Genetic characterization of chayote [*Sechium edule* (Jacq.) Swartz.] landraces of North Eastern Hills of India and conservation measure

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Abstract Chayote or chow–chow is an underutilized cucurbit vegetable crop, widely cultivated by farmers in the backyards and *Jhum* lands for its tender leaves, fruits and tuberous root. In order to initiate crop improvement program in this crop, the present study was undertaken to assess the genetic variations in the 74 chow–chow landraces collected from the North Eastern Hill region of India. Wide variations for fruit colors, fruit length (6.5–21.5 cm), fruit width (4.2–10.7 cm), fruit weight (60–560 g), vitamin-C (2.6–13.8 mg/100 g), reducing sugar (0.18–2.77%), total sugar (1.09–2.94%) and phenol content (0.17–3.85 mg/100 g FW) were recorded among the landraces. All the landraces were also characterized using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. In RAPD analyses, out of 28 primers a total of 198 reproducible amplicons were formed at an average of 7.01 per primer and an overall polymorphism of 88.38%. Eight fragments were specific to landraces with light green fruits. Four fragments were observed to be specific to RCSC-22 (dark green fruits) and another four specific to a RCSC-30 (pale yellow fruits). Out of 30 ISSR, only 5 primers generated a total of 32 reproducible amplicons with an average of 6.4 per primer and overall polymorphism of 62.5%. The pair wise similarity coefficient values ranged from 0.55 to 0.96. The grouping of landraces in cluster analysis was found to be independent of their respective geographic locations. The cuttings of suckers and shoot top (2 months old) treated with indole-3-butyric acid (200 mg l⁻¹) provide an

alternative for the conservation of the diverse genetic materials to the researchers.

Keywords *Sechium edule* · Quality · Genetic diversity · Molecular marker · Vegetative propagation

Introduction

Chayote [*Sechium edule* (Jacq.) Sw.] is popularly known as chow–chow and squash in North Eastern India and also known by different dialect such as Piskot (*Khasi*) in Meghalaya, Iskut (*Mizo*) in Mizoram and Lonku in Himachal Pradesh. In sub-family Sicyoideae, chayote is the only cultivated cucurbit. Though, *Sechium* was considered as monotypic in earlier study, but now it comprises eight different species. Chayote was probably domesticated from its wild related species or from *S. compositum* in Mexico and Guatemala but now it is cultivated in several tropical and sub tropical regions (Newstrom 1990). In India, the crop was introduced by the Western Missionaries (Singh et al. 2012) and nowadays it is widely distributed along the Himalayas from Siphir (Mizoram) in East to Mandi, Himachal Pradesh in west and adjoining areas of Myanmar, Bhutan and Nepal. It is also grown in peninsular region of India especially Madurai and Nilgiri hills of Tamil Nadu and parts of Kerala and Karnataka (Sanwal et al. 2008).

Chow–chow is an underutilized vegetable widely grown in the North Eastern Hill (NEH) region of India and is an excellent source of nutrition. It is a herbaceous, perennial, monoecious, vigorous creeper. A thick root generally used for growing this crop which bears fruit (single or rarely in pairs) on a peduncle. It is basically used for human consumption. In addition to the fruits; stems, tender leaves and tuberous roots are also eaten. In recent past, the crop has

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gained importance as it possesses several medicinal properties due to richness in polyphenols (Ibarra-Alvarado et al. 2010) flavonoids especially in leaves (3.5 mg g^{-1} dried leaves), roots (3.05 mg g^{-1}) and stems (1.93 mg g^{-1}) (Siciliano et al. 2004) and Sechumin, an anticancer ribosome-inactivating protein (Th et al. 1998). Besides, fruit extract also has antihypertensive effect (Gordon et al. 2000); antibacterial, antifungal, antioxidant (Ordóñez et al. 2003, 2009; Sibi et al. 2013); anti-proliferative properties against cervical carcinoma, mouse lung fibrosarcoma and mouse macrophage leukaemia (Cadena-Iniguez et al. 2013); antihyperglycemic (Tiwari et al. 2013); anticonvulsant and central nervous system depressant activity (Firdous et al. 2012).

Agricultural research has traditionally given little attention to minor (or underutilized or neglected) crops. These underutilized crops are generally grown in marginal land and found to be more adaptable for harsh agro-ecological conditions as compared to major crops. Several nutritionally richer underutilized crops found to be popular staple food crop at regional level (e.g. *S. edule* in North-East India) but are neglected at national level. The research and developmental activities are very limited in underutilized crops which limit the developmental and conservation activities. The *S. edule* production is affected by different biotic e.g. downy mildew (Baiswar et al. 2010) and abiotic stresses during pre- and post-harvest period which limits its commercial production by marginal tribal farmers. Therefore, a comprehensive improvement program needs to be initiated to overcome the different limitation of *S. edule* production.

For effective conservation and improvement of a plant species, assessment of extant genetic variability is essential (Hamrick and Godt 1996). Characterization of germplasm based on morphological characters and molecular markers give the insight into the genetic variation present in the collection. Though a number of cultivated and wild type landraces of genus *Sechium* are available in different parts of the India, no information is available on their genetic base. Earlier, the evaluation and characterization studies have been conducted by some workers based on quantitative traits (Singh et al. 2002; Rai et al. 2005; Yadav et al. 2005; Sanwal et al. 2008; Cadena-Iniguez et al. 2013) and using biochemical markers (Abdelnour and Rocha 2008; Avendano-Arrazate et al. 2012). Morphological and biochemical markers are generally influenced by environment and stage of development, hence it is ideal to use DNA based molecular marker for the assessment of genetic diversity present in a plant species. In India, systematic effort to assess the genetic variability of *S. edule* using molecular makers, has yet to be made.

NEH region is endowed with diverse climatic conditions and it receives rainfall ranging from 150 to 250 cm. The

occurrence of high rainfall especially during flowering period limits the conservation of germplasm by selfing and bagging. Besides, conservation of chow-chow germplasm is also very tedious due to highly cross pollination (entomophilous), smaller size of flowers, and poor setting of single seeded fruits. Thus, vegetative propagation provides a better alternative means for the conservation of the valuable diverse germplasm of the region.

In a plant, auxin has an important role as a growth regulator. Indole-3-butyric acid (IBA) is a plant growth regulator in the auxin family and was found as the leading plant hormone for promoting root formation in plants growing from cuttings (Rao et al. 2005). Significant effect of IBA on survival and rooting has also been observed by El-Abd (1997) and Ali and Abed (2013) in cucumber and El-Eslamboly (2014) in watermelon.

Keeping above facts in view, the present investigation was carried out with the following objectives: to study the genetic diversity in the collected landraces of chow-chow by using fruits, quality attributes and molecular (RAPD and ISSR) markers and to study the response of cuttings to plant growth regulators for conservation of diverse genetic resources.

Materials and methods

Plant materials

The fruits of 74 landraces of chow-chow with different characteristics were collected from the different parts of Sikkim, West Bengal, Meghalaya, Arunachal Pradesh and Nepal during September–October 2013–2014 (Tables 1, 2).

Characterization of landraces for fruit and quality traits

To evaluate the crop for different fruit and quality traits, the collected single sprouted fruits of each accession were planted at Experimental Farm of ICAR Research Complex for NEH Region, Umiam, Meghalaya situated at about 980 m above mean sea level. The fruits were planted in pits ($60 \times 60 \times 60 \text{ cm}$ size) at $3.0 \times 2.5 \text{ m}$ spacing between plant to plant and row to row, respectively in a complete randomized block design with three replications during March–November 2014 and 2015 following standard cultural practices. Three plants were randomly chosen for recording data on fruits and quality traits. The observations for quantitative traits such as average fruit length (cm), basal fruit width (cm), fruit weight (g) were taken from the six fruits in each replication and qualitative traits such as vitamin-C (mg/100 g), reducing sugar (%), total sugar (%)

Table 1 Collection sites/sources of chow–chow germplasm in North Eastern Hill regions of India

Sl. no.	Genotype	Village	District	State/country	Fruit shape	Fruit colour	Ridge
1	RCSC-1	Bhojogri	East Sikkim	Sikkim	Pyriform	Light green	Moderate
2	RCSC-3	Chhabbis Busti	Darjeeling	West Bengal	Pyriform	Light green	Prominent
3	RCSC-4	Chhabbis Busti	Darjeeling	West Bengal	Pyriform	Light green	Prominent
4	RCSC-5	Chhabbis Busti	Darjeeling	West Bengal	Pyriform	Light green	Prominent
5	RCSC-6	Chhabbis Busti	Darjeeling	West Bengal	Elongated pyriform	Green	Moderate
6	RCSC-7	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Moderate
7	RCSC-8	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Prominent
8	RCSC-9	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Moderate
9	RCSC-10	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Prominent
10	RCSC-11	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Prominent
11	RCSC-12	Lingchom	West Sikkim	Sikkim	Ovoid	Light green	Moderate
12	RCSC-13	Lingchom	West Sikkim	Sikkim	Globose	Light green	Prominent
13	RCSC-14	Lingchom	West Sikkim	Sikkim	Elongated pyriform	Light green	Prominent
14	RCSC-15	Singtom	Darjeeling	West Bengal	Elongated pyriform	Light green	Prominent
15	RCSC-16	Singtom	Darjeeling	West Bengal	Pyriform	Light green	Moderate
16	RCSC-17	Pongthong	North Sikkim	Sikkim	Pyriform	Light green	Moderate
17	RCSC-18	Singtom	Darjeeling	West Bengal	Globose	Light green	Moderate
18	RCSC-19	Pongthong	North Sikkim	Sikkim	Pyriform	Pale yellow	Moderate
19	RCSC-20	Kalimpong	Darjeeling	West Bengal	Pyriform	Pale yellow	Prominent
20	RCSC-21	Kalimpong	Darjeeling	West Bengal	Pyriform	Pale yellow	Moderate
21	RCSC-22	Lapchu	Darjeeling	West Bengal	Pyriform	Dark Green	Prominent
22	RCSC-23	Lapchu	Darjeeling	West Bengal	Pyriform	Light green	Moderate
23	RCSC-24	Pongthong	North Sikkim	Sikkim	Pyriform	Light green	Prominent
24	RCSC-25	Pongthong	North Sikkim	Sikkim	Pyriform	Light green	Moderate
25	RCSC-26	Pongthong	North Sikkim	Sikkim	Pyriform	Pale yellow	Prominent
26	RCSC-27	Pongthong	North Sikkim	Sikkim	Ovoid	Light green	Nil
27	RCSC-28	MG Market	North Sikkim	Sikkim	Pyriform	Pale yellow	Prominent
28	RCSC-29	MG Market	North Sikkim	Sikkim	Pyriform	Light green	Moderate
29	RCSC-30	MG Market	North Sikkim	Sikkim	Pyriform	Pale yellow	Prominent
30	RCSC-31	MG Market	North Sikkim	Sikkim	Ovoid	Light green	Prominent
31	RCSC-32	MG Market	North Sikkim	Sikkim	Elongated pyriform	Light green	Moderate
32	RCSC-33	MG Market	North Sikkim	Sikkim	Pyriform	Light green	Moderate
33	RCSC-34	MG Market	North Sikkim	Sikkim	Pyriform	Light green	Moderate
34	RCSC-35	MG Market	North Sikkim	Sikkim	Pyriform	Light green	Moderate
35	RCSC-36	MG Market	North Sikkim	Sikkim	Globose	Light green	Moderate
36	RCSC-37	MG Market	North Sikkim	Sikkim	Globose	Pale yellow	Prominent
37	RCSC-38	Lapchu	Darjeeling	West Bengal	Elongated pyriform	Light green	Moderate
38	RCSC-39	Lapchu	Darjeeling	West Bengal	Pyriform	Dark green	Moderate
39	RCSC-40	Pasupati	–	Nepal	Elongated pyriform	Light green	Prominent
40	RCSC-41	Sadam	South Sikkim	Sikkim	Pyriform	Green	Prominent

and phenol content (mg/100 g FW) were analyzed from the single fruit in each replication.

The estimation of the vitamin-C, reducing sugar, total sugar and phenol content in fruits were based on the procedure described by Ranganna (1979). The ascorbic acid was determined by the direct colourimetric method. It was

based on the measurement of the extent of decolorization of 2, 6-dichlorophenol indo-phenol solution (dye) by ascorbic acid available in sample extracts and standard ascorbic acid solution. Sugar content was estimated by determining the volume of unknown sugar solution of fruit pulp required for complete reduction of standard Fehling's

Table 2 Collection sites/sources of chow–chow germplasm in North Eastern Hill regions of India

Sl. No.	Genotype	Village	District	State/country	Fruit shape	Fruit colour	Ridge
41	RCSC-42	Rocky Villa	Darjeeling	West Bengal	Elongated pyriform	Light green	Prominent
42	RCSC-43	Sadam	Soth Sikkim	Sikkim	Elongated pyriform	Light green	Moderate
43	RCSC-44	Sadam	Soth Sikkim	Sikkim	Pyriform	Light green	Prominent
44	RCSC-45	Soreng	Darjeeling	West Bengal	Elongated pyriform	Light green	Prominent
45	RCSC-46	Soreng	Darjeeling	West Bengal	Pyriform	Pale yellow	Prominent
46	RCSC-47	Soreng	Darjeeling	West Bengal	Elongated pyriform	Light green	Moderate
47	RCSC-48	Soreng	Darjeeling	West Bengal	Elongated pyriform	Light green	Moderate
48	RCSC-49	Soreng	Darjeeling	West Bengal	Pyriform	Light green	Moderate
49	RCSC-50	Mangan	North Sikkim	Sikkim	Elongated pyriform	Green	Prominent
50	RCSC-51	Mangan	North Sikkim	Sikkim	Pyriform	Green	Moderate
51	RCSC-52	Mangan	North Sikkim	Sikkim	Ovoid	Light green	Moderate
52	RCSC-53	Mangan	North Sikkim	Sikkim	Elongated pyriform	Green	Moderate
53	RCSC-54	Tura	West Garo Hill	Meghalaya	Elongated pyriform	Light green	Prominent
54	RCSC-55	–	–	Arunchal Pradesh	Pyriform	Light green	Prominent
55	RCSC-56	–	–	Arunchal Pradesh	Pyriform	Light green	Prominent
56	RCSC-57	Laitkynsaw	East Khasi Hill	Meghalaya	Pyriform	Light green	Prominent
57	RCSC-58	Nongpoh	Ri-Bhoi	Meghalaya	Elongated pyriform	Light green	Moderate
58	RCSC-59	Shillong	East Khasi Hill	Meghalaya	Pyriform	Light green	Moderate
59	RCSC-60	Umroi	Ri-Bhoi	Meghalaya	Pyriform	Light green	Moderate
60	RCSC-61	Umroi	Ri-Bhoi	Meghalaya	Ovoid	Dark green	Nil
61	RCSC-62	Shillong	East Khasi Hill	Meghalaya	Ovoid	Pale yellow	Prominent
62	RCSC-63	Bhojogri	East Sikkim	Sikkim	Pyriform	Light green	Prominent
63	RCSC-64	Chhabbis Busti	Darjeeling	West Bengal	Pyriform	Green	Prominent
64	RCSC-65	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Light green	Prominent
65	RCSC-66	Pakyong	East Sikkim	Sikkim	Pyriform	Light green	Moderate
66	RCSC-67	Pakyong	East Sikkim	Sikkim	Globose	Dark Green	Prominent
67	RCSC-68	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Pale yellow	Moderate
68	RCSC-69	Pakyong	East Sikkim	Sikkim	Pyriform	Light green	Moderate
69	RCSC-70	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Light green	Prominent
70	RCSC-71	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Pale yellow	Prominent
71	RCSC-72	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Light green	Moderate
72	RCSC-73	Pakyong	East Sikkim	Sikkim	Pyriform	Light green	Moderate
73	RCSC-74	Pakyong	East Sikkim	Sikkim	Pyriform	Dark Green	Moderate
74	RCSC-75	Pakyong	East Sikkim	Sikkim	Elongated pyriform	Light green	Moderate

solution. The amount of total phenolics in extracts was determined with the Folin–Ciocalteu reagent (Lim et al. 2006). Gallic acid was used as a standard compound and the total phenols were expressed as mg g^{-1} gallic acid equivalent using the standard curve equation: $y = 0.006x + 0.039$, $R^2 = 0.998$, where y is absorbance at 760 nm and x is total phenolic content in chow–chow expressed in mg g^{-1} FW. The concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg ml^{-1} of gallic acid were prepared in methanol. Concentration of 0.1 and 1 mg ml^{-1} of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with

2.5 ml of a tenfold dilute Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature before the absorbance was at read at 760 nm spectrometrically.

DNA isolation and PCR amplification

DNA extraction and quantification

Young actively growing leaves of 1 month old plants were collected and used for DNA extraction. Total genomic

DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai-Marooft et al. 1984). The DNA quantification was performed by using Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). These estimates were confirmed by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 80 V for 1 h in Tris acetate EDTA (TAE) buffer (Promega, USA) using 100 bp DNA Ladder (Thermo Fisher Scientific, Benagluru, India).

Optimization of PCR and selection of primer

Different concentrations of template DNA (15–40 ng), Taq DNA polymerase (1–2 U) and MgCl₂ salt (0–4 mM) were used to optimize reaction conditions of the polymerase chain reaction (PCR). Five randomly selected cultivars were chosen for primer survey. Seventy RAPD primers (University of British Columbia, Canada and Operon Technologies, USA) and 30 ISSR (University of British Columbia, Canada) were surveyed with these five cultivars to identify primers that were reproducible and generated the most polymorphic patterns.

PCR and gel electrophoresis

PCR reactions were carried out in a DNA Thermal Cycler (Veriti, Applied Biosystem, CA, USA). In RAPD each 25 ml reaction mixture contained 1 × reaction buffer (10 mM Tris–HCl, pH 8.3 and 50 mM KCl), 2.5 mM MgCl₂, 1 U of Taq DNA polymerase; 200 mM each of dATP, dTTP, dCTP and dGTP (all reagents from Thermo Fisher Scientific, Benagluru, India); 0.6 mM of primer and about 30 ng of template DNA. In PCR amplification, the initial step of denaturation was at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, primer annealing at 38 °C for 45 s and primer elongation at 72 °C for 1 min, followed by an extended elongation step at 72 °C for 7 min. Like RAPD, same protocol was also followed for the ISSR analysis. Reaction products were mixed with 2 µl of 6X loading dye (ThermoFisher Scientific, Benagluru, India) and spun briefly in a microfuge before loading. The amplification products were electrophoresed on 1.8% agarose gel at 60 volts. Gels were stained with ethidium bromide and documented using a chemidoc (BioRad, California, USA).

Data analysis

Each amplification product was considered a DNA marker and was scored across all samples. Bands were recorded as present (1) or absent (0). Very faint bands were not included in final scoring. Molecular weights of the amplification products were estimated by using 100 bp DNA

ladder (Thermo Fisher Scientific, Benagluru, India) as standards. All amplifications were repeated at least twice and only reproducible bands were considered for analyses. The data set of cultivars and reproducible bands were used to calculate pair-wise similarity coefficients following Jaccard (1908). This matrix of similarity coefficients was subjected to unweighted pair group method with arithmetic mean analysis (UPGMA) to generate a dendrogram and calculating the principal components using the NTSYS-pc, version 2.1 (Exeter Software, New York). The chow–chow accessions was grouped into 4 categories (pale yellow, light green, green and dark green) depending on their fruit color (Fig. 1). These 4 groups were analyzed using POP-GENE 32 software (Yeh et al. 2000) for estimating effective number of alleles (Kimura and Crow 1964), Nei's (1973) gene diversity and Shannon's Information index (Lewontin 1972). The morphological data were analyzed by using XLSTAT (<http://www.xlstat.com/>) software. The basic information that determines their application in genetic mapping both the marker system was calculated by using the polymorphism information content (Lynch and Walsh 1998). Polymorphism information content (PIC) express the discriminating power of the locus by taking into account not only the number of alleles that are expressed, but also their relative frequencies and frequency of alleles per locus, expressed as: $PIC = 1 - \sum p_i^2$, where p_i is the frequency of the i th allele.

Vegetative propagation

To identify the suitable propagating materials, the suckers (2 months), bottom portion of vine (6–7 months old), middle portion of vine (3–4 months) and top of the defoliated shoots (2 months old) with 3 nodes of 0.5–0.6 cm diameter were selected and treated with IBA 200 mg l⁻¹. This experiment was conducted for 2 years during October to February 2013 and 2014. To study the response of plant growth regulators IBA (100, 200 and 300 mg l⁻¹ in quick dip method for 10 s) and Rooton-1 (a commercial root hormone powder, manufactured by Indo-Holland Gardening, Varanasi, India) were applied to the cuttings of suckers and further planted in black polybags having pre-sterilized moist growing medium comprising of sand: leaf mould: FYM in 1:1:1 (v/v each) ratio. Each treatment comprised of 15 cuttings (one cutting per polybag). The experiment was arranged in a randomized complete block design with three replications. The poly bags were placed in naturally ventilated polyhouse with natural light and irrigated on alternate days after planting using fogger system. The average, maximum and minimum air temperatures for the entire experiment were 23.5, 31.6 and 15.23 °C, respectively and relative humidity (RH) of 75–80%. The observations were recorded for days taken to root initiation using research

Fig. 1 Major group of chow-chow germplasm based on fruits colours. **a** Pale yellow, **b** light green, **c** green and **d** dark green

microscope (Olympus BX-53, Japan), number of roots per cutting and root length (cm) at 20 days after planting and final survival percentage in each treatment. To study the relationship between chemical properties of cuttings and survival percentage, observations on dry matter (%), chlorophyll a (mg/100 g), chlorophyll b (mg/100 g), total sugar (%), reducing sugar (%), and total nitrogen content (%) were analyzed as described by Ranganna (1979). The Pearson's correlation coefficients (r) were tested between survival (%) to chemical properties of the cuttings and rooting. The mean data was analyzed using SAS software and the means were compared using least significant difference (LSD) at $P < 0.05$.

Results

Characterization of landraces for fruit and quality traits

Out of 74 landraces, the fruits of 5 accessions were of dark green, 6 green, 53 light green and 10 pale yellow in color. Among the accessions, RCSC-27 was found to be unique having fruits fully covered with spines. The wide range of variations (Table 3) were also recorded for fruit length (6.5–21.5 cm), fruit diameter (4.2–10.7 cm), average fruit weight (60–560 g), vitamin-C (2.6–13.8 mg/100 g), reducing sugar (0.18%), total sugar (1.09–2.94%) and

phenol content (0.17–3.85 mg/100 g FW). Fruit length, fruit width and fruit weight found significantly negative correlated with reducing sugar and total sugar content. Among quality traits, significant positive correlation was observed between reducing sugar and Vitamin-C content. Phenol content and vitamin-C content was found higher in accessions with dark green fruits with significant and positive correlation of $r = 0.40$ and 0.29 , respectively (Fig. 2).

In clustering pattern, all 74 accessions were grouped in 5 different clusters (Table 4). Maximum numbers of accessions were retained in cluster I (66) followed by cluster II (5) and rest were mono-genotypic. However, the maximum fruit length (21.5 cm), and fruit weight (550 g) was recorded from the collection RCSC-40 of Nepal. The highest fruit weight (525 g) with phenol content (2.66 mg/100 g) was recorded from the landrace RCSC-41 (green fruit) of the cluster-III. The maximum contribution towards divergence was due to phenol content followed by vitamin-C and total sugar content.

Principal components analysis performed on quantitative traits revealed that the first three most informative components accounted for 77.74% variance. Important characters with greater weightage in principal component axis I included fruit length, fruit width and fruit colour. Important characters with greater weightage in principal component axis II comprised of quality traits i.e., vitamin-C content, reducing sugar and total sugar. The groupings

Table 3 Variations in fruits and quality traits among chow–chow landraces

Characters	Minimum	Maximum	Mean	Standard deviation	Skewness	Kurtosis
Fruit length (cm)	6.5	21.5	12.24	2.26	0.36	2.59
Fruit width (cm)	4.2	10.7	7.26	1.14	−0.06	0.83
Fruit weight (g)	60	560	270.7	102.9	0.41	0.47
Vitamin-C (mg/100 g)	2.6	13.8	7.73	2.29	0.6	0.11
Reducing sugar (%)	0.18	2.77	1.83	0.39	−0.63	2.91
Total sugar (%)	1.09	2.94	2.01	0.39	0.2	−0.44
Phenol (mg/100 g)	0.17	3.85	1.4	0.96	0.52	−0.8

Fig. 2 Relationships among vitamin-C and phenol content with fruit color**Table 4** Cluster means for different characteristics in chow–chow landraces

Cluster	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Vitamin-C (mg/100 g)	Reducing sugar (%)	Total sugar (%)	Phenol (mg g ^{−1})
I	12.3	7.37	274	7.81	1.82	1.99	1.33
II	8.5	4.92	95	7.7	2.49	2.66	1.99
III	16.2	10.7	525	5.2	1.32	1.46	2.66
IV	14.5	8.3	380	5.2	0.18	2.02	1.61
V	21.5	7.7	550	7.8	1.21	1.32	1.44

obtained through cluster analysis in general, confirmed by the principal components analyses (Fig. 3).

Molecular characterization

Template concentration of 30 ng was found to be the suitable concentration as it gave maximum number of reproducible bands. Similar variation in the concentration of Taq DNA polymerase and MgCl₂ showed best results with 1U of Taq DNA polymerase and 2.5 mM of MgCl₂. Out of the 70 RAPD and 30 ISSR primers surveyed, 28 RAPD and 5 ISSR primers were selected for the analyses

as others were found as monomorphic. All the 74 landraces were subjected to PCR amplifications using these 40 primers. A total of 198 amplification products were scored with different RAPD primers, which exhibited an overall 88.38% polymorphism (Table 5). The average numbers of amplification products formed were 7.01 with a maximum of 13 in UBC-155 and a minimum of 3 in OPAY-06 and OPA-11. The size of the amplification products varied in case of each primer and the range was 0.15–1.5 kb. In general, the extent of polymorphism was found to be moderate. 15 out of 28 RAPD primers showed 100% polymorphism and one primer (OPAY-06) showed less

Fig. 3 Principal component (PCA) scatter plot of 74 chow-chow germplasm based on fruits and quality traits

than 50% polymorphism. Highest number of polymorphic fragments were observed in primer OPAB-09, UBC-155 and UBC-228 (11 polymorphic products for each) whereas least number of polymorphic fragments were observed in OPE-9, OPB-04 and OPA-15 (2 polymorphic products for each). The PIC of the RAPD primers was in the range 0.14 (OPAB-14) to 0.70 (OPA-09) with an average of 0.42.

A total of 32 amplification products were scored in the 74 landraces with different ISSR primers, which exhibited an overall 62.5% polymorphism (Table 5). The size of the amplification products varied in case of each primer and the range was 0.12–1.0 kb. The average numbers of amplification products formed were 6.4 with a maximum of 8 in ISSR-822 and a minimum of 5 in ISSR-824. In general, the extent of polymorphism found was moderate. Highest number of polymorphic fragments were observed in Primer ISSR-822 (5 polymorphic products) whereas least number of polymorphic fragments was observed in ISSR-814 (3 polymorphic products). The PIC of the ISSR primers was in the range 0.03 (ISSR-822) to 0.37 (ISSR-824) with an average of 0.22.

In the group based diversity analysis (fruit color) using RAPD and ISSR markers, the highest percentage of polymorphic fragments were observed in light green group (Table 6). Eight markers i.e., OPC-08 (1275 bp), OPC-08 (825 bp), OPA-2 (900 bp), OPAB-14 (650 bp), UBC-135

(1000 bp), UBC-135 (800 bp), OPB-04 (600 bp) and OPL-16 (1175 bp) were found specific to landraces with light green fruits. Four markers (UBC-155 (1400 bp), OPC-02 (1300 bp), UBC-228 (900 bp) and OPF-06 (875 bp)) were found specific to RCSC-22 (dark green fruit) and another 4 (OPAB-09 (1300 bp), UBC-228 (1200 bp) UBC-135 (950 bp) and OPA-12 (1200 bp)) to RCSC-30 (pale yellow fruit). Based on Nei's (1973) original measures of genetic distance among different groups based on RAPD and ISSR markers, light green fruits were found closer to pale yellow color fruit than the dark green and green fruit (Table 7).

The data obtained from RAPD and ISSR were subjected to UPGMA analysis to find out the relationship between the accessions analyzed. The value of Jaccard's similarity coefficient ranged from 0.55 to 0.96. In cluster analysis, 56 landraces form a large cluster whereas rest of 18 landraces was present in cluster of one or two accessions (Fig. 4). The large cluster may be subdivided in two sub-clusters and have accessions from the entire region included in this study. Two landraces from South Sikkim (RCSC-7 and RCSC-11) appear to be distinct from all others.

The grouping of accessions based on principal component analyses were found similar to the UPGMA cluster. A total of 61 amplification products were required to explain up to 99% of the total variations observed among the cultivars. This was indicative of the presence of high

Table 5 Details of primers used for genetic diversity analysis of chow–chow landraces

Primer name	TNA	NPA	Polymorphism (%)	PIC	Size range (bp)
<i>RAPD</i>					
OPC-08	8	8	100	0.58	400–1275
OPR-06	5	3	60	0.53	575–1300
UBC 135	4	4	100	0.65	750–1000
OPAJ-14	5	3	60	0.40	400–1125
OPE-09	3	2	67	0.23	500–1250
UBC-253	8	8	100	0.29	450–1400
OPH-03	7	5	71	0.15	775–1300
OPL-04	7	7	100	0.39	475–1275
OPX-17	5	5	100	0.65	250–1200
OPF-06	4	4	100	0.59	250–875
OPAB-14	7	5	71	0.14	650–1250
UBC-296	5	5	100	0.21	450–1075
OPAD-12	12	10	83	0.33	150–1150
OPA-02	9	9	100	0.50	600–1200
OPV-19	10	10	100	0.44	250–1050
OPA-09	7	7	100	0.70	500–1125
OPAY-06	3	1	33	0.31	750–1100
OPAB-09	12	11	92	0.47	500–1300
UBC-155	13	11	85	0.50	250–1400
UBC-228	11	11	100	0.55	400–1200
OPL-16	12	10	83	0.34	300–1300
OPC-02	10	10	100	0.35	350–1300
OPB-07	8	8	100	0.46	400–1500
OPB-04	4	2	50	0.50	550–1100
OPA-11	3	3	100	0.44	900–1175
OPA-12	4	3	75	0.53	500–1200
OPA-15	4	2	50	0.26	375–825
OPAK-04	8	8	100	0.31	300–1200
<i>ISSR</i>					
ISSR-813	6	4	67	0.17	325–1000
ISSR-814	6	3	50	0.33	275–625
ISSR-822	8	5	63	0.03	250–650
ISSR-824	5	4	80	0.37	125–525
ISSR-827	7	4	57	0.21	250–600
Total	230	195	85	–	–

TNA total number of amplicon, *NPA* number of polymorphic amplicons, *PIC* polymorphic information content (mean)

Table 6 Group based diversity (summary) of chow–chow landraces using RAPD and ISSR markers

Group	na	ne	h	I	Polymorphic loci	Polymorphic %
Light Green	1.78 ± 0.41	1.46 ± 0.37	0.27 ± 0.19	0.40 ± 0.27	181	78.7
Pale Yellow	1.53 ± 0.50	1.36 ± 0.39	0.20 ± 0.21	0.30 ± 0.17	121	52.61
Green	1.25 ± 0.43	1.19 ± 0.35	0.18 ± 0.10	0.26 ± 0.15	59	25.65
Dark Green	1.49 ± 0.44	1.35 ± 0.41	0.26 ± 0.17	0.38 ± 0.26	113	49.13

na, observed number of alleles; ne, effective number of alleles (Kimura and Crow 1964); h, Nei's (1973) gene diversity; I, Shannon's Information index (Lewontin 1972)

genetic diversity at various RAPD loci in the accessions analyzed. The first 3 most informative principal components explained only 26.52% of the total variation.

Vegetative propagation

The results on vegetative propagation through cuttings have shown the significant effect of plant growth regulator (auxin) on rooting and survival percentage of the cuttings. Of the treatments, root initiation was observed between 15 and 18 days after planting. The 45–60 days old cuttings/seedlings become ready for transplanting (Fig. 5). Among the plant growth regulators, the maximum number of roots, root length and survival percentage was recorded from the cuttings treated with IBA 300 mg l⁻¹ and it was at par with IBA 200 mg l⁻¹ (Table 8).

Among the cuttings, the highest survival was recorded in the cuttings from suckers followed by top shoot and middle shoots (Table 9). The results on chemical analyses of

different cuttings revealed the highest chlorophyll a and b content, reducing sugar and total nitrogen content was recorded in the cuttings from suckers followed by top shoot and least in cuttings of bottom portion of vines (Table 9). The results have also shown the positive and significant correlation between survival percentage to chlorophyll a ($r = 0.58$) and b ($r = 0.63$), reducing sugar ($r = 0.65$) and total nitrogen ($r = 0.79$) content. However, the negative correlation was observed with dry matter (-0.80) and total sugar ($r = -0.12$).

Discussion

In present study, wide variations for fruit and quality traits were recorded among landraces. Based on cluster analysis, maximum variation for fruit and quality traits was observed in the collection of Sikkim and Darjeeling (West Bengal) accessions. However, accessions from different states grouped in the same cluster (I and II) revealed that germ-plasm distribution did not necessarily determine genetic divergence. Similar findings were also reported by Sanwal et al. (2008) in chow-chow. The genotypic differences among the landraces were possibly due to their wide geographic distribution and considerable morphological variation with respect to fruit shape, size and color (Dey et al. 2006). The crop is propagated through fruits and the possible reason for grouping of accessions of different states in

Table 7 Nei's original measures of genetic distance among different group based on RAPD and ISSR markers

Group	Light green	Pale yellow	Green
Pale yellow	0.0295		
Green	0.078	0.0812	
Dark green	0.0568	0.0847	0.1192

Fig. 4 Genetic similarity relationships among 74 chow-chow landraces based on Jaccard's coefficient of similarity (Jaccard 1908) using RAPD and ISSR markers

Fig. 5 Developmental stages in chow-chow cuttings. **a** Section of vascular bundle of vine, **b** development of root hair in vascular bundle, **c** section of root hair, **d** transplanting

Table 8 Chemical properties of chow-chow cuttings and response to IBA (200 mg l^{-1}) on survival per cent

Cuttings	Dry matter (%)	Chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Total sugar (%)	Reducing sugar (%)	Nitrogen (%)	Survival (%)
Bottom	10.75 ^a	0.03 ^b	0.02 ^b	4.78 ^c	0.80 ^c	0.81 ^d	2.93 ^d
Middle	10.01 ^b	0.04 ^b	0.02 ^b	5.37 ^a	0.76 ^c	1.05 ^c	46.66 ^c
Top shoot	7.49 ^c	0.04 ^b	0.02 ^b	5.24 ^b	1.31 ^b	1.39 ^b	66.77 ^b
Suckers	7.34 ^c	0.13 ^a	0.05 ^a	4.1 ^d	2.56 ^a	2.68 ^a	69.55 ^a
LSD	0.57	0.01	0.01	0.08	0.32	0.10	1.80
<i>(P < 0.05)</i>							

Means with the same letter in column are not significantly different at $P < 0.05$ level

Table 9 Effect of plant growth regulators on rooting and survival of cuttings (suckers) of chow–chow

PGR	Root numbers	Root length (cm)	Survival (%)
IBA-100 mg l ⁻¹	5.0 ^c	9.0	53.6 ^c
IBA-200 mg l ⁻¹	7.1 ^{ab}	10.5	67.0 ^a
IBA 300 mg l ⁻¹	7.7 ^a	10.2	68.3 ^a
Rooton	5.3 ^c	9.6	63.3 ^b
LSD (<i>P</i> < 0.05)	1.8	NS	2.57

Means with the same letter in column are not significantly different at *P* < 0.05 level

one cluster could be the free movement of the popular landraces from one growing region to another by the traders due to difference in the availability period of the fruits within the North Eastern states. In chow–chow, fruit traits like length, width and weight being some highly heritable traits (Sanwal et al. 2008), the characterization data of fruit of different landraces will be useful for selecting suitable parents for hybridization program. Estimation of mean value for different clusters helps in identifying parents for crossing to generate variability in breeding population. The crop has gained its importance as it possesses several medicinal properties due to richness in polyphenol (Ibarra-Alvarado et al. 2010), flavonoids especially in leaves followed by roots and stems (Siciliano et al. 2004). The wide range of variation was observed for phenol content in fruits. Among the landraces, RCSC-41 (Cluster V) having high phenol content (2.66 mg/100 g) with higher fruit weight (525 g) may be used as a parent for selection and hybridization program.

For assessing the genetic diversity in underutilized crops, where no or very little genetic information available, random molecular markers like RAPD and ISSR are very useful for studying pattern of genetic diversity. The RAPD and ISSR markers used in this study were found to be informative in distinguishing the landraces collected from the NEH region of India. The range of polymorphism in RAPD and ISSR was 33–100% and 50–80%, respectively. Likewise, the range of similarity coefficient was 0.55–0.96 between the landraces based on molecular markers indicating moderate level of genetic diversity in the landraces. This may be due to free movement of the materials across the locations having differences in the time of availability of fruits and cross pollination. The moderate genetic diversity has also been observed in other minor cucurbits such as *Benincasa hispida* (0.69–0.93) by Verma et al. (2007) and 0.47 in *Momordica dioica* and 0.42 in *Momordica subangulata* by Bharathi et al. (2012) by using of RAPD and ISSR markers.

The results on responses of plant growth regulators (IBA and Rooton-1) have shown the significant increase in the rooting of cuttings of suckers with increase in

concentration of IBA. The highest survival of cuttings of suckers treated with IBA (300 mg l⁻¹) is probably due to better rooting as it has shown significant positive correlation between number of roots and survival percent (*r* = 0.828). The increase in rooting in cuttings of shoots and suckers may also be due to increase in the auxin biosynthesis over the other plant parts as the apical meristems are the primary sites of auxin biosynthesis (Taiz and Zeiger 2002). Similar finding was also reported by Elhaak et al. (2015) in rosemary. Also, in earlier report the use of IBA in stimulating rooting was appreciated as it is not toxic to plants at different concentrations and also it improves root initiation with uniformity (Hartmann et al. 1990).

Among the propagating materials, the highest survival in suckers and top shoot may be due to better rooting and also due to positive correlation with chlorophyll a and b, reducing sugar and nitrogen content and higher accumulation in the cuttings of suckers and top shoots over the other plant parts (Table 8). These results were in agreement with that recorded by El-Abd (1997) in cucumber and El-Eslamboly (2014) in watermelon. The positive effect of nitrogen content and reducing sugar is crucial in determining the rooting in cuttings of chrysanthemum (Druege et al. 2000) and *Euphorbia pulcherima* (Zerche and Druege 2009). The root formation is essential for establishing a cutting of plant. The successful initiation and formation of root in a plant cutting primary depends on plant growth regulators, nutrients availability and growth stage (Hartmann et al. 2002).

Conclusions

The results on genetic diversity analysis of the chow–chow accessions of NEH region has shown moderate to high genetic similarity as shown by values of Jaccard's similarity coefficient and PCA. Fifty-six landraces from the entire region included in this study form a large cluster. The grouping of accessions observed was not found according to their geographic distribution. Since the genetic drift and selection in different ecological niche may create greater genetic variability; it could be a reason for clustering of accessions from different geographic regions in same. The markers associated with nutritionally superior dark green accessions (Verma et al. 2014) may be further used for the hybridization and selection. The unique and diverse genetic resources of chow–chow can be conserved by using suckers and top shoots as a propagating materials treated with 200 mg l⁻¹ of IBA. Further, vegetative propagation will not only help in conserving the genetic resources but also in saving the produce, off season production and exploitation of heterosis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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