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Genetic Diversity in Local Chow-Chow (*Sechium edule* Sw.) Germplasm of Sikkim

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ABSTRACT

Sixteen chow-chow accessions collected from different locations of Sikkim were studied for fifteen morphological and biochemical characters. Highest fruit weight was recorded in entry S8 (461g) followed by S9 (416g) and S1 (399.09g). Highest dry matter content was found in the entry S5. Entries S2, S3, S10 and S11 contained higher ascorbic acid content in their fruits. Twelve polymorphic RAPD markers were used for differentiating the 16 accessions, generated a total of 25 bands (2 bands per primer). The UPGMA dendrogram obtained from the cluster analysis using Jaccard's similarity coefficient divided the accessions into four clusters. The morphological or biochemical characters of the accessions did not show association with the RAPD data.

Keywords: Chow-chow, Genetic diversity, North-East, RAPD

INTRODUCTION

Chow-Chow (*Sechium edule* (Jacq.) Sw. is a popular underutilized Cucurbitaceae vegetable of hilly regions of North Eastern India, Himachal Pradesh, J&K and Uttarakhand. It is known by different names like chow-chow, Isqush (Nepali) and Chayote (Hindi). It is very popular among the tribals of these regions owing to its hardiness, profuse fruiting with minimum care and its multiple uses. It grows luxuriantly under high rainfall conditions. Considerable variation in colour, size and fruit habit exists in these regions. So far, it remained a neglected underutilized crop, and few studies have been carried out. Its high-yield potential, nutritional aspects, tolerance to biotic and abiotic stresses and very low input requirement makes it a potential crop in changing climatic scenario. Considerable diversity of isqush is found in North Eastern Hilly region, particularly, Meghalaya, Sikkim and Mizoram (Rai et al. 2002). In Sikkim, it is a popular vegetable crop grown in the traditional way in backyards and kitchen gardens. This vegetable also fetches good price in the market due to its demand in the local market.

The morphological traits along with a molecular approach for identification of plant varieties/ genotypes and characterizing them according to the quantitative and qualitative characters they possess are more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants. Genetic analysis using genetic markers is a useful tool for *ex-situ* conservation as it may contribute to the characterization and evaluation of similar accessions to avoid duplication of plant material. PCR-based molecular markers have been widely used in many plant species for identification, phylogenetic analysis, population studies and genetic linkage mapping (Williams et al. 1990). Among the different types of molecular markers, randomly amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity

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(Williams et al. 1990) owing to their simplicity, speed and relatively low-cost compared to other types of molecular markers. RAPD markers have been used extensively in cucurbits to classify accessions of *Cucumis sativus* (Horejsi and Staub 1998), *Citrullus lanatus* (Lewi et al. 2002) and to identify cultivars and hybrids. For any crop improvement programme exploring the existing genetic diversity is a prerequisite for exploring their unique characteristics and evaluation at different regions for their suitability. Keeping these factors in view the present study was undertaken to analyze the genetic diversity of local Chow-Chow germplasm of Sikkim using morphological, biochemical and RAPD markers.

MATERIALS AND METHODS

The experimental material consisted of 16 Chow-Chow accessions collected from different locations of Sikkim (Fig 1). These collections have been planted and maintained at the research farm of ICAR Research Complex for NEH region Sikkim Centre, Tadong, Gangtok situated at 1320m amsl. These collections have been coded serially S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15 and S16 for keeping their identity. These germplasm were studied for fruit colour, spiny habit, fruit weight, fruit length (cm), fruit diameter (cm), petiole colour, fresh fruit weight (g), dry weight(g), dry matter(%), juice%, Total Soluble Solids(%), Acidity(%) and Ascorbic acid content (mg/100g). Total Soluble Salts (TSS) was determined by Zeiss hand refractometer. Freeds (1966) visual titration method was followed in estimating the ascorbic acid content of the fruit pulp. For extraction of titrable acidity, pulp was grinded and 100 ml of distilled water was added and filtered. 10 ml of filtrate was titrated against 0.1 N NaoH using phenolphthalein as an indicator. Rests of the morphological characters were measured by standard protocol/methods.

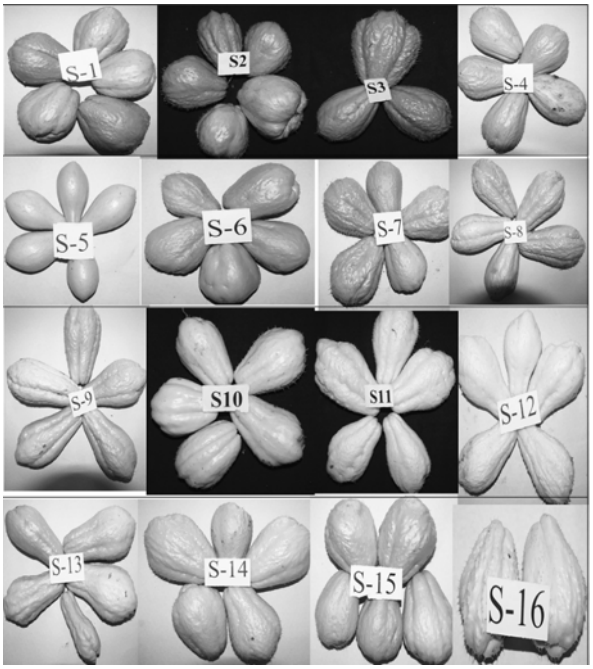

Fig 1: Local chow-chow germplasm of Sikkim Serial. No S1-S16.

For studying the RAPD pattern, DNA was extracted from young tender leaves from each of sixteen isqush plantations using modified Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Thirty primers (from Clonitec) were initially screened for their ability to amplify the DNA. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species/varieties. To ensure reproducibility, the primers generating weak, or complex patterns were discarded. Out of the thirty primers, twelve were selected. Standardization of the PCR reaction was done until clear bands appeared. DNA amplification was carried out by making final reaction volume to 25µl containing Taq buffer (1x), dNTPs (10mM), $MgCl_2$ (25mM), Primer (100 µÌ), Taq Polymerase (1U) and Template DNA (25ng). Amplification was carried out in thermal cycler (Applied Biosystems) programmed for an initial denaturation of 95°C (3min), 44 cycles of denaturation for 1 min (94°C), annealing at 37°C for 1 min. and extension at 72°C for 2 min. Then final extension at 72°C for 7 min. and storage at 4°C for 8. Amplified products were separated alongside a molecular weight marker (1.0kb plus ladder, Promega) by 1.5% agarose gel in 0.5xTBE (Tris borate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were taken using Gel Doc System (Gel Doc. 2000, Bio-Rad, California, USA). RAPD profiles of 16 chow-chow accessions were analyzed by scoring the presence or absence of each RAPD band. A binary data matrix with '1' indicating the presence of band and '0' indicating its absence was constructed. The binary data was used to generate a similarity matrix using Jaccard's similarity coefficient, $Jij = Cij/(ni+nj-cij)$ where *'Cij'* is the

number of positive matches between two genotypes, while ni and nj is the total number of bands in the genotype i and J respectively in SIMQUAL programme of NTSYS-pc package version 1.80 (Rohlf 1993).The The data were subsequently used to construct a dendrogram using unweighted pair group method with arithmetical averages(UPGMA) in SAHN programme of NTSYS-pc package.

RESULTS AND DISCUSSION

Our studies found considerable differences in sixteen isqush accessions for various quantitative and biochemical characters studied (Table 1). The fruits exhibited dark green, light green, yellow and white colour. Spiny habit was present in all the collections except S5 which exhibited smooth surface. The petiole colour in the fruits was dark green, light green and white. The fruit shapes varied from pyriform, round to ovoid. The mean fruit weight was highest for S8 (461g) followed by S9 (416g) and S1 (399.09g). S8 and S9 also showed maximum fruit length (16.76 and 15.43cm respectively). The accessions No. S8 and S9 were having large size fruits, which directly affect the yield. These entries can be commercially exploited in other areas/location of the state or other isqush growing areas for its cultivation on a larger scale. The dry matter content was highest for S5 (12.32%), followed by S4 and S8 (10.75% each). S9 showed highest juice content (75%) followed by S11 (68%). Ascorbic acid content was found to be highest for S2 (28.0mg/100g) followed by S3 and S10 (26.0mg/100g each). Acidity per cent was highest for S3 (0.32%). Total soluble salts content highest for S10 and S11. Considerable variation was found in respect of various fruit characteristics and other

Table 1 : Quantitative and biochemical characters of 16 chow-chow accessions

Accession No.	Single fruit weight (g) (g)	Fruit length (cm)	Fruit diam. (cm)	Fresh weight (g)	Dry weight(g)	Dry matter (%)	Juice (%)	TSS (%)	Acidity (%)	Ascorbic acid(mg/100g)	Fruit colour	Spine	Petiole colour	Juice colour	Fruit form
S1	399.93	10.53	8.45	90.2	5.8	6.43	50.9	4.2	0.10	8.0	DG	P	DG	DG	OV
S2	292.23	8.97	8.2	83.2	6	7.21	56.2	4.8	0.29	28.0	DG	P	DG	DG	R
S3	352.96	11.06	8.29	80.5	6.6	8.19	61.7	5	0.32	26.0	DG	P	DG	DG	OV
S4	342.73	11.96	7.62	115.3	12.4	10.75	40.2	4	0.10	10.0	DG	P	LG	DG	OV
S5	74.1	9.09	4.55	56.8	7	12.32	31.2	4	0.10	6.0	LG	A	LG	DG	PYR
S6	205.4	8.82	6.72	67.8	4.2	6.19	53.4	4.1	0.06	10.0	DG	P	DG	LG	R
S7	245.66	10.09	6.91	62.1	4.2	6.76	47.1	4.2	0.13	8.0	DG	P	DG	LG	OV
S8	461	16.76	8.28	49.3	5.3	10.75	41.1	4.3	0.06	8.0	LG	P	DG	LG	PYR
S9	416	15.43	7.54	54.3	5	9.21	75	4.4	0.06	10.0	LG	P	DG	DG	PYR
S10	177.13	8.43	6.42	64.6	3.9	6.04	56.8	5.2	0.22	26.0	Y	P	LG	W	OV
S11	318.16	13.13	7.78	113.6	5.7	5.02	68	5.1	0.19	22.0	W	P	LG	PW	PYR
S12	246.1	12.3	6.85	54.4	3.3	6.07	54.7	4	0.10	12.0	W	P	LG	W	PYR
S13	327.13	14.9	6.87	52	4.2	8.08	61.8	4.4	0.06	10.0	LG	P	DG	DG	PYR
S14	380.9	11.73	7.91	67.9	4.8	7.07	50.2	4	0.06	8.0	DG	P	LG	LG	OV
S15	231.53	9.56	7.36	69.7	5.9	8.46	66.79	4.1	0.13	10.0	DG	P	DG	LG	R
S16	198.3	8.95	4.28	61.7	6.1	9.89	28.2	4.8	0.06	12.0	Y	P	W	PW	R
Mean	291.82	11.35	7.12	71.46	5.65	8.02	52.70	4.41	0.13	13.38					
Range	74.1 -461.0	8.43-16.76	4.28-8.45	49.3-115.3	3.3-12.4	5.02-12.32	28.2-75	4.0-5.2	0.06-0.32	6.0-28.0					

biochemical traits in indigenous chow-chow collections (Singh et al. 2002; Yadav et al. 2005; Rai et al. 2006; Sanwal et al. 2008). Dry matter content is an important characteristic of a vegetable crop as it includes all the important nutrients like carbohydrates, proteins, vitamins and minerals available for absorption. Accession No. S5 had highest dry matter percent signifying a relatively higher per cent of nutrients as compared to other entries. Accession S2, S3, S10 and S11 contain highest ascorbic acid content, a form of Vitamin C. Vitamin C is a powerful antioxidant, which can be utilized preferably for enriching fodder or human vegetable food rich in Vitamin C. Mostly the dark green coloured fruits are preferred by the people of the region. The variability in colour can be utilized to meet different preferences for fruit colour ranging from dark green, light green white and yellow. The variation in colour may be attributed to changes in pigment content and type such as chlorophylls and xanthophylls (Hendry 1993).

Twelve RAPD primers chosen for amplification showed 100% polymorphism. However, the total numbers of bands amplified by the primers were low as these bands were selected on the basis of their reproducibility. Only the reproducible bands were selected, and weak and non-reproducible bands were discarded. The 12 RAPD primers amplified a total of 25 markers with an average of 2 bands per primer (Table 2). Band size of amplicons ranged from 1000bp-9000bp. Maximum number of bands were produced by primer No.174 (5 bands) whereas primer No. 161, 162, 165, 168, 178 and 188 produced one band each. RAPD data divided 16 germplasm in to four main clusters (Fig 2). Group 1 consists of sample S1, S9, S14 and S6. Group 2 contains S2, S12, S8 and S10 whereas samples S13, S15 and S16 clustered together in group 3. Group 4 consisted of S3, S4, S5, S7 and S11. As per Jaccard's similarity coefficient table constructed the coefficient ranged from 0.05 to 0.75. The similarity coefficient was highest between S4 and S5 (75%) followed by S8 and S10 (57%) & S10 and S13 & S13 and S14 (50%). The similarity coefficient of S2 and S10 & S2 and S12 is 43% and 40% respectively. The lowest similarity percentage was obtained between S10 and S13. Several primers were identified as good markers in the study. Primer No. 168 and 188 showed specific band size of 5000 bp and 7000bp for the samples S6 and S1, respectively, so these amplified products can be used as fingerprint of these accessions. The characters of the accessions in each cluster did not show association with the RAPD data generated i.e the accessions did not differ as accordingly to the RAPD clustering pattern. Abdelnour and Oscar (2008) revealed a high degree of genetic diversity in the chayote accessions using isozyme markers.

Table 2: List of random primers, amplified bands and percent polymorphism used in RAPD analysis

S. No	Primer No.	Sequence (5'— 3')	Total no. of bands	Poly-morphic bands	Percent poly-morphism
1	161	CGTTATCTCG	1	1	100
2	162	AACTTACCGC	1	1	100
3	163	CCCCCCAGAT	2	2	100
4	165	GAAGGCACTG	1	1	100
5	168	CTAGATGTGC	1	1	100
6	171	TGACCCCTCC	3	3	100
7	172	ACCGTCGTAG	4	4	100
8	174	AACGGGCAGC	5	5	100
9	177	TCAGGCAGTC	2	2	100
10	178	CCCTCATTGG	1	1	100
11	188	GCTGGACATC	1	1	100
12	196	CTCCTCCCCC	3	3	100
		TOTAL	25	25	100

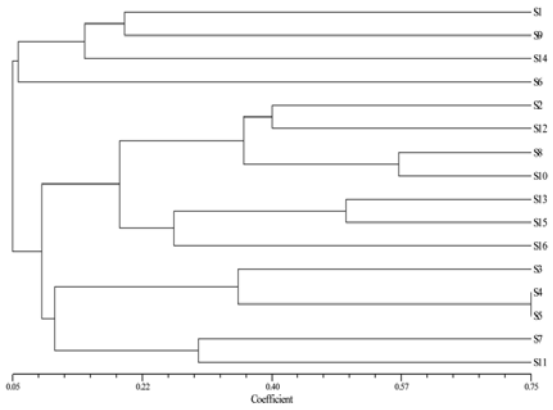

Fig. 2: Dendrogram of 16 chow-chow accessions obtained after NTSYS analysis with RAPDmarkers

The chow-chow accessions of Sikkim varied in their morphological characters and also differ in their biochemical composition. The RAPD profiling figured out genetic differences in the accessions. Conservation efforts need to be strengthened for sustainable utilization of these underutilized resources by characterizing them and exploring their useful traits.

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