RESEARCH ARTICLE

Genetic diversity of cucumber estimated by morpho-physiological and EST-SSR markers

Sudhakar Pandey¹ · Waquar Akhter Ansari¹ · Maneesh Pandey¹ · Bijendra Singh¹

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Abstract In the present study, genetic variation among 40 cucumber genotypes was analyzed by means of morpho-physiological traits and 21 EST-SSR markers. Diversity was observed for morpho-physiological characters like days to 50% female flowering (37–46.9, number of fruits/plant (1.33–5.80), average fruit weight (41–333), vine length (36–364), relative water content (58.5–92.7), electrolyte leakage (15.9–37.1), photosynthetic efficiency (0.40–0.75) and chlorophyll concentration index (11.1–28.6). The pair wise Jaccard similarity coefficient ranged from 0.00 to 0.27 for quantitative traits and 0.24 to 0.96 for EST-SSR markers indicating that the accessions represent genetically diverse populations. With twenty-one EST-SSR markers, polymorphism revealed among 40 cucumber genotypes, number of alleles varied 2–6 with an average 3.05. Polymorphism information content varied from 0.002 to 0.989 (mean = 0.308). The number of effective allele (N_e), expected heterozygosity (H_e) and unbiased expected heterozygosity (uH_e) of these EST-SSRs were 1.079–1.753, 0.074–0.428 and 0.074–0.434, respectively. Same 21 EST-SSR markers transferability checked in four other *Cucumis* species: snapmelon (*Cucumis melo* var. *momordica*), muskmelon (*Cucumis melo* L.), pickling melon (*Cucumis melo* var. *conomon*) and wild muskmelon (*Cucumis melo* var. *agrestis*) with frequency of 61.9, 95.2, 76.2, and 76.2%, respectively. Present study provides useful information on variability, which can assist geneticists with desirable traits for cucumber germplasm

utilization. Observed physiological parameters may assists in selection of genotype for abiotic stress tolerance also, EST-SSR markers may be useful for genetic studies in related species.

Keywords EST-SSR · Morpho-physiological markers · Genetic relationship · Transferability · *Cucumis sativus*

Introduction

Cucumber (*Cucumis sativus* L. $2n = 2x = 14$), is one of the most important and widely cultivated vegetables crops, it originates from India, and China is extensively considered as its secondary centre of origin (Staub et al. 1997). Total world production of cucumber in India was 171 mt tons from 26,982 ha area (FAOSTAT 2014). The productivity of cucumber in India is 6.34 mt/ha as compare to world which is 30.23 mt/ha (Pandey et al. 2013). Productivity of cucumber in India is about 5 times lower as compare to world productivity. In spite of its considerable morphological variability, cucumber showed a narrow genetic base (Staub et al. 1997), which restricts development of new cucumber cultivars by cross-breeding. Being a largely consumed vegetable cucumber had great scope to improve the production productivity to meet the requirement by adoption of improved varieties/hybrids (Singh et al. 2016, Pandey et al. 2016). In India, 10 cucumber varieties and hybrids has been released for commercial cultivation based on All India Crop Research Programme on Vegetable Crop (AICRP-VC) results (Singh et al. 2015). Choice of selection of good parents for hybridization depends upon the variation available in the gene pool. Identification of the parents is the most important step for

✉ Sudhakar Pandey
sudhakariivr@gmail.com; Sudhakar.Pandey@icar.gov.in

¹ ICAR-Indian Institute of Vegetable Research, Post Box-01,
P.O.-Jakhani (Shahanshahpur), Varanasi,
Uttar Pradesh 221305, India

breeding programme, which is at present entirely based on morphological trials in cucumber (Pandey et al. 2013).

Diversity is the foundation of novel genes for countering pressure to agricultural production rising due to various biotic or abiotic factors (Gepts 2006). Cucumber survives in varying climate conditions and also act as a very important food source in difficult times. These fascinating properties make cucumber an interesting crop for a detailed and widespread characterization of its germplasm.

Recent advancement in plant genomics especially in the area of comparative genomics, is offering new opportunities to assess the genetic diversity at molecular level. The level of genetic dissimilarity in cucumber was assessed by many researchers using a number of DNA markers (Zhuang et al. 2008, Sikdar et al. 2010), which present functional genetic knowledge for cucumber cross-breeding. However, these markers are not expected to have close linkage to the transcribed sequences. Among the molecular markers, EST-SSRs is a new marker system which may be functionally more informative than genomic SSR (gSSR), that can be applied for analysis of functional diversity present in gene rich regions of genome and is found more conserved (Zhang et al. 2005). EST-microsatellite (eSSR) polymorphism is associated with transcribed regions of the genome and reflects the genetic diversity inside or adjacent to the genes (Varshney et al. 2005). EST-SSRs are generally developed by EST database electronic search (Yasodha et al. 2008). EST-SSRs have been developed and used successfully in watermelon (Hwang et al. 2011) cucumber and melon (Levi et al. 2006; Kong et al. 2007; Hu et al. 2010a, b, 2011), for molecular mapping of important traits and DNA fingerprinting. But we are not aware of any report on the assessment of genetic diversity in Indian collection of cucumber, using EST-SSR markers.

In this study, we analyzed the genetic diversity of released Indian cucumber varieties and germplasm, collected from different regions. Molecular study was based on EST-SSRs, while morpho-physiological observation was recorded using yield related traits, relative water content (RWC), electrolyte leakage (EL), photosynthetic efficiency (F_v/F_m) and chlorophyll concentration index (CCI).

Materials and methods

Plant materials and DNA extraction

To study the genetic diversity, a total of 40 cucumber accessions were used (Table 1). Four related species of *Cucumis* i.e., *Cucumis melo* var. *momordica* (snampelon), *Cucumi smelo* (muskmelon), *Cucumis melo* var. *conomon*

(pickling melon) and *Cucumis melo* var. *agrestis* (wild muskmelon), were used for studying the transferability of cucumber EST-SSRs. Seed samples were obtained from gene pool maintained at Indian Council of Agricultural Research (ICAR)-Indian Institute of Vegetable Research (IIVR), Varanasi, India. Ten seeds of each accession were planted in mixture of vermiculite and coco peat in portray. The genomic DNA was extracted from young leaf tissues at the true 4–5 leaf stage. Extraction was performed by using CTAB method with minor modification (Doyle and Doyle 1990).

Field evaluation and data collection

The experiment was conducted on experimental site of ICAR-IIVR, Varanasi. The soil type of the experiment was sandy loam with average pH in the range of 7.0–8.0. Ten plant of each genotype were sown, with a row spacing of 100 cm and 60 cm spacing between the plants, in a complete randomized block design (CRBD) with three replications. Five plants were randomly chosen and data were recorded on 7 quantitative traits, i.e. (1) days of 50% female flowering, (2) number of branches per plant, (3) vine length (cm), (4) number of fruit per plant, (5) average fruit weight (g), (6) fruit polar length (cm) and (7) fruit equatorial length (cm). The 2-year pooled data for quantitative traits were analyzed for CRBD (Gomez and Gomez 1984).

In same experiment the RWC and EL of leaf tissues were measured according to Khare et al. (2010). To measure the RWC, 10 leaf discs were freshly weighed (FW). The same tissues were rehydrated in water for 6 h until fully turgid, surface-dried and reweighed [turgid weight (TW)] followed by oven-drying at 80 °C for 24 h, and reweighed [dry weight (DW)]. Calculation of RWC was carried by the following equation: $RWC \% = (FW - DW / TW - DW) \times 100$. Conductivity meter (CM-180, Elico, India) were used for the measurement of EL: 12 leaf discs were placed in 20 ml Milli Q water and kept at room temperature for 4 h (a) and then after autoclaving at 121 °C for 30 min (b). The EL was calculated as: $EL (\%) = a / b \times (100)$. F_v/F_m was recorded using a portable Handy Plant Efficiency Analyzer (Hansatech Instruments, King's Lynn, Norfolk, UK). The leaves were exposed to dark for 30 min using leaf clips on the adaxial side. Red light was used to irradiate the leaf surface; the fluorescence signal was collected at excitation irradiance, set at 3000 $\mu\text{mol}/\text{m}^2 \text{ s}$ from the same surface. Minimum (F_0) and maximum (F_m) chlorophyll fluorescence of the dark-adapted leaf was recorded and maximum quantum efficiency of photosystem II (PSII) was calculated according to: $F_v/F_m = (F_m - F_0) / F_m$ (Maxwell and Johnson 2000). CCI of the leaf was

Table 1 Details of *cucumber* genotypes used in the present study

S. no.	Germplasm name	Country	Source (place of collection)	Agro-climatic zone in India ^a
1	K-75	India	Himachal Pradesh	I
2	Kalyanpur Green	India	Uttar Pradesh	IV
3	Swarna Sheetal	India	Jharkhand	IV
4	Swarna Poorna	India	Jharkhand	IV
5	Pant Khirai	India	Uttanchal	I
6	Punjab Naveen	India	Punjab	IV
7	VRC-26	India	Uttar Pradesh	IV
8	Cucumber Long Green	India	Jammu	I
9	K-90	India	Himachal Pradesh	I
10	IIHR-374	India	Jharkhand	IV
11	IIHR-285	India	Karnatka	VIII
12	IIHR-34	India	Maharastra	VII
13	IIHR-81	India	Uttar Pradesh	IV
14	IIHR-414	India	Chatishgarh	V
15	IIHR-258	India	Odisha	V
16	IIHR-407	India	Maharastra	VII
17	IIHR-271	India	Himachal Pradesh	I
18	IIHR-82	India	Uttar Pradesh	IV
19	IIHR-337	India	Jharkhand	IV
20	IIHR-409	India	Maharastra	VII
21	IIHR-341	India	Jharkhand	IV
22	IIHR-264	India	Odisha	V
23	IIHR-3	Canada	Canada	–
24	IIHR-266	Japan	Japan	–
25	SPP-98	India	Uttar Pradesh	IV
26	SPP-31	India	Uttar Pradesh	IV
27	CH-122	India	Jharkhand	IV
28	SKY/DR/RS-131	India	Uttar Pradesh	IV
29	SKY/DR/RS-133	India	Uttar Pradesh	IV
30	VRC-19	India	Uttar Pradesh	IV
31	Pilibhet Local	India	Uttar Pradesh	IV
32	BSC-4	India	Uttar Pradesh	IV
33	Patna-3	India	Bihar	IV
34	VR-101	India	North Eastern Hill Region	III
35	SPP-56	India	Uttar Pradesh	IV
36	CU-5	India	Uttar Pradesh	IV
37	SPP-26	India	Uttar Pradesh	IV
38	SPP-63	India	Uttar Pradesh	IV
39	VRC-27	India	Uttar Pradesh	IV
40	Hobankha	India	Uttar Pradesh	IV

^a As per classification of All India Coordinated Improvement Project-Vegetable Crops

recorded using CCM-200 Portable Chlorophyll Meter (Opti-Sciences, Tyngsboro, MA) at the ratio of 655/940 nm. Five plants were randomly selected and their 1st, 2nd and 3rd fully expanded leaf from the top of the main stems was used for measurement during 10.00–11.00 h (Bahadur et al. 2009).

EST-SSR marker selection, physical position on chromosome and functional annotation

21 EST-SSRs from *C. sativus*, developed by Hu et al. (2010a, b) and proven to be polymorphic, were used. Chromosome number has been assigned to each EST-SSR through Gy14 assembly also functional class was assigned

to each EST-unigene based on BLASTx analysis (<http://blast.ncbi.nlm.nih.gov/blast.cgi>), according to degree of similarity (E-value) with the closest counterpart sequence found in other plant species.

EST-SSR amplification

The PCR reaction mixture in 15 µl volume was composed of 1.5 µl 10 × buffer, 0.6 µl MgCl₂ (25 mM), 0.18 µl dNTP (25 mM), Taq polymerase 0.3 µl (5U/µl), 9.42 µl dH₂O, 1 µl forward primers (10 pm), 1 µl reverse primer (10 pm) and 1 µl (40 ng) DNA. All the reagents used belong to (MBI Fermentas, USA). The amplification reactions were carried out in a thermo cycler (Bio-rad, Dyad, USA) at initial denaturing step at 94 °C for 4 min followed by 36 cycles of 94 °C for 1 min, 52–56 °C for 1 min and 72 °C for 1 min. In the last cycle, primer extension at 72 °C for 10 min and storage 4 °C forever. The expected size of fragments resulting from amplification was observed on 2.5% agarose gel. Amplification products were visualized in a gel documentation system (Alfa Imager 2200, Alfa Innotech Corporation, California). The 100-bp DNA ladder (MBI Fermentas, USA) was used as molecular size marker. The amplification was repeated 2–3 times to ensure that the amplification obtained with the primers is reproducible and consistent.

EST-SSR analysis

The genomic DNA fragments from EST-SSR primers generated clear and unambiguous bands of various molecular weight sizes were scored as present (1) and absence (0) for each of the 40 genotypes in the form of binary matrix. NTSYS-pc version 2.11 W (Rohlf 1998) were used to analyze data matrix. The Jaccard's coefficient was calculated using SIMQUAL program. The similarity matrix was computed using EST-SSR markers following the UPGMA methods using SHAN programme of NYSYSpc for 40 cucumber genotypes, to estimate similarity indices and genetic relatedness among the genotypes. A dendrogram was then constructed applying the unweighted pair group method with arithmetic mean (UPGMA) cluster algorithm. Polymorphic information content (PIC) value of each EST-SSR markers was calculated as per the formula (Anderson et al. 1993): $PIC_i = 1 - \sum P_{ij}^2$ Where PIC_i is the polymorphic information content of a marker i ; P_{ij} is the frequency of the j th pattern for marker i and the summation extends over n patterns. Numbers of effective alleles (N_e), expected heterozygosity or observed heterozygosity (H_e) and unbiased expected heterozygosity (uH_e) were analyzed using GenAEx 6.1 (Peakall and Smouse 2006).

Results

Genotype performance based on morphological parameters

The mean value of yield and yield-associated traits of the genotypes are given in Table 2. The days to 50% female flowering indicates the earliness in fruiting of the genotypes. The genotype BSC-4 was very early in appearance of 50% female flowering (37 days after seed sowing), whereas, IIHR-81 was recorded to be the latest (49 days after seed sowing) in 50% female flowering. The number of primary branches was maximum (4.0) in the genotype IIHR-407 while this value found lowest for the genotype SKY/DR/RS-131 (2.60). Vine length (364 cm) was maximum in the variety VRC-26 while lowest (36.0 cm) for BSC-4. Genotype IIHR-264 exhibited maximum average fruit weight (333 g), while maximum number of fruits per plant (5.80) was recorded in variety Swarna Poorna. The number of fruits was lowest in the genotype IIHR-271 (1.33). The polar (51.4 cm) and equatorial (29.8 cm) circumference of fruit was maximum in IIHR-264 and Kalyanpur Green, respectively. Variability in cucumber germplasm was also observed for days taken to appearance of first male and female flowering, 50% male flowering and root length (data not presented).

Genotype performance based on physiological parameters

RWC, EL, F_v/F_m , CCI, values of the 40 cucumber genotypes are shown in Table 3. RWC found highest (92.7%) of genotype SPP-56 followed by IIHR-264 (88.5%), whereas it was lowest in the genotype BSC-4 (58.5%). EL of the genotypes ranged from 15.9 (IIHR-341) to 37.1% (IIHR-414). Leaf chlorophyll fluorescence (F_v/F_m), was highest of the genotype IIHR-264 (0.75), while this value was lowest for the genotype IIHR-82 (0.40). CCI of the genotype ranged from 11.1 (CU-5) to 28.6 (Kalyanpur green).

Genotype cluster analysis based on morpho-physiological traits

The taxonomic distance matrix of 10 quantitative traits was employed for cluster analysis of the 40 genotypes (Fig. 1). All the cucumber genotypes grouped into three major clusters, the longest branch separated IIHR-258 Swarna Poorna from the rest of the accessions at a taxonomic distance of 0.27 and this accession was the lone member of the cluster III. The first major cluster (cluster I) consisted of twenty-four genotypes, which is further grouped into three sub-cluster i.e. IA (5 genotypes), IB (12 genotypes),

Table 2 Mean of the 7 quantitative characters of 40 cucumber genotypes

S. no.	Genotype	Days of 50% female flowering	No. of primary branching	Vine length (cm)	No. of fruits/plant	Average fruit wt (g)	Fruit polar length (cm)	Fruit equatorial width (cm)
1	K-75	46.00	3.25	234.60	3.00	153.25	37.50	18.20
2	Kalyanpur Green	40.00	3.00	198.50	3.40	155.00	47.30	29.80
3	Swarna Sheetal	42.00	3.10	154.40	4.86	131.60	41.80	24.00
4	Swarna Poorna	45.00	3.33	206.80	5.80	174.50	32.20	23.00
5	Pant Khirai	40.00	3.00	207.30	2.88	159.33	38.40	27.40
6	Punjab Naveen	42.00	3.10	183.00	4.68	158.40	35.60	28.40
7	VRC-26	41.00	3.00	364.25	2.30	156.00	29.32	17.80
8	Cucumber Long Green	41.00	3.20	193.80	3.50	139.00	49.80	18.20
9	K-90	48.00	3.40	224.00	3.66	130.00	44.25	23.40
10	IIHR-374	41.00	3.50	124.00	2.50	64.80	23.40	15.60
11	IIHR-285	38.00	3.40	180.00	2.67	77.00	26.20	18.00
12	IIHR-34	41.00	3.00	146.00	2.00	41.00	24.25	13.00
13	IIHR-81	49.00	3.20	131.66	2.00	112.00	24.50	17.20
14	IIHR-414	43.00	3.25	116.00	2.50	198.20	47.00	22.60
15	IIHR-258	45.00	3.33	101.66	2.75	280.60	49.50	26.40
16	IIHR-407	42.00	4.00	75.00	3.50	41.80	24.20	11.40
17	IIHR-271	39.00	3.25	95.00	1.33	80.00	25.00	11.00
18	IIHR-82	39.00	3.00	207.33	4.00	104.00	28.20	17.00
19	IIHR-337	46.00	3.00	73.33	2.50	65.20	21.20	15.40
20	IIHR-409	46.00	3.00	198.66	2.00	76.60	26.50	15.50
21	IIHR-341	38.00	3.20	62.25	2.00	78.25	17.50	13.25
22	IIHR-264	42.00	3.50	157.00	2.60	333.20	51.40	18.60
23	IIHR-3	43.00	3.66	79.66	1.67	132.00	23.60	15.20
24	IIHR-266	41.00	3.60	151.66	1.40	110.00	29.20	18.20
25	SPP-98	39.00	3.00	46.00	1.67	69.20	24.20	15.40
26	SPP-31	40.00	3.50	52.00	4.72	115.00	26.25	15.50
27	CH-122	39.00	3.20	137.50	3.50	65.20	18.00	13.00
28	SKY/DR/RS-131	38.00	2.60	95.00	1.29	235.00	27.00	19.85
29	SKY/DR/RS-133	42.00	3.00	234.33	1.50	65.40	24.40	12.20
30	VRC-19	39.00	3.00	72.33	2.00	85.20	19.50	14.00
31	Pilibhet local	38.00	3.66	57.66	2.67	151.20	39.00	22.00
32	BSC-4	37.00	3.40	36.00	2.00	85.50	27.00	16.00
33	Patna-3	41.00	3.50	107.00	2.50	60.00	19.20	13.20
34	VR-101	38.00	2.80	78.33	4.00	75.50	23.50	15.25
35	SPP-56	43.00	3.50	140.00	3.25	225.00	28.50	19.75
36	CU-5	44.00	3.20	47.00	1.50	78.00	17.00	22.00
37	SPP-26	39.00	3.00	78.00	4.50	111.00	28.20	20.00
38	SPP-63	43.00	3.00	95.00	2.75	140.80	46.00	23.20
39	VRC-27	41.00	3.40	107.00	3.00	217.00	36.00	18.00
40	Hobankha	42.00	3.20	75.00	2.00	90.40	22.00	16.00
CD (< 0.05%)		0.926	0.086	22.231	0.350	20.884	3.195	1.514

Table 3 Relative water content (RWC %), electrolyte leakage (EL %), photosynthetic efficiency (F_v/F_m) and chlorophyll concentration index (CCI)

S. no.	Genotype	RWC %	EL %	F_v/F_m	CCI
1	K-75	73.25	26.45	0.67	23.40
2	Kalyanpur Green	69.66	28.37	0.59	28.60
3	Swarna Sheetal	73.55	23.9	0.63	24.20
4	Swarna Purna	77.48	28.25	0.63	27.70
5	Pant Khirai	74.59	23.88	0.57	21.80
6	Punjab Naveen	78.43	26.66	0.65	23.20
7	VRC-26	73.56	24.48	0.69	25.90
8	Cucumber Long Green	79.70	30.6	0.62	24.60
9	K-90	75.40	32.34	0.64	21.00
10	IIHR-374	67.65	29.22	0.62	20.80
11	IIHR-285	70.68	26.48	0.58	23.10
12	IIHR-34	75.25	28.65	0.58	22.30
13	IIHR-81	82.38	30.34	0.69	21.60
14	IIHR-414	79.59	37.13	0.60	20.10
15	IIHR-258	62.79	24.88	0.71	26.40
16	IIHR-407	68.85	24.87	0.56	26.60
17	IIHR-271	77.50	21.17	0.58	14.10
18	IIHR-82	67.60	23.8	0.40	23.20
19	IIHR-337	66.49	21.31	0.49	17.20
20	IIHR-409	75.26	18.56	0.68	24.80
21	IIHR-341	62.06	15.89	0.72	21.30
22	IIHR-264	88.55	23.73	0.75	20.30
23	IIHR-3	64.50	28.77	0.70	18.50
24	IIHR-266	70.73	26.78	0.59	23.10
25	SPP-98	68.78	28.48	0.64	26.00
26	SPP-31	65.22	18.46	0.62	21.70
27	CH-122	67.22	21.49	0.63	25.20
28	SKY/DR/RS-131	79.00	18.89	0.53	25.20
29	SKY/DR/RS-133	70.88	18.37	0.58	22.30
30	VRC-19	73.03	23.33	0.66	18.40
31	Pilibhet local	71.07	23.07	0.57	22.20
32	BSC-4	58.53	29.16	0.71	25.50
33	Patna-3	70.62	22.22	0.61	27.00
34	VR-101	70.18	18.81	0.74	25.10
35	SPP-56	92.66	24.55	0.69	18.80
36	CU-5	73.20	28.09	0.57	11.10
37	SPP-26	72.72	22.67	0.69	18.90
38	SPP-63	73.65	33.98	0.59	22.70
39	VRC-27	64.69	19.23	0.55	26.10
40	Hobankha	63.13	24.48	0.72	23.90
CD (< 0.05%)		2.201	1.486	0.022	1.152

and IC (7 genotypes). The second major cluster (cluster II) consisted of 14 genotypes. This cluster could be further divided into three sub-clusters, sub-cluster IIA separated alone (1 genotypes) sub-cluster IIB (09 genotypes) and

sub-cluster IIC (04 genotypes). The third cluster (cluster III) had 2 genotypes. Dendrogram showed that in major cluster I genotype IIHR-34 and SKY/DR/RS-133 are very similar to each other, similarity genotypes IIHR-374 and Patna-3 showed close similarity.

Characterization of the EST-SSRs

A total of 21 primer pairs were used for genotyping 40 cucumber genotype. Out of 21 EST-SSRs maximum showed sequence homology with functionally known genes from different plant species, as shown in Table 4. Out of twenty-one EST-SSRs, no any hit found for the eight EST-SSRs, five markers were categorized as unknown protein, two predicted protein and six found to be involved in biological processes. Also, chromosome number has been assigned to each EST-SSRs through Gy14 assembly, out of 21 EST-SSRs, 3 found on chromosome 1, 4 on chromosome 2, 5 on chromosome 3, 1 on chromosome 5, 4 on chromosome 6 and 1 for chromosome 7. The EST-SSRs, EC31, EC47 and EC56 not linked with any chromosome based on GY14 assembly data. The selected 21 EST-SSR is distributed in all chromosome except chromosome 4.

Among the 40 cucumber genotype, all 21 EST-SSR loci revealed allelic variation. Total of 64 alleles scored for 21 EST-SSR loci with a range of 2–6 alleles per locus with an average of 3.05 alleles and the size of the band range from 105 to 498 bp. Primers EC50, EC52 and EC19 generated the highest number of alleles (6, 6, 5, respectively), while ten primers generated two alleles. PIC values had a wide range from 0.002 (EC19) to 0.989 (EC39) with a mean of 0.308 (Table 5). Expected heterozygosity and unbiased expected heterozygosity ranged from 0.074 (EC39) to 0.428 (EC22) and 0.074 (EC39) to 0.434 (EC22), respectively. Mean expected heterozygosity and mean unbiased expected heterozygosity were 0.255 and 0.258, respectively. Whereas, number of effective allele ranged from 1.08 (EC39) to 1.75 (EC22), with mean value of 1.44.

Cluster analysis based on EST-SSR markers

Among all the accession pairwise comparison was performed. Jaccard's similarity coefficients of EST-SSR varied from 0.24 to 0.96 with a mean value of 0.60. The maximum similarity coefficient (0.93) was observed between genotype IIHR-81 and genotype IIHR-407. While lowest similarity coefficient was revealed between the genotype VRC-26 and genotype Pilibhet Local. Based on Jaccard's similarity coefficient data, a dendrogram of 40 genotypes was constructed (Fig. 2). These 40 genotypes grouped into three main clusters consisting of 4, 02 and 34 genotype in major cluster I, II and III respectively. The genotypes of major cluster III further grouped into 4 sub

Fig. 1 Genetic relationship 40 cucumber genotypes based on 10 quantitative traits using UPGMA cluster analysis of the distance matrix. Number indicates genotypes as per listed in Table 1

cluster IIIA (1 genotypes), IIIB (2 genotypes), IIIC (2 genotypes) and IIID (29 genotypes). There was no any further sub grouping in major cluster I and II. The dendrogram presents VRC-26 in sub-cluster IIIA as separate genotype alone. Dendrogram showed that genotypes K-75, IIHR-3 and CH-122 singly separate in there respective groups. The EST-SSR markers based clustering pattern of cucumber genotypes was not in consonance with the groupings based on quantitative traits.

Transferability of EST-SSR markers among *Cucumis* species

Transferability of the 21 EST-SSR markers was examined within four other *Cucumis* spp. represented by two genotypes of each: *Cucumis melo* var. *momordica*, *Cucumis melo*, *Cucumis melo* var. *conomon* and *Cucumis melo* var. *agrestis*. Maximum transferability found in muskmelon (95.2%) followed by pickling melon (76.2%), wild muskmelon (76.2%) and snapmelon (61.9%), respectively.

Discussion

For efficient preservation and to exploit the cucumber genetic resources, assessment of the genetic variation within collections using molecular markers in combination

with morpho-physiological traits is very useful for germ-plasm curators and plant geneticist (Pandey et al. 2008). Earlier morphological markers have been used for assessment of relationship among Indian cucumber genotypes (Yadav et al. 2012). In the present study, morpho-physiological trait along with molecular markers were used to assess the genetic diversity among 40 cucumber genotypes representing 12 major states of India and covering 6 agro-climatic zones. The genotype used in present study showed a wide phenotypic variation in flowering behavior, fruit shape, size and number of fruits, male flower appearance was early when compared to the female flowers. Three types of growth habit observed based on vine length, viz.; short, medium and tall. As expected the more number of fruit per plant was contributed towards total yield. The variety Swarna Poorna showed maximum yield and could be utilized in future breeding programmes for development of high yielding cultivars. Yield per plant showed maximum deviation from their mean and some of the trait like number of fruits, average fruit weight and earliness showed significant variation within the genotype indicating the role of environment in expression of these traits. In case of cucumber, earliness is important trait, because early produce in Indian market gives better price to the farmers. The genotype BSC-4 recorded very early due to 50% female flowering after 37 days of sowing. The expression of genetic diversity in cucumber genotypes makes it possible

Table 4 Details of 21 cucumber EST-SSR primer pairs, their GenBank accession number, repeat motifs, chromosome number, expected product size, range of product size obtained, putative function, organism and e-value

S. no.	Gene bank accession number	Markers	Repeat motif	Chromosome number (Gy14 assembly)	Expected product size (bp)	Observed allele size (bp)/range	Putative function	Organism	E-value
1	DN910157	EC11	(TCT)10	Chr2	199	160–215	AT1G22230.1 unknown protein	Arabidopsis	7e–006
2	DN910504	EC12	(TCTT)6	Chr6	156	155–165	No hit found		
3	DN909514	EC13	(TTC)9	Chr2	244	220–245	XP_002307286 predicted protein	Populus trichocarpa	9e–015
4	DN909840	EC15	(AGA)6	Chr6	267	265–290	ABK96787 unknown	Populus trichocarpa x Populus deltoides	3e–018
5	DN910067	EC18	(CCT)6	Chr1	218	195–220	No hit found		
6	CK700725	EC19	(TCC)7	Chr3	168	160–175	No hit found		
7	DN910665	EC20	(CT)8	Chr2	181	170–190	XP_002527362 E3 ubiquitin protein ligase upl2, putative	Ricinus communis	7e–074
8	DN910469	EC22	(AAG)7	Chr6	192	180–200	XP_002528321 LOB domain-containing protein, putative	Ricinus communis	1e–047
9	DN910437	EC24	(TTA)7	Chr3	234	180–300	ABK92498 unknown	Populus trichocarpa	4e–094
10	DN909941	EC27	(ATC)7	Chr3	287	220–370	XP_002309627 predicted protein	Populus trichocarpa	1e–076
11	DN910959	EC28	(CAA)8	Chr1	237	225–240	NP_566273 heavy-metal-associated domain-containing protein	Arabidopsis thaliana	9e–029
12	CK758579	EC31	(TTC)7	No hit	164	165–180	BAA96893 serine carboxypeptidase	Arabidopsis thaliana	4e–030
13	AF202378	EC34	(TA)15	Chr1	191	180–200	No hit found		
14	CV000928	EC35	(TTTTC)5	Chr5	237	235–250	No hits found		
15	BI740103	EC39	(TCA)7(CTT)5	Chr2	155	160–190	No hit found		
16	EW968287	EC41	(AGA)5	Chr7	234	235–260	ABW75770 cyclin D3.2	Cucumis sativus	2e–077
17	AY942801	EC47	(CT)8	No hit	258	245–260	AAL35980 unknown	Cucumis sativus	6e–059
18	CK758649	EC49	(TCTTTC)6	Chr3	219	215–240	No hits found		
19	CK758649	EC50	(GAA)5	Chr3	223	105–250	No hits found		
20	CO998209	EC52	(TAA)6	Chr6	186	180–498	ACU24100 unknown	Glycine max	3e–031
21	AB112672	EC56	(AGA)16	No hit	162	150–180	AT1G19220.1 ARF19 (Auxin Response Factor 19); DNA binding/transcription factor	Arabidopsis	1e–185

A functional class was assigned to each EST-unigene based on the degree of similarity (e-value) with the closest counterpart sequence found in other plant species

to develop breeding strategies for high yielding cultivar of cucumber (Pandey et al. 2013).

RWC, which measures the maximum amount of water that a tissue can hold is the most appropriate measure of plant water status in terms of the physiological consequences of cellular water deficit, in the present experiment the genotype

SPP-56 and IIHR-264 showed maximum RWC. The genotype with higher RWC even under abiotic stress condition showed tolerance against the respective abiotic stress condition (Ansari et al. 2017). EL of the genotype IIHR-341 was lowest, while the genotype IIHR-414 showed maximum EL, genotypes with lower electrolyte leakage under abiotic stresses

Table 5 Characteristics of EST-SSR used for the diversity analysis

Marker	Primer sequence (5'–3')	No. of alleles	Ne	He	uHe	PIC
EC11	F: TCTTCGCAGTCACCATTTTC R: CCTTCCTCTGTTTCTGTTC	3	1.588	0.318	0.322	0.016
EC12	F: TATTCTTCTTTGCTACCCAT R: TAAGTTTATTTTCTGCTGTCTA	3	1.271	0.161	0.163	0.048
EC13	F: GCAATGAATCATGACCTCCA R: CTGAGAATTGGGAAGGGACA	3	1.380	0.213	0.216	0.344
EC15	F: ACCAAAAACAGACCCCTATG R: GAAAGGGAAAAACAAACGAGG	4	1.292	0.198	0.201	0.046
EC18	F: TGCCATTTCATCGACTCTTC R: GCATTTCTGCTGTGGCTTAG	2	1.526	0.330	0.334	0.662
EC19	F: TTTCTCTCCAACCTCACCTG R: TACATCGGCTTTGCTCATCT	5	1.499	0.284	0.287	0.002
EC20	F: AAAGTTGCTCTTGTGTGTC R: GAGGTGAATGGTGGTGGCT	2	1.515	0.272	0.275	0.358
EC22	F: CAGCAGAGAACTCAATCCA R: CTGTTTACGGCTGCATTGGT	2	1.753	0.428	0.434	0.009
EC24	F: ACAACACAACCGTTCTCGT R: TGAGCCCAAGCACATAACAG	4	1.411	0.237	0.240	0.049
EC27	F: GTTGGAAGGCACACAAAGTC R: CGAGATGATTGGAGGATGATG	2	1.315	0.272	0.275	0.358
EC28	F: CTGAGTTATGGGAAAGCAA R: TGTTAGTGATGTTGTTGGACC	2	1.465	0.250	0.253	0.609
EC31	F: CTAACCAGCAGAACCCAATG R: GTATCCTGTTTCCAGCGAGA	2	1.510	0.262	0.265	0.474
EC34	F: GATCCCCATCATAATCACCC R: CAAAGGGCTACAATAACAAAC	4	1.418	0.233	0.236	0.234
EC35	F: ATCCACAACACAAAAACCAC R: AAGAAGAACAGCCAAGAATG	3	1.351	0.195	0.198	0.314
EC39	F: CCAAGTTTAAGTTATTTAGGG R: GAAGAGGACGATAAAGATGA	2	1.079	0.074	0.074	0.989
EC41	F: AGCATGTGGAGGAGAAAGCA R: TTCATCATCGAGTGGGTCTG	3	1.342	0.225	0.228	0.697
EC47	F: CGATCTTTGTCATCCGACCT R: AGAACGAGCACGTTTTGAGC	2	1.575	0.320	0.324	0.488
EC49	F: CGTGTTCCTCAGATTTCCCA R: CACTTCCCTTATCAACCCCA	2	1.552	0.298	0.302	0.464
EC50	F: GGAACAGGGAAATCCACCAT R: TCGCTTCATCTCCCTCCTCC	6	1.338	0.202	0.205	0.016
EC52	F: TCAAACACGAACCCGAAACG R: CAAGAAATTGCCAGGACGAG	6	1.429	0.258	0.262	0.039
EC56	F: TTTTGGGGGTTTGTGAGAG R: AGCTTTGTTCCCTATCTTCC	2	1.552	0.321	0.325	0.247
Mean		3.048	1.438	0.255	0.258	0.3078

Ne number of effective alleles, *He* expected heterozygosity, *uHe* unbiased expected heterozygosity, *PIC* polymorphic information content

would assist as materials for future breeding aspects under changing climate scenario (Ansari et al. 2017). F_v/F_m and CCI, are the two consistent parameters for abiotic stress screening

of a large number of genotypes. Higher F_v/F_m may be the probable reason for better yield of genotypes even under abiotic stress condition (Pandey et al. 2016). These

Fig. 2 UPGMA dendrogram of 40 genotypes of cucumber based on the 21 polymorphic EST-SSR markers. Number indicates genotypes as per listed in Table 1

physiological parameters clearly indicate that cucumber genotype have significant variability.

Although EST-SSR has been used for cucumber genetic diversity study, but still there is no any report on Indian cucumber genotype diversity based on EST-SSRs, also no report correlating EST-SSR with morpho-physiological traits. A total of 64 alleles were found with the mean of 3.05, which is approximately similar as reported earlier by Hu et al. (2011), but lower than gSSR, (Hu et al. 2011). Product size range is similar to the size obtained through EST-SSR in 29 cucumber accessions (Hu et al. 2010a, b). The level of polymorphism observed in this study is lower in compare as reported by Hu et al. (2010a, b), the region may be that the Indian genotype selected may have little diversity in compare to Chinese cucumber genotypes. To assess the usefulness of the EST-SSRs marker, number of effective alleles (N_e), gene diversity or expected heterozygosity (H_e) and unbiased expected heterozygosity (uH_e) were calculated for each EST-SSRs marker. N_e of the marker EC22 was highest, while marker EC39 with

lowest N_e value. H_e and uH_e were found highest for the same marker EC22, also the lowest H_e and uH_e , observed for the marker EC39.

The coefficient of Jaccard's similarity based on EST-SSRs in the present study was very near as reported by Hu et al. (2011). It was observed that mean value of similarity coefficient of EST-SSRs is higher compared to ISSR markers (Parvathaneni et al. 2011), and SSR markers, which was observed in our earlier study also (Pandey et al. 2013). This is probably due to the differences between the different samples or marker system tested. The genotype Pilibhet local, CU-5, VRC-27 and Hobankha separated from VRC-26, IIHR-264 and IIHR-409 as they fall on either side of the dendrogram, indicating a high genetic diversity among these 7 genotypes. Grouping of genotypes according to geographical origin was random, all the released cucumber variety of India grouped into sub-cluster IIID except the variety VRC-26 which grouped separately into sub-cluster IIIA. It can be seen from the dendrogram that most of the varieties are very close and grouping

together, varieties like Punjab Naveen, Cucumber Long Green and K-90 grouped together. The variety Swarna Poorna and Pant Khira 1 grouped together and three genotypes which are grouping separately are, K-75 and VRC-26 was of Indian origin, and the third genotype IIHR-3 originated from Canada. If geographical origin is considered, all the four genotypes clustered in group I and group II belongs to Uttar Pradesh, although other genotypes of Uttar Pradesh also grouped in major group III. Maximum similarity was found between the genotype IIHR-81 and IIHR-407 which belong to Uttar Pradesh and Maharashtra. Most dissimilar genotypes were VRC-26 and Pilibhet local, both belonging to Uttar Pradesh, indicating the large diversity in cucumber accessions belonging to Uttar Pradesh. One genotype each of Bihar and north eastern hill region grouped together in sub group IIID, also genotype of Japan grouped with genotype from Uttar Pradesh in subgroup IIID. In 1992, US-India joint expedition collected cucumber germplasm from Rajasthan, Uttar Pradesh and Madhya Pradesh states of India (Staub et al. 1997). The present results also indicate that accessions of Punjab, Jammu and Himachal Pradesh are very close as they grouped together. The Swarna Poorna, IIHR-285, VRC-19, BSC-4, K-75, IIHR-3 and VRC-26 came out to be most diverse based on EST-SSR markers and clearly separated. K-75, IIHR-258 and VRC-26 were found to be the most diverse genotypes based on quantitative traits and EST-SSR markers, respectively. A non-significant correlation was observed between the Jaccard's similarity based on EST-SSR and average taxonomic distance based on quantitative traits, and grouping obtained from molecular analysis did not match with the grouping obtained from the quantitative traits. This is in concurrence with the reports that morphological traits based dissimilarity and Jaccard's similarity based on molecular markers usually showed non-significant correlation (Riday et al. 2003; Pandey et al. 2008). The reason of mismatch between clustering based on EST-SSRs and clustering based on quantitative traits may be that most of the morphological trait are controlled by a number of genes and are highly influenced by the environment (Pandey et al. 2008; Williams et al. 1990; Dahlberg 2000 and Joyee et al. 1999). The selection of quantitative trait is also very important to evaluate the genetic variability. Besides, morphological and physiological trait, biochemical traits should be given importance during variability analysis, which might explain genetic diversity more precisely. The protection of a variety often requires a rigorous declaration of uniqueness and in the case of species with a narrow genetic base such as cucumber, application and cost of development is often an important consideration.

Highest transferability of 21 EST-SSRs were observed in muskmelon (95.2%), which is very similar as reported

by Hu et al. (2011). Transferability in snapmelon, pickling melon and wild muskmelon is reported here first time. Earlier, cross species transferability with cucumber gSSR has been reported by Watcharawongpaiboon and Chunwongse (2008). Cross species transferability of molecular markers is beneficial for species that lack sequence information, species like snapmelon, pickling melon and wild muskmelon.

Genetic diversity study is a pre-requisite for any crop improvement plan as it helps in estimating and establishing of genetic correlation in germplasm collection, identifying diverse parental combinations to generate segregating progenies having highest genetic variability and superior recombinations for further selection and introgressing gene of interest from diverse germplasm (Pandey et al. 2013). This study provides useful information on variability, which can assist geneticists with desirable traits for cucumber germplasm utilization. The observed physiological parameters may assist in selection of genotype for abiotic stress tolerance whereas the EST-SSR markers may be useful for genetic studies in related species.

Conclusion

Genetic variation in cucumber collections was assessed using morpho-physiological and EST-SSR markers and this showed that Indian collections displayed considerable diversity in morpho-physiological traits. The genetic diversity at molecular level was relatively low. A threat to biodiversity is increasing in India due to cultivation of improved varieties, and F1 hybrids have resulted in better productivity and quality. However this process narrowed the genetic basis. Diverse genotype for number of fruits, earliness and high yield provides an opportunity to enrich the gene pool of cucumber with novel allele. This can improve productivity and quality. The diverse genotypes of this result may be selected for cross breeding and development of mapping population in cucumber breeding programme.

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