containing 2% SDS and 5% Mercaptoethanol) and few drops of N- hexane was added. N-hexane helps in dissolving the lipids that interfere during protein isolation[18]. Homogenized protein sample were centrifuged at 10000rpm for 15 min at 4°C. The collected supernatant was used for subsequent analyses.

Protein electrophoresis

SDS- PAGE was performed according to Sadashivam and Manickam[14] using 15% separating gel and 4% stacking gel. Protein samples mixed with equal amount of loading buffer (10 mg Bromophenol blue, 10% Glycerol, 1 M Tris- Cl pH 6.8-6.25 ml, 2% SDS, water-12.05ml) presence of bromophenol blue facilitates easy loading of samples. Equal amount of protein (50µl) was loaded per well. Electrophoresis was performed at 50V in stacking gel and 100V in resolving gel. Gel were stained in coomassie brilliant blue R-250 solution (0.1g coomassie brilliant blue R-250 in 40ml methanol, 10ml glacial acetic acid and 50ml water) overnight and destained with destaining solution (40ml methanol, 10ml glacial acetic acid and 50ml water) until the background was clear.

After completion of staining and destaining 'Rm' was calculated by using following formula:

$$\mathbf{Rm} = \frac{\textbf{Distance between the origin and band}}{\textbf{Distance between the origin and tracking dye}}$$

Then, similarity index after electrophoresis was calculated by Jaccard's similarity coefficient (J) when J value is one then both the Types are considered identical and when zero then they are different (Jaccard, 1908). J value is calculated by using following formula:

$$\mathbf{J} = \frac{\mathbf{2m[ab]}}{\mathbf{m[a]+m[b]}}$$

Where, m [a] and m [b] represents the total numbers of bands present in genotype [a] and [b] respectively and m[ab] the number of common bands present in genotypes a and b.

RESULT

The data of all the morphological characters of fruits measured is summarized in TABLE 1

A Morphometric fruit difference in *C. trigonus* with regard to total seed protein profile was clearly indicated in the present studies TABLE 2.

It is evident from TABLE 1 that protein band varied from 4-14 with maximum number in Type: 4. Four mobility groups are recognised from the species studied according to their 'Rm' values.

A. Slow mobility group-'Rm' ranges from 0.02-0.11.
B. Medium mobility group-'Rm' ranges from 0.11-0.25.
C. Fast mobility group-'Rm' ranges from 0.25-0.45.
D. Fastest mobility group-'Rm' ranges from 0.45-0.65.

It is revealed from the 'Rm' values for various bands there is variation in the number, position and intensity of protein bands. A major band of 'Rm' value 0.51 and 0.58 were observed in all Types of *C. trigonus* except Type: 5. Bands having 'Rm' value 0.7 present in the

TABLE 1 : Morphological variation in *Cucumis trigonus* fruits

Fruit colour (Skin)	Average fruit (cm)	Fruit length(cm)	C:L	C/L Ratio	Average no. of seeds per fruit	Seed colour	Seed length (cm)	Seed breadth (cm)	L/B (m)
Type:1 (Brownish yellow)	16.3	11.2	1.45	1.45	400	Cream	0.6	0.3	2.00
Type :2 (Light green with mosaic strips of dark green)	10.3	5.9	1.74-1.77	1.74	412	Cream	0.6	0.3	2.00
Type:3 (Dark green patches with light green background)	10.6	5.5	1.92	1.92	406	Cream	0.6	0.2	3.00
Type:4 (Light green with dark green patches)	10.2	5.1	2.00-2.15	2.00	235	Whitish	0.5	0.2	2.5
Type:5 (Brown & light green patches)	8.4	5.8	1.50-1.56	1.45	300	Cream	0.4	0.2	2

Note : All fruit are mature; C- Circumference; L- Length; B- Breadth

TABLE 2 : Relative mobility values for various bands elecrophored in seed protein extract of *C. trigonus*

Mobility group	Band No.	'Rm' value	*C.trigonus*				
			Type:1	Type:2	Type:3	Type:4	Type:5
A	1	0.02	-	-	-	0.02	-
	2	0.06	-	-	-	0.06	-
	3	0.1	-	0.1	-	0.1	-
	4	0.4	0.4	-	0.4	0.4	0.4
	5	0.7	-	0.7	-	0.7	-
	6	0.11	-	-	-	0.11	-
	7	0.13	-	-	-	-	0.13
	8	0.15	0.15	-	-	-	0.15
B	9	0.17	-	-	-	0.17	-
	10	0.18	-	-	-	-	0.18
	11	0.25	0.25	-	0.25	-	-
	12	0.28	-	-	-	0.28	-
	13	0.32	-	-	-	0.32	-
C	14	0.35	0.35	-	-	0.35	0.35
	15	0.44	-	-	-	0.44	-
	16	0.45	-	-	-	-	0.45
	17	0.47	0.47	-	-	0.47	-
	18	0.51	0.51	0.51	0.51	0.51	-
D	19	0.54	-	-	-	-	0.54
	20	0.58	0.50	0.58	0.58	0.58	-
	21	0.65	-	-	-	-	0.65

Type: 1, 2, 4 while a band of 'Rm' value 0.44 and 0.25 unique to the Type: 4, 6. The slowest moving band ('Rm' value 0.1) was observed in the Type: 2. Of *C. trigonus* Type: 4 shows maximum number of protein band while Type : 2 shows minimum number of protein bands.

The species were also compared for the similarity index to study phonetic resemblance by Jaccard's Similarity Coefficient (J). Type:3 shows closer resemblance with Type: 4 and Type : 5 while Type: 2 and Type : 5 shows very distant relationship. They are not genetically identical as their J value is zero. Jaccard's similarity Coefficients based on SDS-PAGE analysis depicted in the TABLE 3.

TABLE 3 : Similarity indices of seed proteins in *C. trigonus*

C. trigonus	*C. trigonus*				
	Type :1	Type :2	Type :3	Type :4	Type :5
Type : 1	-	0.5	0.66	0.54	0.37
Type :2	-	-	0.5	0.44	0.00
Type :3	-	-	-	0.37	0.16
Type :4	-	-	-	1.00	0.18
Type : 5	-	-	-	-	1.00

It is revealed that Type: 1, Type: 4and Type: 5 are sharing higher homogeneity while Type: 2, Type: 5 are showing totally different protein profile. They are not genetically identical as their J value is zero.

DISCUSSION

Macromolecular chemosystematics which deals with polymeric compounds such as nucleic acids and proteins is of greater value in studying the evolutionary relationship. The protein profile analysis helps in calculating the degree of genetic similarity between different species or within single species which shows phenotypic variation. Valuable wild cucurbit, *C. trigonus* was analysed for their protein polymorphism with hope that information will be use of finding out differentiation as well as to study physiological behaviour and eco-diversity.

FULL PAPER

CONCLUSION

There is a variation in proteins of all five samples of *C. trigonus*. Changes in protein profile shows differences in physiological behaviour of *C. trigonus*. Based on morphometric variations and protein profile studies, it can be concluded that there are five different ecotypes of *C. trigonus*.

REFERENCES

[1] M.Asif-ullah, K.S.Kim, Y.G.Yu; Phytochemistry, **67(9)**, 870-875 **(2006)**.

[2] H.S.Chawala; Laboratory Manual for plant biotechnology, Published by Oxford & IBH Publishing Co, 2003 **16**, 129-136 **(2004)**.

[3] R.N.Chopra, S.L.Nayar, I.C.Chopra; Glossary of Indian Medicinal Plants, CSIR, New Dehli 41 **(1986)**.

[4] R.J.Cooke; J.Chromatogr., **698**, 282-299 **(1995)**.

[5] J.V.Dogra, D.Jha, M.Tiwari; Phytochemistry and Plant Taxonomy **2,** 193-197 **(1989)**.

[6] S.Jain, G.Padamaja; Indian Journal of Plant Physiology, 343-347 **(2004)**.

[7] D.A.Johnson; Plant Embryology : Walthom Moss, U. S. A., **(1950)**.

[8] D.A.Levin; 'Plasticity, canalization and evolutionary status in plants. In: Plant population ecology, A.J.davy, M.S.Hutching, A.R.Wakkinson; (eds.) 28th Symposium of the British Ecological Society **(1988)**.

[9] M.B.Mulimani, N.S.Chavan; Nature Enviournment and Pollution Technology., **6(1)**, 177-182 **(2007a)**.

[10] M.B.Mulimani; Studies on Economically importnat wild cucurbits., Ph.D.Thesis, Shivaji University, kolhapur **(2007b)**.

[11] V.R.Naik, N.V.Agshikar, G.J.S.Abraham; Pharmacology, **20**, 52-60 **(1980)**.

[12] B.M.Naveena, S.K.Mendiratta, A.S.Anjaneyulu; Meat Science, **68(3)**, 363-369 **(2004)**.

[13] C.Rhem; Journal of Science Food Agriculture., **12**, 679 **(1957)**.

[14] S.Sadashivam, A.Manickam; 'Biochemical methods'. Second edition, New Age Publishers, Coimbatore **(1992)**.

[15] K.Schnarf; Vergleichende Embryology der Angiosperm Berlin, **(1931)**.

[16] D.Singh; Bulletin of the Indian National Science Academy, **41**, 212-219 **(1970)**.

[17] K.R.Sporne; Biol.Rev., **31**, 1-29 **(1949)**.

[18] H.Stegmann, G.Pietsh; 'Methods for characterization of seed proteins in cereals and legumes', In: W.Gottschack, H.P.Muller (eds.), Seed proteins: Biochemistry, genetics, Nutritive value, Martinus Nijhoff, The Netherlands, 45-75 **(1983)**.

[19] A.Ulubelen, T.Baytop, B.Cubukcu; Planta Medicine, **30(2)**, 144-145 **(1976)**.

[20] S.F.Uppin; Karnataka sasya sampada @ Karnataka Vishvidyalaya Dharwad Karanataka University, Dharwad, **(1974)**.

[21] C.I.Wright, L.Van-Buren, C.I.Kroner, M.Koning; Journal of Ethnopharmacology, **114(1)**, 1-31 **(2007)**.