GENETIC DIVERSITY OF CUCUMBER GENOTYPES REVEALED BY SSR MARKERS AND AGRONOMIC TRAITS

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Understanding the level of genetic diversity and structure of landraces is essential for economical use of genetic resources. In the present study, we investigated the genetic diversity of 36 representative sample of cucumber genotypes based on 10 quantitative traits and 17 polymorphic simple sequence repeat (SSR) markers. Our result revealed variability in flowering behavior, growth habit and fruit characters. We found that the traits, days to opening of first female flower varied from 39.03 to 51.94, fruit length 6.09 to 30.01(cm), fruit weight 104.39 to 277.05(g) and yield per plant 699.38 to 1670.93(g). Genetic distance based on 17 SSR markers among 36 genotypes was quantified ranging from 0.03 to 0.70 indicated a wide diversity among the genotypes selected for evaluation in the present study. These primers produced 2-4 number of alleles with an average 2.76. Polymorphism information content varied from 0.005 to 0.550 with an average of 0.348. The observed heterozygosity mean was 0.098, while gene diversity or expected heterozygosity was 0.625 to 0.057. The distinct genotypes found in this study based on

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morpho-molecular characters will great interest to cucumber breeder for selection of diverse parent or production of mapping population.

Keywords: Cucumber, Genetic diversity, Simple sequence repeat (SSR), Agronomic traits

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is major vegetable as well as one of the most important and quickly maturing vegetables grown all over the world (PATI *et al.,* 2015a). Its tender fruits are in great demand for salad and pickles round the year in almost every part of the world. Cucumber is the fourth most important vegetable worldwide (TATLIOGLU, 1997). Cucumber is indigenous to India (SEBASTIAN *et al.,* 2010). India being native palce of cucumber possesses vast genetic variability for its vegetative and fruit characters (PATI *et al.,* 2015b). The fruits are also used as an astringent, antipyretic and are good for people suffering from constipation, jaundice and indigestion. Sex form of cucumber is also important and intensity of sex expression is also important for commercial cucumber cultivar (PATI *et al.,* 2015). Agronomic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant. In contrast, molecular markers, based on DNA sequence polymorphisms, are independent of environmental conditions and show higher level of polymorphism. In cucurbits, reports based on the uses of inter-simple sequence repeat (ISSR) markers (PRAKASH *et al.,* 2014) and SSR markers (BHWANA *et al.,* 2015, 2015a) for analysing genetic diversity. Among the molecular markers available, Simple Sequence Repeat (SSR) markers are one of the best choices for studying the genetic variability. Simple sequence repeat (SSR) or microsatellite markers occur frequently in most eukaryotic genomes and can be very informative, reliable (reproducible), codominant, multiallelic and highly polymorphic, making them well suited for detecting variation among closely relative varieties (FORMISANO *et al.,* 2012; GARCIA *et al.,* 2004). Genetic diversity was assessed previously by using 23 SSR primer pairs in cucumber (DAR *et al*., 2017). Although, the degree of genetic diversity in cucumber has been assessed with a number of DNA markers (STAUB *et al.,* 2005; ZHUANG *et al.,* 2008; SIKDAR *et al.,* 2010). However, there are limited published reports on diversity analysis or characterization of cultivars/genotypes by using both agronomic traits and SSR markers. In the current study, we focused on examining the genetic structure of the 36 cucumber genotype by using 44 SSR markers coupled with 10 agronomic traits.

MATERIALS AND METHODS

Genotypes, field evaluation and data collection

Seeds from 36 indigenous genotypes of cucumber were collected from different parts of India (Table 1). The selection of genotypes was based on genetic as well as eco-geographical diversity. The seeds were evaluated at the research farm, Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. The experiment was laid out in a Randomized Complete Block Design with three replications for phenotypic evaluation. Seeds were sown in both sides of channel with a spacing of 1.5 m between channel and 60 cm between plants. All the recommended agronomic practices along with plant protection measures were followed to raise an ideal crop. The fruits were harvested at marketable stage. Five Plants were selected, after discarding the border plants at both ends, and were examined for 10 quantitative traits: (I) Node number of first female flower (NNFFF), (II) days to opening of first female flower (DTFFF), (III**)** Days to fruit set from opening of first female flower(DTFSOFFF), (IV) Days to first fruit harvest (DTFFH), (V) number of fruits per plant, (VI) fruit length (cm), (VII) fruit diameter (cm), (VIII) Fruit index (length x diameter), (IX) fruit weight(g), (X) yield per plant(g). Means across three replications were calculated for each trait and used for data analysis (Table 2).

| Sl.No | Varieties/lines | Source of collection |
|--------------|---------------------|-------------------------------|
| $\mathbf{1}$ | CHC-1 | Ranchi, Jharkhand |
| 2 | SH-K-50 | Srinagar, Jammu and Kashmir |
| 3 | Sel-97-7 | Ludhiana, Punjab |
| 4 | Poinsette | New Delhi |
| 5 | C. hardwickii | Dehradun, Uttarakhand |
| 6 | $WBC-1$ | New Jalpaiguri, West Bengal |
| 7 | Pant Khira-1 | Pantnagar, Uttarakhand |
| 8 | Himangi | Rahuri, Maharashtra |
| 9 | Raja | New Delhi |
| 10 | $WBC-41$ | Hooghly, West Bengal |
| 11 | Jharkhand Local-1 | Ranchi, Jharkhand |
| 12 | Japanese Long Green | Katrain, Himachal Pradesh |
| 13 | $SH-K-1$ | Srinagar, Jammu and Kashmir |
| 14 | $CHC-2$ | Ranchi, Jharkhand |
| 15 | $DC-1-3$ | New Delhi |
| 16 | $CRC-5$ | New Delhi |
| 17 | Poona Khira | Pune, Maharashtra |
| 18 | Khira-90 | Solan, Himachal Pradesh |
| 19 | $DC-27$ | New Delhi |
| 20 | $WBC-23$ | South 24 pargana, West Bengal |
| 21 | Tapan | New Delhi |
| 22 | Swarna Poorna | Ranchi, Jharkhand |
| 23 | CRC-8 | IARI, New Delhi |
| 24 | 338A | IARI, New Delhi |
| 25 | WBC-29 | Hooghly, West Bengal |
| 26 | DC-305 | New Delhi |
| 27 | WBC-24 | Nadia, West Bengal |
| 28 | Sel-75-2-10 | Solan, Himachal Pradesh |
| 29 | WBC-43 | North 24 pargana, West Bengal |
| 30 | $DC-21$ | New Delhi |
| 31 | $WBC-11$ | South 24 pargana, West Bengal |
| 32 | WBC-40 | Howrah, West Bengal |
| 33 | DC-319c | New Delhi |
| 34 | Pusa Uday | New Delhi |
| 35 | Green Long -60 | Faizabad, Uttar Pradesh |
| 36 | PL-165 | Kanpur, Uttar Pradesh |

Table 1. Details of cucumber genotypes taken for study

| S.No | Genotypes | *NNOF | DTOO | DTSF | DTF | No. of | Fruit | Fruit | Fruit | Fruit | Yield |
|----------------|-----------------------|-------------|-------------|-------------|------------|---------|--------|----------|--------|--------|---------|
| | | $_{\rm FF}$ | FFF | SFOO | FH | fruits/ | length | diamete | index | weight | /plant |
| | $CHC-1$ | | | FFF | | plant | (cm) | r (cm) | | (g) | (g) |
| $\mathbf{1}$ | | 4.85 | 48.75 | 7.02 | 59.67 | 4.69 | 16.17 | 4.03 | 64.98 | 202.75 | 947.74 |
| $\mathbf{2}$ | SH-K-50 | 5.77 | 45.14 | 5.55 | 65.33 | 4.83 | 30.01 | 3.19 | 95.52 | 171.92 | 830.98 |
| 3 | Sel-97-7 | 5.45 | 47.88 | 6.52 | 58.67 | 5.60 | 17.93 | 5.51 | 98.51 | 182.98 | 1023.8 |
| $\overline{4}$ | Poinsette | 4.85 | 48.34 | 6.55 | 62.00 | 6.55 | 17.11 | 4.26 | 72.78 | 153.94 | 1005.90 |
| 5 | C.hardwickii | 4.55 | 41.23 | 4.89 | 55.00 | 6.72 | 6.09 | 3.26 | 19.75 | 104.39 | 699.38 |
| 6 | WBC-1 | 6.37 | 44.53 | 5.80 | 58.67 | 5.29 | 16.69 | 4.08 | 67.80 | 170.67 | 903.75 |
| $\overline{7}$ | Pant Khira-1 | 5.15 | 48.60 | 5.41 | 65.33 | 6.38 | 14.67 | 4.82 | 70.50 | 183.50 | 1169.32 |
| 8 | Himangi | 3.92 | 43.99 | 5.19 | 56.00 | 5.28 | 12.44 | 3.73 | 46.36 | 141.93 | 747.05 |
| \mathbf{q} | Raja | 5.53 | 44.47 | 4.59 | 69.67 | 4.36 | 17.00 | 4.77 | 81.17 | 165.47 | 716.17 |
| 10 | WBC-41 | 6.05 | 47.98 | 5.58 | 62.00 | 5.39 | 15.40 | 4.45 | 68.61 | 182.81 | 985.00 |
| 11 | Jharkhand Local-1 | 6.11 | 47.75 | 5.32 | 65.33 | 6.00 | 15.17 | 4.63 | 70.06 | 241.27 | 1448.91 |
| 12 | JapaneseLong Green | 4.47 | 45.46 | 6.21 | 65.33 | 4.36 | 20.63 | 5.49 | 113.58 | 136.05 | 594.09 |
| 13 | $SH-K-1$ | 5.49 | 42.80 | 6.03 | 65.00 | 6.51 | 12.69 | 3.71 | 47.03 | 132.83 | 863.62 |
| 14 | CHC-2 | 4.46 | 45.36 | 5.74 | 66.33 | 5.44 | 14.81 | 4.94 | 72.85 | 248.39 | 1352.15 |
| 15 | $DC-1-3$ | 5.33 | 47.44 | 6.60 | 61.00 | 7.30 | 16.69 | 5.28 | 88.18 | 175.19 | 1278.94 |
| 16 | $CRC-5$ | 5.54 | 51.94 | 7.58 | 65.33 | 5.28 | 14.70 | 4.26 | 62.58 | 244.34 | 1289.90 |
| 17 | Poona Khira | 6.64 | 46.66 | 5.08 | 66.67 | 4.31 | 17.25 | 5.13 | 88.47 | 190.40 | 820.83 |
| 18 | Khira-90 | 4.94 | 47.32 | 5.89 | 68.33 | 5.05 | 16.33 | 4.87 | 79.46 | 196.17 | 993.08 |
| 19 | $DC-27$ | 5.80 | 44.28 | 5.49 | 57.00 | 4.38 | 22.08 | 5.62 | 124.03 | 245.44 | 1071.85 |
| 20 | WBC-23 | 5.42 | 46.58 | 5.94 | 69.00 | 7.30 | 15.15 | 4.35 | 65.59 | 167.50 | 1224.96 |
| 21 | Tapan | 4.76 | 46.53 | 5.12 | 66.33 | 4.19 | 15.86 | 4.03 | 63.93 | 190.40 | 798.81 |
| 22 | Swarna Poorna | 5.41 | 43.44 | 5.35 | 58.67 | 7.78 | 17.47 | 4.37 | 76.36 | 191.21 | 1485.41 |
| 23 | $CRC-8$ | 5.76 | 44.94 | 5.78 | 58.33 | 6.33 | 14.11 | 5.43 | 76.56 | 148.17 | 938.92 |
| 24 | 338A | 4.80 | 39.03 | 5.55 | 57.33 | 5.44 | 15.18 | 4.61 | 69.92 | 151.40 | 826.41 |
| 25 | WBC-29 | 4.73 | 44.36 | 6.44 | 60.67 | 5.69 | 15.12 | 3.80 | 57.30 | 127.33 | 726.44 |
| 26 | DC-305 | 4.74 | 40.47 | 5.82 | 57.00 | 7.05 | 16.30 | 4.61 | 75.12 | 236.83 | 1670.93 |
| 27 | WBC-24 | 4.44 | 50.69 | 6.78 | 62.00 | 7.35 | 14.17 | 3.84 | 54.38 | 184.07 | 1349.34 |
| 28 | Sel-75-2-10 | 5.44 | 47.25 | 5.75 | 67.33 | 3.53 | 12.45 | 3.33 | 41.39 | 112.05 | 1395.96 |
| 29 | WBC-43 | 6.54 | 51.86 | 5.14 | 62.00 | 6.67 | 15.96 | 4.26 | 68.03 | 183.00 | 1220.84 |
| 30 | $DC-21$ | 4.31 | 45.22 | 4.11 | 56.33 | 5.55 | 16.44 | 5.40 | 88.73 | 180.59 | 1002.83 |
| 31 | $WBC-11$ | 4.32 | 42.44 | 4.67 | 58.33 | 5.59 | 15.07 | 5.23 | 78.86 | 170.07 | 952.63 |
| 32 | WBC-40 | 5.21 | 47.47 | 4.55 | 66.00 | 4.46 | 17.28 | 3.70 | 63.90 | 180.67 | 805.54 |
| 33 | DC-319c | 4.70 | 44.41 | 6.11 | 60.00 | 3.75 | 14.36 | 4.21 | 60.45 | 127.16 | 477.57 |
| 34 | Pusa Uday | 5.30 | 45.43 | 5.44 | 61.33 | 4.94 | 15.16 | 5.03 | 76.63 | 203.00 | 1003.29 |
| 35 | Green Long -60 | 5.45 | 45.03 | 5.69 | 58.33 | 5.27 | 18.05 | 5.83 | 104.54 | 277.05 | 1458.45 |
| 36 | PL-165 | 5.49 | 47.05 | 5.25 | 60.67 | 4.58 | 17.44 | 5.70 | 99.46 | 243.61 | 1115.41 |
| | Mean | 5.22 | 45.89 | 5.68 | 62.01 | 5.53 | 16.10 | 4.55 | 73.70 | 181.79 | 1005.44 |
| | $SEm+$ | 0.37 | 2.13 | 0.60 | 2.91 | 0.24 | 0.93 | 0.19 | 4.28 | 6.53 | 78.27 |
| | CD at 5% | 0.74 | 4.26 | 1.2 | 5.82 | 0.48 | 1.86 | 0.38 | 8.56 | 13.06 | 156.54 |

Table 2. Perse performance of 36 genotypes of cucumber.

*NNFFF: Node number of first female flower. DTFFF: Days to opening of first female flower. DTFSOFFF: Days to fruit set from opening of first female flower. DTFFH: Days to first fruit harvest

DNA isolation and PCR amplification

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DNA was extracted from young healthy leaves using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (SAGHAI MAROOF *et al.,*1984) with minor modifications. The Purified DNA was subjected to amplification for SSR in a 25µl reaction mixture containing 40 ng genomic DNA, 1 U *Taq* DNA polymerase (MBI Fermentas), 3mM MgCl₂, 200 μM dNTPs, 0.4 µM primer and 1 x Taq buffer (MBI Fermentas). PCR amplification were carried out in 96 well thermocycler (Perkin Elmer, Model 9600) programmed for an initial denaturation at 94° C for 4 min, followed by 40 cycles of 94⁰ C for 1 min., 65⁰C for 1 min (65⁰C for most of the primers used in the study) 72° C for 1 min. and finally, a 10 min. extension at 72° C. At the time of electrophoresis, PCR product was added with 5μ l of 6× loading dye containing Bromophenol blue. The amplified products were resolved on a 1.5% agarose gel containing ethidium bromide at a constant voltage of 60 V for 3 h in gel electrophoresis unit (BioRad Gel Electrophoresis Unit). The amplified fragments were visualized and photographed under UV light using a gel documentation system (Syngene, UK).

SSR data analysis

In the present study, 44 SSR primers were screened. Seventeen of them produced polymorphic bands were used for the analysis (Table 3). DNA fragment size on agarose gel were estimated by comparisons with 50 bpDNA marker run on the same gel. The amplified fragments were scored '1' for presence and '0' for absence in DNA samples amplified to create a binary data matrix. The binary matrix was used to estimate Jacard's genetic similarity coefficient (JACARD, 1908) for SSR. Dendogram was constructed by using unweighted pair group method with arithmetic averages (UPGMA) using NTSYS-pc software, version 2.1 (ROHLF, 1998). Major allele frequency, average number of allele per locus, gene diversity, heterozygosity and polymorphism information content were calculated using Power Maker V 3.25 (LIU and MUSE, 2005).

RESULTS

Morphological diversity

The morphological variations observed in cucumber genotypes with respect to yield and yield related traits were given in Table 2. Lowest node number of first female flower appearance was observed in Himangi (3.92) and the maximum was observed in Poona Khira (6.64). 338A took minimum (39.03) days and CRC-5 took maximum (51.94) days for opening of first female flower. DC-21 took (4.11) days for fruit set from opening of first female flower. For fruit harvest *C. hardwickii* took (55) days, whereas genotype Raja took (69.67) days. The genotype Swarna Poorna exhibited highest number of fruits per plant (7.78). Fruit length was found maximum in the genotype SH-K-50 (30.01cm), while the fruit diameter was found maximum in Green Long- 60 (5.83cm). Fruit index was found highest in the genotype DC-27(124.03), while lowest in *C. hardwickii* (19.75). The maximum fruit weight was recorded in Green Long -60 (277.05g), while yield per plant was recorded highest in DC-305(1670.93g). Thirty five cucumber genotypes grouped into two major clusters and CRC-5 is an outlier (Fig. 2). In first major cluster DC-305 was quite diverse from rest of the 9 genotypes and other was subgrouped into two subclusters. The first subcluster consisted of Japanese Long Green, Sel 75- 2-10 and DC 319 and other had 22 genotypes. WBC-23 and WBC-43, SH-K-50 and Poona Khira, CRC-8 and WBC-11 genotypes were very closely related with one another.

SSR based genetic diversity

Amplified products were observed for 36 genotypes of cucumber using 44 SSR primers. A total of 17 SSR primers were polymorphic based on preliminary screening were CMTC47, CMCCA145, CMCT160a, CMTC160a+b, CMCTC51, CSAT425, CSWCT13Balt, CSWCT16B, CSWCT25, CSWCT28, CSWGCA01, CSWTA08B, CSWTAAA01, CMBR41, CMBR65, CMBR88 and CMBR84 (Table 3). Major allele frequency was observed, minimum for primer CSWCT13Balt (0.443) and maximum for primer CMBR65 (0.97) with a mean of 0.70. The observed heterozygosity (*Ho*) mean was 0.098, while gene diversity or expected heterozygosity (*He*) was maximum for CSWCT16B (0.625) and minimum for CMBR65 (0.057) with a mean of (0.403). The major allele frequency was calculated for all the primers ranged from 0.443 to 0.97 with an average of 0.701 (Table 3). The gel representation of 36 genotypes with primer CMBR41 and CMBR88 is presented in Fig. 1. The results of the consensus tree indicated that 36 genotypes were grouped into two major clusters with one major cluster had 35 genotypes and other had only one genotype Pusa Uday (Fig. 3). The first major cluster again divided into six subclusters. The subclusters I divided from the rest of the subclusters at the similarity coefficient of 0.56. The subcluster I was represented by 4 genotypes namely 319D, CRC-8, DC-21 and *C. hardwickii*. The subcluster II was further divided from the rest of subclusters at similarity coefficient of 0.59. The subcluster II consist of 3 genotypes namely Raja, Himangi and Poinsette. The subcluster III divided from the rest of the subcluster at similarity coefficient of 0.59 and contained 6 genotypes namely Sel-75-2-10, 338A, WBC-43, WBC-40, WBC-24 and Poona Khira. The subcluster IV divided from the rest of the subclusters at similarity coefficient of 0.65. The subcluster IV represented by 3 genotypes namely SH-K-1, Japanese Long Green and SH-K-50. The subcluster V further divided from the rest of the subcluster at similarity coefficient of 0.66. The subcluster V consist of 7 genotypes namely DC 319, WBC-29, DC-27, Khira -90, WBC-41, Pant Khira and WBC-1. The genotypes Pant Khira and WBC-1 are more similar than the rest of all the genotypes. The sixth subcluster having 12 genotypes was further divided into 2 subclusters having 8 and 4 genotypes at similarity coefficient of 0.70. The first subcluster represented by 8 genotypes (DC-1-3, DC-305, PL-165, Green Long- 60, CRC-5, CHC-2, Tapan and Sel-97-7) and other having 4 genotypes (Swarna Poorna, WBC-23, Jharkhand Local-1 and CHC-1).

Fig. 1. DNA profile of 36 genotype of cucumber showing polymorphism with SSR primer CMBR41(Top: Lane 1-24 & Mid Lane 25-36) and CMBR41 (Mid Lane 1-12 & Bottom Lane 13-36, M is 100 bp ladder.)

Fig 2. Similarity tree of cucumber (*Cucumis sativus* L.) genotypes based on ten morphological traits.

Fig 3. UPGMA dendrogram of 36 genotypes of cucumber based on the 17 SSR primers.

| Primer name | Primer sequence | Major Allele | Allele N _o | (Ho) | (He) | PIC |
|---------------------|----------------------------------|-----------------|--------------------------|--------|--------|--------|
| CMTC47 | GCATAAAAGAATTTGCAGAC | Frequency | | | | |
| | | | | | | |
| | AGAATTGAGAAGAGATAGAG | 0.8429 | 3.0000 | 0.0286 | 0.2747 | 0.2535 |
| CMCCA145 | GAGGGAAGGCAGAAACCAAAG | | | | | |
| | GCTACTTTTGTGGTGGTGG | 0.4444 | 3.0000 | 0.0000 | 0.6096 | 0.5267 |
| CMCT160a | GTCTCTCTCCCTTATCTTCCA | | | | | |
| | ACGGTGTTTGGTGTGAGAAG | 0.7639 | 3.0000 | 0.0278 | 0.3862 | 0.3505 |
| $CMTC160a+b$ | GTCCTCTCCCTTATCTTCCA | | | | | |
| | GATGGTGCCTTAGTTGTTCCG | 0.7778 | 3.0000 | 0.1667 | 0.3642 | 0.3267 |
| CMCTC51 | TTGGGGTTTCTTTGAAGGTGA | | | | | |
| | CATGTCTAAAAACTCCATGTGG | 0.7361 | 4.0000 | 0.4167 | 0.4078 | 0.3531 |
| CSAT ₄₂₅ | AGGGCAGGTATTATTTTCAG | | | | | |
| | CGGACTGATTTAGTAATAGGC | 0.5968 | 3.0000 | 0.1613 | 0.5250 | 0.4393 |
| CSWCT13Balt | ATAGGGCAATTTGTCTCT | | | | | |
| | CACTGGGATCCTAACAAC | 0.4429 | 3.0000 | 0.0286 | 0.6192 | 0.5388 |
| CSWCT16B | CTTATGGTCGGAGAA | | | | | |
| | CTCAGATAACCCAAAATA | 0.4714 | 3.0000 | 0.0286 | 0.6257 | 0.5500 |
| CSWCT25 | AAAGAAATTAAGTCAATCAAACCG | | | | | |
| | CCCACCAATAGTAAAATTATACAT | 0.5000 | 2.0000 | 0.0286 | 0.5000 | 0.3750 |
| CSWCT28 | GAATTCAAAAGCATTTCAAAACTA | | | | | |
| | GAATTCAATTGGGTTTTTGAACCC | 0.6912 | 2.0000 | 0.0294 | 0.4269 | 0.3358 |
| CSWGCA01 | AGTGATGGTGCAGGGCTATCTTAT | | | | | |
| | TTGTCTTCCCTCCTCTTCTCGTCT | 0.6528 | 3.0000 | 0.2222 | 0.5127 | 0.4589 |
| CSWTA08B | TTGCATTAATGCTATAAACTTACC | | | | | |
| | GAAATTAATATTTAGGCATTG | 0.6970 | 2.0000 | 0.0000 | 0.4224 | 0.3332 |
| CSWTAAA01 | CAATGCCTCAATCTGATAGGAATG | | | | | |
| | ACTGGCTCTCTACATATTGTGAGG | 0.7639 | 4.0000 | 0.1389 | 0.3939 | 0.3673 |
| CMBR41 | GTACCGCCTAGGGTTTCTCC | | | | | |
| | CGAGGAAGAGAGAGAAGGGG | 0.8571 | 3.0000 | 0.0571 | 0.2514 | 0.2310 |
| CMBR65 | TTTCCTTATGAGTTAGGGTTTC | | | | | |
| | TGAAGAGACTACCATCCCCA | 0.9706 | 2.0000 | 0.0000 | 0.0571 | 0.0555 |
| CMBR88 | CCACTAAAGTTTCCTTATGTTTTGG | | | | | |
| | TGGTTGAGGAAGACTACCATCC | 0.8714 | 2.0000 | 0.0286 | 0.2241 | 0.1990 |
| CMBR84 | GCGATGATCAACAGAAACAGG | | | | | |
| | ACCATTCAGGCTGACACTCC | 0.8472 | 2.0000 | 0.3056 | 0.2589 | 0.2254 |
| | Mean | 0.7016 | 2.7647 | 0.0981 | 0.4035 | 0.3482 |

Table 3. Summary statistics of the ssr markers used in this study of 36 accessions of cucumber

DISCUSSIONS

Genetic diversity using morphological and molecular markers is useful for germplasm curators and plant geneticist (MONOHAR and MURTHY, 2012) The current study estimated the genetic diversity among 36 cucumber genotypes representing nine major states of India revealed a considerable variability in flowering behaviour, growth habit and fruit characters based on the morphological traits. The observation suggest that morphological variability can be used as a tool for collection of germplasm. More number of fruits, fruit length and diameter contributed towards total yield. The variety CRC-305 showed maximum yield followed by Swarna Poorna and could be utilized in future breeding programmes for development of high yielding variety. The earliness of cucumber is judged through appearance of female flower and days required for first picking which was essential for getting the better market price. Yield per plant showed maximum variation from their mean and other morphological traits like fruit weight, number of fruits, days to first fruit harvest, days to opening of first female flower can be used as tool for initial field level decision of explorer in tapping cucumber variability.

Based on SSR polymorphism the primers CMCTC51 and CSWTAAA01 showed maximum numbers of allele (4) and primers CSWCT25, CSWCT28, CSWTA08B, CMBR65, CMBR88, and CMBR84 showed minimum number of allele (2). The average number of alleles per primer was 2.76. PANDEY *et al.,* (2013) used SSR markers and reported mean number of alleles per locus was 3.05 in cucumber. BHAWNA *et al.,* (2015a) used 40 microsatellite markers in their study on bottle gourd. The alleles identified varied from 2-5 in seven nobel polymorphic marker pairs with an average of 2.85 alleles per locus. KONG *et al.,* (2007) used EST-SSR markers and found that the number of alleles ranged from 2 to 5 with an average of 2.9 alleles per locus in *C. melo*. TZITZIKAS *et al.,* (2009) investigate the genetic diversity and population structure of traditional Greek and Cypriot melon cultigens by SSR markers. They found a total number of 81 alleles, averaging 4.7 alleles per locus. This study shown that besides morphological traits, molecular markers are a powerful tool to discriminate for genetic diversity, especially the microsatellite (SSRs) are more effective for genotyping the accession examined.

Our results showed average of PIC value (0.384), was similar when compared with previous findings in Indian cucumber (0.333) (DAR *et al.,* 2017) and Chinese cucumber (0.388) (HUA *et al.,* 2010). A wide range of genetic distance was found among the genotypes ranging from 0.03 to 0.70 which indicated a large diversity among the genotypes selected in the present study. WENG (2010) reported higher range of genetic distance between melon and cucumber (0.933), *C. metuliferous* and melon (0.897) and between *C. metuliferous* and cucumber (0.954). In the present study, morphological diversity based on 10 quantitative traits and molecular diversity based on 17 SSR primers did not show any correspondence and the grouping obtained from molecular analysis not matched with grouping obtained from quantitative traits. PANDEY *et al.,* (2013) also did not observe any correlation between the grouping obtained with SSR markers and morphological traits in cucumber. The main reason of mismatch between clustering based on molecular markers and quantitative traits may be that most of the quantitative traits are controlled by a large number of genes (polygene) and these traits are highly influenced by environment (DEY *et al.,* 2006). The spatial structure of the genetic diversity can be related to evolution of species and the discordance between the morphological and molecular structure may result from similar selection pressure at different places leads to similar forms with different genetic background (PISSARD *et al.,* 2008).

UPGMA based cluster analysis (Fig. 3) showed that 36 genotypes were grouped in two major groups at the similarity coefficient of 0.53. First group had 35 genotypes except Pusa Uday. Pusa Uday showed most diverse genotype and it may be used as best parent for crop improvement programme. It was revealed from the dendrogram of SSR and quantitative traits that the genotypes Green Long-60 and PL-165 were not distinguished by SSR markers but these genotypes were separated by quantitative traits. Similar result also found in case of WBC-41 and Khira-90 were not distinguished by quantitative traits but these genotypes separated based on the dendrogram for SSR markers. This may be due to the quantitative traits which were selected to evaluate the genetic diversity might not explain the genetic variation completely; there could be other traits physiologically and biochemically more important which might explain molecular genetic diversity more precisely (DEY *et al.,* 2006). The information generated by using 36 genotypes of cucumber in the present study offers an important resource for conserving these

valuable genetics resources and exploited in future breeding programmes and cultivar development.

CONCLUSION

Genetic diversity in cucumber genotype reveled by SSR markers and agronomic traits showed that considerable genetic diversity. Diverse genotype for fruit weight, number of fruits, days to first fruit harvest, days to opening of first female flower provides an opportunity for tapping cucumber variability. Diverse genotype can be exploited for future breeding programme and variety development in cucumber.

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GENETIČKI DIVERZITET GENOTIPOVA KRASTAVCA UTVRĐEN NA OSNOVU MOLEKULARNIH MARKERA I AGRONOMSKIH SVOJSTAVA

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Izvod

Razumevanje nivoa genetske raznolikosti i strukture populacija od ključnog je značaja za ekonomično korišćenje genetičkih resursa. U ovom istraživanju smo ispitivali genetičku raznolikost 36 reprezentativnih uzoraka genotipova krastavaca na osnovu 10 kvantitativnih osobina i 17 polimorfnih SSR markera. Utvrđena je varijabilnost kod cvetanja, načina porasta i osobina ploda. Otkrili smo da je osobina broj dana do otvaranja prvog ženskog cveta, varirala od 39,03 do 51,94, dužine ploda od 6,09 do 30,01 (cm), težine ploda od 104,39 do 277,05 (g) i prinosa po biljci od 699,38 do 1670,93 (g). Genetička udaljenost bazirana na 17 SSR markera među 36 genotipova je bila u rasponu od 0,03 do 0,70, što ukazuje na veliku raznolikost među genotipovima odabranim za evaluaciju u ovom radu. Ovi prajmeri su proizveli prosečno 2.76 alela. PIC je varirao od 0.005 do 0.550 sa prosekom od 0.348. Utvrđena srednja vrednost heterozigotnosti bila je 0.098, dok je genska raznolikost ili očekivana heterozigotnost bila od 0.625 do 0.057. Genetički udaljeni genotipovi na osnovu morfo-molekularnih karakteristika će biti od velikog interesa za oplemenjivače krastavaca i za selekciju udaljenih roditelja ili proizvodnju mapirajućih populacija.

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