Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genetic Diversity of bottle gourd [Lagenaria siceraria (Mol.) Standl.] genotypes of Eastern India through agro-morphological traits and ISSR markers: Implication for future breeding

Rajesh Kumar (rkumar@cus.ac.in)
Sikkim University https://orcid.org/0000-0002-9275-8518
Randhir Kumar
Bihar Agricultural University
Bishun Deo Prasad
Rajendra Agricultural University: Dr Rajendra Prasad Central Agricultural University
Jitesh Kumar
Bihar Agricultural University
Bahadur Singh Bamaniya
Sikkim University

Research Article

Keywords:

Posted Date: May 22nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2927537/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on July 20th, 2023. See the published version at https://doi.org/10.1007/s10722-023-01670-0.

Abstract

Genetic characterization of plant genetic resources is a pre-requisite for a strategic breeding program. Bottle gourd is an important cucurbit grown throughout India for its tender fruits used for culinary purpose. For the present investigation twenty-four genotypes of bottle gourd were collected from Eastern India to examine the magnitude of genetic diversity based upon 16 quantitative traits and seven ISSR (Inter simple sequence repeats) markers. In morphological study genotypes performed significantly in all traits, however BRBG-65 (591.10 q/ha) was proved to be high yielder with other attributing traits in pooled analysis of two growing seasons than compare to the all three check varieties of the region. Almost all the traits under study showed high percentage of genotypic coefficient of variation, phenotypic coefficient of variation and heritability with genetic advancement. Quantitative traits like fruit weight, number of fruits per vine and yield of marketable fruits per vine shown strong character association with yield per hectare. Principal component analysis on quantitative traits depicted six principal components, accounted for 79.33% of the total variations. The first two components accounted for 44.92 cumulative percent of the total variation, which signifies a high degree of correlation between the analyzed traits. In the molecular study, average polymorphism percentage was obtained by ISSR marker pattern studied by seven primers. Total 48 reproducible amplicons were generated of an average around 6.85 amplicon per primer. The values of average polymorphic information content, major allele frequency and major gene diversity were obtained in wide range. Wide range of value regarding Jaccard's similarity coefficient was also ensured through ISSR markers. Dendrogram and similarity matrix produced from ISSR data then compared and concluded the discrimination among genotypes within these clusters. Most genotypes studied in the present experiment differed with their geographic origin. The investigation highlights the potential utilization of these germplasm for further genetic analysis.

Introduction

Among cucurbits, bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is an annual monoecious crop synonymously referred to as calabash or white floral gourd with chromosome no. 2n = 22 (Kumar et al. 2011). The centre of origin of calabash is thought to be Africa and America in line with Cutler and Whitaker (1967). In India, it is acknowledged for its high yield potential and adaptability to the various agro-climatic conditions grown for its tender fruits primarily used as cooking purpose. Fruits are used as a nutritious agent possessing different medicinal properties including cardio protective, cardio-tonic and diuretic and aphrodisiac properties. It has the property to cure pain, ulcers and fever and potentially used against pectoral cough, bronchial asthma and different cartilaginous tube disorders. The fruit is also known to be source of essential amino acids like phenylalanine, cystine and valine, in conjunction with B-complex vitamin, thiamine, vitamin B_2 and C.

In India, bottle gourd is being cultivated within the area 0.193 million hectares with the whole production of 3.171 million tons (Anonymous, 2022). Still the quantity of supply is not sufficient to meet the requirement of huge population in the country. To initiate any effective breeding programme, diversity assessment is one amongst the vital tools to quantify variability in each cross and self-fertilized crops for crop improvement additionally as cultivar development programme (Kundu et al. 2012). Morphological characterization of germplasm not solely helps within the identification of lines with distinctive characteristics however conjointly within the designing of inheritance analysis (Sidhu and Pathak, 2016). Phenotypical variation is completely coupled with genetic diversity, however is well dependent upon environmental factors furthermore as on interaction between environment and genotypes (Moose and Mumm, 2008). Hence, deciding genetic diversity among the genotypes or populations is an important method for plant breeding programs (Bamaniya et al. 2018). Selection and hybridization are the two basic strategies for upgrading crop plants. It depends upon the amount of genetic variation found within the base material. Therefore, for higher level of improvement at quicker rate, a broad genetic base material might be used (Hailu, 2011). Genetic diversity is crucial to fulfil the varied goals of plant breeding like breeding for increasing yield, wider adaptation, fascinating quality, insect-pest and disease resistance (Ferdous et al. 2011).

Heritability is an index for assessing the influence of climate on genotypical expression. Broad sense heritability estimates the proportion of phenotypical variance ensuing because of genetic cause (Lush, 1949). Genetic advance denotes the improvement within the genotypic value of the chosen individuals in comparison to the initial population. The potentiality of genetic advance is proportional to phenotypical variation present within the original population and maintained in consequent selection cycles. An estimate of genetic advance in conjunction with heritability is useful in assessing the dependableness of characters for selection (Lynch and Walsh, 1998). Correlation and path co-efficient analysis determines nature as well as magnitude of association among variables and is that the measuring of direct influence of a variable upon the other. All measures are therefore necessary for the identification of genetically distant parental combinations, planning to use distinct cistron sets in crossings for getting superior hybrids and segregates, to judge the degree of genetic erosion, or perhaps to work out the extent of the genetic base of cultivated forms to develop heterotic combination (Gonçalves et al. 2008; Munhoz et al. 2009).

The crop improvement programme chiefly depends upon the choice against superior parents for crossbreeding and information of combining ability and relative magnitude of gene action concerned in expression of necessary agriculture traits. Because the accessions don't seem to be fairly characterized it is essential in the beginning of breeding programme to discriminate among accessible genotypes to determine the extent of genetic divergence and thereby, identity the foremost appropriate materials for hybridization (Debnath et al. 2020). The study of genetic diversity is critical indeed for efficient form of utilization, conservation and management of genotypes collections in bottle gourd.

The variation in inheritable traits might express itself within the kind of altered morphological, anatomical, physiological behaviour or biochemical characters. Diversity in plant genetic pool paves the method for breeders to develop distinctive and genetically improved variety with fascinating characters (Debnath et al. 2021), that take into account each farmer most well-liked traits (greater yield potential, large and bold seed etc.) and breeder most well-liked traits (disease and insect-pest resistance and photoperiod sensitivity etc.). With ever-changing environmental condition situation, breeding for climate resilient cultivars is turning into a lot of importance. Genetic diversity permits breeders to develop varieties for specific traits like quality and tolerance to abiotic as well as biotic stresses. Diversity is additionally of best-known importance with reference to adjustability of crop to varied climatic conditions with special relevance ever-changing climate (Islam et al. 2010). Genetic diversity among individuals or population scan is set using morpho-molecular markers. In contrast, molecular

markers based upon deoxyribonucleic acid sequence polymorphism, are freelance of environmental conditions (Kumar et al., 2021). Identification of genotypes based upon morphological markers implies culture examination at completely different stages and isn't so reliable due to several traits of interest having low heritability and are genetically more complicated. Molecular markers offer a fast and reliable methodology for estimating genetic relationships amongst genotypes (Thormann et al., 1994).

In plants, inter simple sequence repeats or ISSR markers are a lot of in demand, as a result of they are abundant in quantity, highly reproducible and polymorphic, and relatively more informative (Zietkiewicz *et al*., 1994). ISSR markers are present in nuclear genome, provide a genotyping system with stability, reliability and dominancy (Peng et al., 2006). ISSR utilizes presence throughout the entire genome of simple sequence repeats (SSR) with tandem repeats motifs composed of 1 to 7 bases and reveal information that indicates the length variation amongst adjacent microsatellites. Unlike SSR, its implementation does not need any prior knowledge of sequence information (Zietkiewicz et al. 1994). These are useful novel DNA markers due to fast inexpensive genotyping technique that produces more reproducible bands than RAPD markers due to its relatively high annealing temperature (Bornet and Branchard, 2001; Chowdhury et al. 2002) and may be used in other techniques including phylogenetic analysis, DNA fingerprinting and gene tagging. The ISSR-PCR uses one primer which composed of a di-nucleotide or tri-nucleotide simple sequence repeats and uses primers which anchored at five or three ends of repeat regions and extended towards flanking region. The benefits of this ISSR method embrace multiple polymorphic loci, high output and low cost. This system has provided a robust tool for the investigating genetic variability amongst the genotypes. Despite huge economic importance and health benefit properties of bottle gourd, limited study has been done to evaluate its genetic diversity with the help of molecular markers. However, there are impressive numbers of the studies available to examine the genetic diversity of bottle gourd by using morphological traits. When it comes to molecular validation, only few studies available using RAPD (random amplified polymorphic DNA) marker (Decker-Walters et al., 2004) and SSR marker (Xu et al., 2014) to assess its genetic diversity and population structure.

Eastern part of India is blessed with huge genetic diversity of different commercial and underutilized vegetable crops. The soil and climatic condition of this region is suitable for cultivation of large number of vegetable crops. Despite of all suitable growing condition, the production of different vegetable crops is being declined including bottle gourd. Due to unavailability of suitable varieties/hybrids suitable for this region, farmers are tending to adjust the cropping pattern or shift to other crops. The genotypes of this region are poorly characterized, hence during our study the number of accessions was assessed for genetic variability for economic traits and molecular validation was done using ISSR marker for genetic diversity estimation to review geographical distribution and genetic divergence of the genotypes. The study facilitates speedy screening of enormous range of genotypes for polymorphic loci for future breeding.

Material and Methods

Experimental site and location

The investigation was carried out at Main Experimental Station, Vegetable research farm, Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural University, Sabour in the spring-summer seasons of year 2017 and 2018 respectively. Geographically, Sabour situated under humid sub-tropical climate and located in between 24.470 and 26.560 N latitude and 82.120 and 83.980 E longitude at altitude of 75 m above MSL in the Indo-Gangetic Plains of Bihar, Eastern India.

Soil physico-chemical properties

Soil of experimental site was tested for different properties i.e., soil pH by digital pH meter (Jackson, 1973), EC (electric conductivity) by digital conductivity meter (Jackson, 1973), organic carbon% (Walkley and Black, 1934), available N in Kg/ha (Subbiah and Asija, 1956), P_2O_5 in Kg/ha by UV/VIS spectrophotometer (Olsen et al., 1954) and K_2O in Kg/ha by Flame photometer (Jackson,1973) using standard protocol for both the year of investigation. Soil was tested in triplicates and results revealed soil pH as 7.37 and 7.24, EC as 0.42 ds/m and 0.40 ds/m, organic carbon% as 0.53% and 0.46%, available N as 213.50 kg/ha and 194.45 kg/ha, available P_2O_5 as 29.75 kg/ha and 27.50 kg/ha, and available K_2O as 165.25 kg/ha and 134.77 kg/ha, respectively for both the year of investigation.

Experimental materials

The experimental materials used for present study consist of twenty-four genotypes including breeding lines maintained at Department of Horticulture (Vegetable and Floriculture) Bihar Agricultural University, Sabour *viz*., BRBG 11 – 1, BRBG 12 – 3, BRBG 21 – 2, BRBG 22 – 2, BRBG 41 – 1, BRBG 42 – 1, BRBG 42 – 2, BRBG 42 – 3, BRBG 51 – 1, BRBGG 51 – 2, BRBG 52, BRBG 52 – 1, BRBG 52 – 2, BRBG 61 – 1, BRBG 62 – 1, BRBG 130, BRBG 140, BRG 190, BRBG 65, BRBG 31 – 1, BRBG 23 and 3 check varieties namely Narendra Rashmi, Pusa Naveen and Rajendra Chamatkar.

Experimental details

The experiment was statistically laid out in RBD with three replications. The experimental field thoroughly ploughed and harrowed bi-times to achieve good tilth and leveled. Simultaneous laddering was done for breaking clods and to remove weeds and other crop residues. Well decomposed FYM and cow dung thoroughly mixed with top soil at rate of 20 kg each per plot. Seeds were laid out on the sides of the sowing channel in well prepared hills with spacing of 3 m between the rows and 75 cm between the plants. Each treatment consisted of one hill and four plants are allowed to grow per hill. The observations were recorded on five randomly selected plants. All the recommended fertilizer doses, sophisticated cultural practices were followed to raise a successful crop.

Morphological and molecular analysis

In this investigation total 16 morphological quantitative descriptors as suggested by National Plant Genetic Resources, New Delhi were recorded namely Days to 50% flowering, Node number to first pistillate flower, days to first fruit harvest, days to last fruit harvest, number of primary branches per vine, internodal length (cm), peduncle length (cm), vine length (m), fruit length (cm), fruit circumference (cm), fruit weight (kg), number of fruits per vine, number of seeds per fruit, yield of marketable fruits per vine (kg), weight of 100 seeds (g) and yield per ha (q).

Genomic DNA of bottle gourd genotypes was isolated from nascent leaves following modified 2X CTAB (cetyl trimethyl ammonium bromide) method. Agarose gel electrophoresis at 0.8% agarose concentration was used for DNA quantification analysis. Besides, DNA samples were quantified by using nanodrop (ND-spectrophotometer) at 260nm. The quality of DNA was estimated at A260/A280 nm. For comparative banding morphotypes testing λ DNA of known quantity (50 ng) was used as a standard. DNA was diluted to MB (molecular biology) grade water to the final concentration of 25 ng/µL, afterwards stored at 4 ^{0}C. ISSR analysis of bottle gourd genotype was conducted by using 21 primers obtained from Operon Technologies, California. ISSR amplification was also done in 10 µl volume containing 2 µl of 1X standard PCR buffer (Tris with 15 mM $MgCl_2$), 2.5 µl of 200 µM dNTPs mixture, 0.5 µl of primer, 1 µl of 25 ng genomic DNA and 0.5 µl of Taq DNA polymerase (0.3 U/ µl) and 3.5 µl of molecular-grade water. The reagents were mixed and then placed upon a Thermal-cycler for cyclic amplification process and the ambient conditions for amplification were programmed. Amplification was performed in an Eppendorf thermal cycler (Eppendorf AG) at 94 ^{0}C for 5 minutes, with 40 cycles of 94 ^{0}C for 1 minute, annealing of primer at 39–44 ^{0}C for 30 seconds (on the basis of individual primer annealing temperature) and an extension at 72 ^{0}C for 1 minute, followed by a final extension at 72 ^{0}C for 3 minutes. Amplified products were visualized under gel electrophoresis in 1.2% agarose gel using 1X TBE buffer, which was detected by ethidium bromide staining, viewed under ultraviolet light lamp and photograph was taken with Gel-documentation system. A standard DNA ladder (1 kbp) was added to each gel as a control for comparison. The allele's length was compared with the standard band of the DNA ladder and subjected to scoring analysis.

Statistical analysis

Morphological data

Data were recorded on 16 quantitative agro-morphological traits for two growing seasons and analysis was done for pooled data of both the seasons. For working out the analysis of variance, the data was analyzed using the model suggested by Panse and Sukhatme (1984). Genotypic coefficient of variation and phenotypic coefficient of variation (GCV and PCV) were estimated using formula of Burton and De Vane (1953). Heritability (broad sense) in percent was estimated as per formula of Burton and De Vane (1953), Johnson et al. (1955) and Hanson et al. (1956). Genetic advance was computed as per formula by Lush (1949). Phenotypic and genotypic coeficientes of correlation were computed by following Al-Jibouri et al. (1958). Correlation coefficients between yield and other characters and among the characters were calculated as per formula given by Searle (1961). Path coefficient analysis was done by the methodology suggested by Wright (1921) and using the formula of Dewey and Lu (1959) to calculate direct influence of one independent variable upon other variable and to divide the total simple correlation into direct and indirect effects. Principal component analysis (PCA) was used to explain the structure of variances and covariances of a matrix composed of p variables. The standardized data, the eigen values and associated eigen vectors were calculated from the covariance matrix (S) between the genotypes. Principal component analysis was performed by using INDOSTAT software (Indostat services, Hyderabad, India).

Molecular data

In molecular analysis the scored band in binary matrix were used to develop similarity matrices with Jaccard's similarity coefficient using SPSS software and dendrogram was generated. In a binary matrix, the presence of a band was marked with 1 and absence was noted with 0. Genetic diversity parameters, such as number of amplified fragments (A_0), number of polymorphic loci, percentage of polymorphic loci, observed number of alleles per locus (Na), number of effective alleles per locus (Ne), major allele frequency (F_x) and Nei's gene diversity (H) and Shannon's information index (I) were calculated using GenAlex version 6.5 (Peakell and Smouse, 2007). PIC was calculated using the formula: PIC = 1 − $\sum$Pij2, where Pij is the frequency of jth allele of the ith locus (Nagy et al., 2012).

Results

Agro-morphological variation among the genotypes

The per se performance of the genotypes, range, standard error of difference and critical difference values under pooled analysis of both the season is presented in the Table 1. The morphological analysis revealed significant variation among the genotypes under investigation (Table 1 and Fig. 1). Days to 50% flowering are directly proportional to early yield. It is observed that the BRBG-21-2 was earliest (44 days) in producing 50% flowering, it was also clear that all genotypes depicted broad range of variation with respect to node number to first pistillate flower appearance. However, first pistillate flower appearance was recorded early in genotype BRBG 190 (8.27th node). It is observed from the table that the BRBG-21-2 was earliest (51.17 days) in producing first fruit harvest, however, BRBG 61 − 1 produced maximum value for days taken to last fruit harvest (119.39 days). Highest number of primary branches per vine was obtained in genotype BRBG 22 − 2 (23.30), whereas minimum inter-nodal length was obtained in the genotype BRBG 190 (15.70 cm). It is quite clear from the study that maximum peduncle length was ascertained in genotype BRBG 42 − 1 (18.78 cm). Whereas, vine length was observed maximum in BRBG 41 − 1 (7.59 m). Data pertaining to fruit length showed that the highest fruit length was obtained in genotype BRBG 52 − 1 (58.06 cm), however maximum fruit circumference was noted in genotype BRBG 62 − 1(41.48 cm). The maximum fruit weight was found in genotype BRBG 52 − 2 (1.31 kg). while maximum value regarding the number of fruits per vine was revealed in genotype BRBG 65 (14.85). It was quite obvious from the study that the genotype BRBG 65 produced maximum value (15.19 kg) regarding the yield of marketable fruits per vine, which was higher than all three check varieties used in this study. It is clearly indicated from the table that the maximum value regarding number of seeds per fruit was observed in BRBG 130 (733.33), however BRBG-23 was recorded with maximum

weight of 100 seeds (16.48 g). It was quite clear from the investigation that the maximum value regarding fruit yield was ascertained in the genotype BRBG 65 (591.10 q/ha). It was quite evident from the table that genotype BRBG 65 showed superiority over all three check varieties used in the investigation.

Table 1
Per se performance of bottle gourd genotypes (pooled analysis of two season)

Genotypes	Days to 50 per cent flowering	Node number to first pistillate flower	Days to first fruit harvest	Days to last fruit harvest	Number of primary branches per vine	Inter-nodal length (cm)	Peduncle length (cm)	Vine length (m)	Fruit length (cm)	Fruit circumference (cm)	Fruit weight (kg)	Number of fruits per vine	Yi m fr vi
BRBG-11-1	51.17	11.77	52.67	105.05	14.79	18.67	12.11	5.27	43.26	23.94	1.06	8.20	7.8
BRBG-12-3	46.17	10.27	52.67	116.67	17.15	19.70	15.00	5.25	45.86	23.57	1.22	9.15	10
BRBG-21-2	44.00	9.05	51.17	108.44	16.23	20.18	13.49	5.26	37.82	25.00	1.29	6.88	8.1
BRBG-22-2	50.17	9.21	56.33	112.67	23.30	17.50	11.16	6.42	43.91	23.13	1.13	9.42	9.6
BRBG-41-1	54.00	9.98	59.83	114.28	16.49	18.97	16.16	7.59	42.55	23.07	0.98	7.65	6.7
BRBG-42-1	54.17	10.75	59.67	119.11	18.00	21.25	18.78	5.56	50.46	25.68	1.24	8.18	8.9
BRBG-42-2	51.17	10.05	56.83	115.20	15.91	18.86	12.66	5.82	23.98	25.85	0.91	10.22	8.4
BRBG-42-3	50.67	10.88	54.67	111.09	16.57	18.04	13.05	5.05	49.12	21.30	1.21	9.78	10
BRBG-51-1	50.83	9.49	58.17	110.54	16.44	16.49	10.22	4.60	43.88	26.63	0.88	8.10	6.3
BRBG-51-2	52.00	11.16	59.33	116.39	21.66	21.34	11.83	5.22	48.97	22.42	1.23	7.68	8.5
BRBG-52	54.17	10.44	59.33	115.89	19.90	23.33	12.68	5.92	43.49	21.41	0.88	6.50	5.3
BRBG-52-1	49.83	9.88	55.83	115.66	18.51	25.40	14.33	6.44	58.06	18.95	1.19	8.80	9.1
BRBG-52-2	47.50	9.77	56.33	109.50	18.88	19.97	13.95	5.53	52.53	23.83	1.31	7.62	8.6
BRBG-61-1	46.33	9.88	54.50	119.39	18.43	18.65	13.17	5.29	48.72	23.79	1.20	8.40	9.5
BRBG-62-1	49.83	10.49	54.33	114.94	19.80	20.45	10.77	5.99	21.42	41.48	1.00	11.27	10
BRBG-130	51.17	10.16	55.00	115.55	18.12	18.98	14.56	5.66	36.02	23.50	0.96	11.62	10
BRBG-140	50.33	10.77	55.00	111.78	18.08	19.97	15.27	5.64	43.34	20.03	0.91	6.03	4.9
BRBG-190	51.33	8.27	55.33	117.78	16.44	15.70	12.26	3.69	22.88	38.92	0.90	4.52	3.6
BRBG-65	50.83	8.99	56.67	115.33	20.07	19.92	13.10	5.93	35.84	22.96	1.14	14.85	15
Narendra Rashmi	51.33	9.44	56.33	115.22	18.18	20.42	12.88	6.07	40.96	21.22	1.06	8.30	7.6
BRBG-31-1	49.83	10.44	55.00	117.39	19.07	19.66	13.72	5.56	54.36	19.25	1.08	8.62	8.3
BRBG-23	48.33	10.66	55.50	117.67	16.60	18.97	16.50	6.00	47.48	21.85	1.20	6.75	7.3
Pusa Naveen	48.67	9.55	54.50	117.44	20.62	18.42	14.27	5.67	32.29	22.98	0.98	7.90	6.9
Rajendra Chamatkar	53.83	9.33	57.00	113.33	19.15	20.25	12.81	5.99	54.94	24.56	1.21	7.75	8.5
Mean	**50.32**	**10.03**	**55.92**	**114.43**	**18.27**	**19.63**	**13.53**	**5.64**	**42.59**	**24.39**	**1.09**	**8.51**	**8.3**
CD (P = 0.05)	1.17	2.62	2.42	3.97	2.09	1.54	1.42	0.67	2.67	2.74	0.13	0.91	1.1
SEm	0.68	0.38	0.70	1.16	0.59	0.43	0.40	0.19	0.75	0.62	0.03	0.21	0.2

Data illustrated in Table 2 clearly indicates that the highest GCV and PCV was recorded for number of seeds per fruit (30.87% and 32.59%) followed by yield of marketable fruits per vine (26.92% and 28.17%), yield per hectare (25.74% and 26.78%), number of fruits per vine (24.24% and 24.95%), fruit length (23.18% and 23.58%) and fruit circumference (21.41% and 22.30%). Whereas, the lowest GCV and PCV were noted for node number to first pistillate flower, days to 50 per cent flowering, days to first fruit harvest, days to last fruit harvest and inter nodal length. Data presented in Table 2 also indicates that, heritability in broad sense was moderate to high (30–60%) for all traits under study indicating the major role of genotypes in expression of these characters. The estimate of heritability for morphological traits was high for fruit length (97%), number of seeds per fruit (90%), yield per hectare (92%), number of fruits per vine (94%), fruit circumference (92%) and yield of marketable fruits per vine (91%).

Table 2

Genetic parameters for yield and attributing traits in bottle gourd (pooled analysis of two season) GCV: genotypic coefficient of variation; PCV; phenotypic coefficient of variation

S.N.	Character	Range		GCV (%)	PCV (%)	Heritability in Broad Sense (%)	Genetic Advance	Genetic Advance (as percent of mean)
		Lowest	Highest					
1.	Node number to first pistillate flower	8.27	11.77	6.92	11.54	36	0.86	8.56
2.	Days to 50 per cent flowering	44.00	54.17	4.92	5.92	69	4.24	8.42
3.	Days to first fruit harvest	51.17	59.83	3.81	4.89	61	3.42	6.12
4.	Days to last fruit harvest	105.05	119.39	2.92	3.83	58	5.25	4.59
5.	Number of primary branches per vine	14.79	23.30	10.41	13.07	63	3.12	17.09
6.	Inter-nodal length (cm)	15.70	25.40	9.98	11.30	78	3.56	18.15
7.	Peduncle length (cm)	10.22	18.78	13.87	15.62	79	3.43	25.35
8.	Vine length (cm)	3.69	7.59	12.24	14.64	70	1.19	21.08
9.	Fruit length (cm)	21.42	58.06	23.18	23.58	97	20.00	46.96
10.	Fruit circumference (cm)	18.95	41.48	21.41	22.30	92	10.33	42.34
11.	Fruit weight (kg)	0.88	1.31	12.54	14.21	78	0.25	22.81
12.	Number of fruits per vine	4.52	14.85	24.24	24.95	94	4.13	48.50
13.	Number of seeds per fruit	224.17	733.33	30.87	32.59	90	226.15	60.25
14.	Yield of marketable fruits per vine (kg)	3.65	5.19	26.92	28.17	91	4.44	52.98
15.	Weight of 100 seeds	11.52	17.94	10.96	12.71	74	2.84	19.48
16.	Yield per hectare (q)	151.55	591.10	25.74	26.78	92	170.80	50.94

GCV: genotypic coefficient of variation; PCV; phenotypic coefficient of variation

To reveal the accuracy of heritable variation, heritability must be examined in association with genetic advance. Genetic advance was estimated at 5% selection intensity and converted into expected genetic gain as per cent of mean. High genetic advance as percent of mean was observed for traits like number of seeds per fruit (60.25%), yield of marketable fruits per vine (52.98%), yield per hectare (50.94%), fruit weight, yield of marketable fruits per hectare (50.94%), number of fruits per vine (48.50%), fruit length (46.96%), fruit circumference (42.34%), peduncle length (25.35%) vine length (21.08%) and fruit weight (22.81%), rest of the characters showed moderate to low values for this estimate.

Character association analysis

Genotypic correlation coefficient

The correlation analysis for all traits under investigation presented in Table 3 revealed that all the traits were positively correlated with yield per hectare at genotypic level except days to 50% flowering (rg=-0.21), days to first fruit harvest (rg=-0.16) and fruit circumference (rg=-0.13). Yield per hectare showed significant and positive correlation with yield of marketable fruits per vine (rg = 0.99), number of fruits per vine (rg = 0.87) and fruit weight (rg = 0.56), indicating that these characters might be useful in increasing the yield. Whereas, yield per hectare was significantly negatively correlated with days to 50% flowering and days to first fruit harvest indicating the existence of early bearing.

Table 3
Genotypic and phenotypic correlation matrix (pooled analysis of two season)

Character		NNFPF	DF	DFFH	DLFH	NPBV	INL	PL	VL	FL	FC	FW	NFV	YMFV	NSF	WS	YPH
NNFPF	G	1.00	0.20	0.03	-0.17	-0.16	0.32	0.26	0.15	0.34	-0.32	0.12	0.00	0.03	0.06	0.11	0.02
	P	1.00	0.12	0.03	-0.08	-0.13	0.20	0.13	0.03	0.21	-0.18	-0.02	0.03	0.01	0.00	0.11	0.01
DF	G		1.00	0.82	0.14	0.12	0.15	0.06	0.26	0.03	0.02	-0.45	0.00	-0.19	0.09	0.14	-0.21
	P		1.00	0.63	0.08	0.07	0.08	-0.02	0.20	0.01	-0.01	-0.32	0.01	-0.14	0.04	0.14	-0.17
DFFH	G			1.00	0.34	0.31	0.22	0.14	0.33	0.20	-0.12	-0.22	-0.05	-0.15	-0.08	0.28	-0.16
	P			1.00	0.21	0.21	0.15	0.07	0.27	0.15	-0.10	-0.13	-0.05	-0.11	-0.08	0.22	-0.13
DLFH	G				1.00	0.34	0.16	0.38	0.07	-0.07	0.08	-0.06	0.04	0.04	0.18	0.23	0.03
	P				1.00	0.20	0.10	0.25	0.02	-0.05	0.06	-0.05	0.03	0.03	0.12	0.11	0.02
NPBV	G					1.00	0.26	-0.23	0.29	0.12	-0.11	0.13	0.25	0.28	-0.06	0.27	0.28
	P					1.00	0.24	-0.17	0.19	0.09	-0.05	0.10	0.16	0.20	-0.05	0.16	0.20
INL	G						1.00	0.28	0.47	0.42	-0.37	0.29	0.07	0.17	-0.06	0.17	0.19
	P						1.00	0.19	0.33	0.37	-0.29	0.23	0.05	0.14	-0.05	0.14	0.16
PL	G							1.00	0.32	0.32	-0.36	0.30	-0.16	-0.02	0.35	0.37	0.00
	P							1.00	0.25	0.27	-0.31	0.20	-0.13	-0.03	0.30	0.27	-0.01
VL	G								1.00	0.21	-0.39	0.04	0.29	0.24	0.42	0.44	0.24
	P								1.00	0.15	-0.32	0.07	0.23	0.21	0.32	0.33	0.19
FL	G									1.00	-0.71	0.60	-0.18	0.10	-0.09	0.52	0.12
	P									1.00	-0.66	0.52	-0.17	0.10	-0.09	0.45	0.12
FC	G										1.00	-0.29	-0.02	-0.13	-0.04	-0.56	-0.13
	P										1.00	-0.22	-0.02	-0.11	-0.04	-0.45	-0.11
FW	G											1.00	0.09	0.50	-0.16	0.29	0.56
	P											1.00	0.08	0.52	-0.13	0.22	0.54
NFV	G												1.00	0.90	0.20	0.06	0.87
	P												1.00	0.88	0.18	0.05	0.85
YMFV	G													1.00	0.09	0.15	0.99
	P													1.00	0.09	0.14	0.98
NSF	G														1.00	-0.12	0.04
	P														1.00	-0.12	0.04
WS	G															1.00	0.15
	P															1.00	0.14
YPH	G																1.00
	P																1.00

NNFPP- Node number to first pistillate flower; DF- Days to 50% flowering; DFFH- Days to first fruit harvest, DLFH- Days to last fruit harvest; NPBV- Number of primary branches per vine; INL- Inter-nodal length; PL- Peduncle length, VL- Vine length; FL- Fruit length; FC- Fruit circumference; FW- Fruit weight; NFV- Number of fruits per vine; YMFV- Yield of marketable fruits per vine; NSF- Number of seeds per fruit; WS- Weight of 100 seeds; YPH- Yield per hectare

Phenotypic correlation coefficient

Correlation analysis for all traits under investigation presented in Table 3 revealed that all the traits were positively correlated with yield per hectare at phenotypic level except days to 50% flowering (rp=-0.17), days to first fruit harvest (rp=-0.13), peduncle length (rp=-0.01) and fruit circumference (rp=-0.11). Fruit yield hectare had significant and positive correlation with yield of marketable fruits per vine (rp = 0.98), number of fruits per vine (rp = 0.85) and fruit weight (rp = 0.54), indicating that these characters might be useful in increasing the yield. Whereas, yield per hectare was significantly negative correlated with days to 50% flowering and days to first fruit harvest indicating the existence of early bearing.

Direct and indirect effect of various characters towards yield per hectare

Direct and indirect effect of various characters towards yield per hectare at genotypic level

Direct and indirect effects of attributing traits on yield per hectare have been computed in Table 4. Number of fruits per vine exhibited highest positive direct effect (1.648) followed by fruit weight (0.962), days to 50% flowering (0.503), vine length (0.399) and days to last fruit harvest (0.349). The lowest positive direct effect on yield per hectare was observed for number of primary branches per vine. Negative direct effect on fruit yield per hectare was recorded upon the traits like yield of marketable fruits per vine, node number to first pistillate flower, inter nodal length, days to first fruit harvest, number of seeds per fruit and weight of 100 seeds. Residual effect of path analysis was very low (0.009) suggesting inclusion of maximum yield influencing characters in the analysis.

Table 4
Direct and indirect effect of various characters on yield of bottle gourd at genotypic level (pooled analysis of two season)

Character	NNFPF	DF	DFFH	DLFH	NPBV	INL	PL	VL	FL	FC	FW	NFV	YMFV	NSF	WS
NNFPF	**-0.105**	-0.021	-0.003	0.018	0.016	-0.033	-0.027	-0.016	-0.036	0.034	-0.013	0.000	-0.003	-0.006	-0.012
DF	0.101	**0.503**	0.410	0.073	0.058	0.073	0.029	0.132	0.013	0.011	-0.225	-0.001	-0.097	0.045	0.069
DFFH	-0.017	-0.465	**-0.570**	-0.196	-0.179	-0.125	-0.078	-0.187	-0.113	0.067	0.127	0.031	0.087	0.046	-0.159
DLFH	-0.058	0.050	0.120	**0.349**	0.120	0.055	0.133	0.023	-0.023	0.027	-0.019	0.015	0.014	0.064	0.081
NPBV	-0.006	0.004	0.012	0.013	**0.039**	0.010	-0.009	0.011	0.005	-0.004	0.005	0.010	0.011	-0.002	0.010
INL	-0.104	-0.048	-0.072	-0.052	-0.085	**-0.328**	-0.091	-0.156	-0.137	0.121	-0.094	-0.024	-0.055	0.019	-0.054
PL	0.019	0.004	0.010	0.029	-0.018	0.021	**0.075**	0.024	0.024	-0.027	0.022	-0.012	-0.001	0.026	0.028
VL	0.059	0.105	0.131	0.027	0.116	0.189	0.127	**0.399**	0.082	-0.155	0.018	0.114	0.094	0.166	0.177
FL	0.066	0.005	0.038	-0.013	0.024	0.080	0.062	0.040	**0.193**	-0.136	0.116	-0.034	0.019	-0.018	0.101
FC	0.052	-0.003	0.019	-0.012	0.018	0.060	0.058	0.063	0.115	**-0.163**	0.047	0.003	0.022	0.007	0.091
FW	0.116	-0.430	-0.215	-0.053	0.121	0.275	0.289	0.043	0.578	-0.278	**0.962**	0.087	0.484	-0.157	0.275
NFV	0.000	-0.002	-0.091	0.072	0.407	0.118	-0.256	0.471	-0.292	-0.026	0.148	**1.648**	1.485	0.324	0.094
YMFV	-0.029	0.184	0.145	-0.039	-0.269	-0.160	0.015	-0.226	-0.095	0.126	-0.478	-0.857	**-0.952**	-0.088	-0.147
NSF	-0.024	-0.039	0.036	-0.081	0.028	0.025	-0.154	-0.183	0.040	0.019	0.072	-0.086	-0.041	**-0.440**	0.054
WS	-0.050	-0.062	-0.126	-0.105	-0.120	-0.075	-0.168	-0.201	-0.236	0.253	-0.129	-0.026	-0.070	0.056	**-0.452**
YPH	**0.020**	**-0.215**	**-0.156**	**0.030**	**0.276**	**0.187**	**0.005**	**0.237**	**0.118**	**-0.131**	**0.559**	**0.868**	**0.997**	**0.043**	**0.154**

Residual effect: 0.009

NNFPP- Node number to first pistillate flower; DF- Days to 50% flowering; DFFH- Days to first fruit harvest, DLFH- Days to last fruit harvest; NPBV- Number of primary branches per vine; INL- Inter-nodal length; PL- Peduncle length, VL- Vine length; FL- Fruit length; FC- Fruit circumference; FW- Fruit weight; NFV- Number of fruits per vine; YMFV- Yield of marketable fruits per vine; NSF- Number of seeds per fruit; WS- Weight of 100 seeds; YPH- Yield per hectare

The indirect path analysis for all traits revealed that trait number of fruits per vine exhibited maximum positive direct effect (1.648) on yield per hectare via positive indirect effect of vine length (0.114) and negative indirect effect of yield of marketable fruits per vine (-0.857). Fruit weight exhibited high positive direct effect (0.962) on yield per hectare via positive indirect effect of days to first fruit harvest (0.127), number of fruits per vine (0.148) and negative indirect effect via yield of marketable fruits per plant (-0.478) and days to 50% flowering (-0.225). It is apparent from the Table 4 that yield of marketable fruits per vine exhibited high negative direct effect (-0.952) via positive indirect effect of fruit weight (0.484) and number of fruits per vine (1.485). Days to first fruit harvest exhibited high negative direct effect (-0.570) via positive indirect effect of days to 50% flowering (0.410), days to last fruit harvest (0.120), vine length (0.131), yield of marketable fruits per vine (0.145) and negative indirect effect via fruit weight (-0.215) and weight of 100 seeds (-0.126).

Direct and indirect effect of various characters towards yield per hectare at phenotypic level

Direct and indirect effects of attributing traits on yield per hectare is presented in Table 5. Yield of marketable fruits per vine exhibited highest positive direct effect (0.930) followed by number of fruits per vine (0.043), peduncle length (0.043) and fruit weight (0.030). The lowest positive direct on yield per hectare was observed for fruit circumference (0.003). Negative direct effect on yield per hectare was recorded upon the traits like node number to first pistillate flower, vine length, days to 50% flowering, days to first fruit harvest, days to last fruit harvest, number of seeds per fruit and weight of 100 seeds. Yield of marketable fruit per vine exhibited high positive direct effect (0.930) via positive indirect effect of fruit weight (0.015). Residual effect of path analysis was very low (0.173) suggesting inclusion of maximum yield influencing characters in analysis.

Table 5
Direct and indirect effect of various characters on yield of bottle gourd at phenotypic level (pooled analysis of two season)

Character	NNFPF	DF	DFFH	DLFH	NPBV	INL	PL	VL	FL	FC	FW	NFV	YMFV	NSF	WS
NNFPF	**-0.007**	-0.001	0.000	0.001	0.001	-0.001	-0.001	0.000	-0.001	0.001	0.000	0.000	0.000	0.000	-0.001
DF	-0.002	**-0.017**	-0.011	-0.001	-0.001	-0.001	0.000	-0.003	0.000	0.000	0.006	0.000	0.002	-0.001	-0.002
DFFH	-0.001	-0.010	**-0.016**	-0.003	-0.003	-0.002	-0.001	-0.004	-0.002	0.001	0.002	0.001	0.002	0.001	-0.004
DLFH	0.001	-0.001	-0.001	**-0.007**	-0.001	-0.001	-0.002	0.000	0.000	0.000	0.000	0.000	0.000	-0.001	-0.001
NPBV	-0.002	0.001	0.003	0.003	**0.016**	0.004	-0.003	0.003	0.001	-0.001	0.002	0.003	0.003	-0.001	0.003
INL	0.003	0.001	0.002	0.001	0.003	**0.013**	0.002	0.004	0.005	-0.004	0.003	0.001	0.002	-0.001	0.002
PL	0.006	-0.001	0.003	0.011	-0.007	0.008	**0.043**	0.011	0.012	-0.013	0.009	-0.006	-0.001	0.013	0.012
VL	0.000	-0.002	-0.002	0.000	-0.002	-0.003	-0.002	**-0.009**	-0.001	0.003	-0.001	-0.002	-0.002	-0.003	-0.003
FL	0.002	0.000	0.001	0.000	0.001	0.003	0.002	0.001	**0.008**	-0.005	0.004	-0.001	0.001	-0.001	0.003
FC	-0.001	0.000	0.000	0.000	0.000	-0.001	-0.001	-0.001	-0.002	**0.003**	-0.001	0.000	0.000	0.000	-0.001
FW	0.000	-0.010	-0.004	-0.001	0.003	0.007	0.006	0.002	0.016	-0.007	**0.030**	0.002	0.015	-0.004	0.006
NFV	0.001	0.001	-0.002	0.001	0.007	0.002	-0.006	0.010	-0.007	-0.001	0.003	**0.043**	0.038	0.008	0.002
YMFV	0.008	-0.128	-0.099	0.024	0.189	0.135	-0.025	0.197	0.094	-0.102	0.481	0.818	**0.930**	0.082	0.133
NSF	0.000	-0.002	0.004	-0.006	0.002	0.002	-0.016	-0.017	0.005	0.002	0.007	-0.009	-0.005	**-0.052**	0.006
WS	-0.002	-0.003	-0.005	-0.002	-0.003	-0.003	-0.006	-0.007	-0.009	0.009	-0.004	-0.001	-0.003	0.002	**-0.020**
YPH	**0.006**	**-0.170**	**-0.127**	**0.019**	**0.204**	**0.161**	**-0.007**	**0.187**	**0.116**	**-0.113**	**0.540**	**0.847**	**0.982**	**0.045**	**0.136**
Residual effect: 0.173															

NNFPP- Node number to first pistillate flower; DF- Days to 50% flowering; DFFH- Days to first fruit harvest, DLFH- Days to last fruit harvest; NPBV- Number of primary branches per vine; INL- Inter-nodal length; PL- Peduncle length, VL- Vine length; FL- Fruit length; FC- Fruit circumference; FW- Fruit weight; NFV- Number of fruits per vine; YMFV- Yield of marketable fruits per vine; NSF- Number of seeds per fruit; WS- Weight of 100 seeds; YPH- Yield per hectare

Principal component analysis

Principal components and corresponding correlation coefficients (eigen vectors) for sixteen quantitative traits are presented in Table 6. The principal component analysis grouped quantitative traits under six principal components (PCs), which accounted for 79.33% of the variation. Peduncle length, fruit length and weight of 100 seeds were positively correlated with PC1, which accounted for 31.19% of the total variation. Fruit weight, yield of marketable fruits per vine were positively correlated, whereas days to 50% flowering and days first fruit harvest were negatively correlated with PC2, which accounted for 13.73% of total variation. Number of primary branches per vine was negatively correlated with PC3, which accounted for 11.67% of total variation. Node number to first pistillate flower, vine length, number of fruits per vine and number of seeds per fruit were positively correlated with PC4, which accounted for 8.76% of total variation. Days to last fruit harvest was negatively correlated with PC5, which accounted for 7.86% of total variation. Internodal length, fruit circumference and yield per hectare were positively correlated with PC6, which accounted for 6.12% of total variation.

Table 6

Principal component analysis explained by six principal components on sixteen quantitative agro-morphological traits of bottle gourd genotypes. Loadings shown strong correlation are highlighted in bold (pooled analysis of two season)

S.N.	Quantitative traits	PC1	PC2	PC3	PC4	PC5	PC6
1	Node number to first pistillate flower	0.17	0.09	0.24	**0.30**	0.23	0.09
2	Days to 50% flowering	0.09	**-0.50**	0.17	-0.03	0.25	-0.01
3	Days to first fruit harvest	0.17	**-0.41**	0.00	-0.41	0.15	0.03
4	Days to last fruit harvest	0.09	-0.36	-0.17	-0.19	**-0.50**	0.34
5	Number of primary branches per vine	0.11	-0.19	**-0.46**	-0.24	0.15	-0.25
6	Internodal length	0.29	-0.07	0.04	0.15	0.30	**0.37**
7	Peduncle length	**0.31**	-0.12	0.19	0.13	-0.37	0.36
8	Vine length	0.26	-0.16	-0.23	**0.41**	0.12	-0.27
9	Fruit length	**0.37**	0.20	0.09	-0.20	0.03	-0.14
10	Fruit circumference	-0.35	-0.22	0.03	0.09	0.02	**0.28**
11	Fruit weight	0.31	**0.41**	-0.10	-0.10	-0.10	0.21
12	Number of fruits per vine	-0.06	-0.10	-0.57	**0.33**	0.15	0.15
13	Yield of marketable fruits per vine	-0.30	**0.16**	-0.34	0.02	-0.21	0.12
14	Number of seeds per fruit	0.14	-0.21	0.09	**0.50**	-0.40	-0.27
15	Weight of 100 seeds	**0.31**	0.00	-0.15	-0.13	-0.29	-0.35
16	Yield per hectare	0.32	0.14	-0.30	0.02	0.15	**0.33**
	Explained variance (Eigen Value)	4.99	2.20	1.87	1.40	1.26	0.98
	Proportion of total variance (%)	31.19	13.73	11.67	8.76	7.86	6.12
	Cumulative variance (%)	31.19	44.92	56.59	65.35	73.21	79.33

Summary of genetic variation statistics for ISSR primers

A total of twenty-one ISSR primers were used in the study in which seven primers were found to be polymorphic. Data generated through ISSR marker is presented in Table 7. A total of 48 amplified fragments were generated by 7 ISSR primers of an average around 6.85 fragments per locus. The number of amplified fragments varied from 5 to 10. The highest number of fragments were found in UBC-861 (10), whereas lowest (5) was in ISSR-8. The size of the amplified products varies from approximately 100 bp to 1000 bp. Number of polymorphic loci were ranged from 19 in ISSR 8, ISSR 22 and UBC-880 to 24 at UBC 861, with a mean of 21.28. Percentage of polymorphic loci also varied accordingly with minimum in ISSR 8 (79.17%), ISSR 22 (79.17%) and UBC 880 (79.17%), while it was maximum in UBC 861 (100%), mean of percentage of polymorphic loci was 88.69% for all the locus. Observed number of alleles per locus was ranged between 1.79 (ISSR 8, ISSR 22 and UBC 880) and 2.00 (UBC 861) with mean value of 1.88 per locus. Effective number of alleles also varied with minimum in UBC 880 (1.33) and maximum in UBC 861 (1.79), with mean value of 1.59 for all locus. The major allele frequency was ranged from 0.50 (ISSR 9) to 0.80 (UBC 880) with an average of 0.59. Nei's gene diversity was ranged from 0.22 (UBC 880) and 0.43 (UBC 861) with an average value of 0.33. Shannon's information index also varied from 0.36 (UBC 880) to 0.62 (UBC 861) with the mean value of 0.50. The PIC (polymorphic information content) value represents the relative information of each marker and, in the present study, the average polymorphic information content value was found to be 0.56 and ranged from 0.29 (UBC 880) to 0.76 (UBC 861). In all the primers UBC 861 (0.76), ISSR 9 (0.72), UBC 808 (0.63), ISSR 44 (0.61) and ISSR 8 (0.50) exhibited higher PIC value. The Jaccard's similarity coefficients of 24 bottle gourd genotypes based on 7 ISSR markers were computed. The similarity coefficient was ranged from 0.37 to 0.91.

Table 7
Summary of genetic variation statistics for all loci of ISSR primers for analyzing 24 genotypes of bottle gourd

S. N.	Primer (Oligo name)	Sequence 5' to 3'	Annealing temperature (^{0}C)	GC content (%)	Total no. of amplified fragments (A_0)	No. of polymorphic loci	Percentage of polymorphic loci (%)	Observed number of alleles per locus (Na)	Effective number of alleles per locus (Ne)	Major allele frequency (F_x)	Nei's gene diversity (H)	Sha info ind
1	ISSR8	$(ATG)_6$	41	33.33	5	19	79.17	1.79	1.59	0.65	0.33	0.48
2	ISSR9	$(GAG)_6$	39	66.66	7	22	91.67	1.91	1.47	0.50	0.29	0.46
3	ISSR22	$(AC)_8AA$	43	44.44	7	19	79.17	1.79	1.45	0.73	0.27	0.42
4	ISSR44	$(AC)_8GA$	39	50.00	7	23	95.83	1.95	1.75	0.56	0.41	0.59
5	UBC861	$(ACC)_6$	42	66.66	10	24	100	2.00	1.79	0.39	0.43	0.62
6	UBC808	$(AG)_8C$	41	47.05	6	23	95.83	1.95	1.78	0.53	0.42	0.60
7	UBC880	$(GGAGA)_3$	44	60.00	6	19	79.17	1.79	1.33	0.80	0.22	0.36
		Sub Total			48	149	620.84	13.18	11.16	4.16	2.37	3.53
		Average			6.85	21.28	88.69	1.88	1.59	0.59	0.33	0.50

Cluster analysis based upon ISSR analysis

The UPGMA dendrogram generated from the Jaccard's similarity values using NTSYS-pc 2.01 software based on 7 ISSR primers is presented in Fig. 2. The association among different genotypes was presented in the form of dendrogram prepared. The resemblances coefficient between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among different clusters. The genotypes were grouped in two major clusters I and II. The cluster I was the largest, comprising of 18 genotypes namely BRBG 11 – 1, BRBG 52, BRBG 12 – 3, BRBG 51 – 2, BRBG 52 – 1, BRBG 41 – 1, BRBG 42 – 1, Narendra Rashmi, BRBG 51 – 1, BRBG 21 – 2, BRBG 22 – 2, BRBG 42 – 2, BRBG 42 – 3, BRBG 23, Pusa Naveen, Rajendra Chamatkar, BRBG 52 – 1 and BRBG 31 – 1. Cluster II was the small cluster comprising of 5 genotypes namely BRBG 61 – 1, BRBG 62 – 1, BRBG 190, BRBG 130 and BRBG 140. In cluster II, BRBG 61 – 1 was presented an outgroup or unique genotype. The genotype BRBG 65 was presented as a unique genotype or outgroup outside of these clusters. Jaccard's similarity coefficient was ranged between 0.37 to 0.91. Maximum similarity was found between genotype BRBG 52 and BRBG 11 – 1 with similarity coefficient of 0.91. The smallest similarity value (0.37) suggested maximum genetic divergence between BRBG 65 and BRBG 11 – 1.

Discussion

Effective use of bottle gourd genetic resources for further breeding requires different genomic tools. Significant achievement has been made in the development of genomic resources in bottle gourd during last one or two decades. These genomic resources provide valuable information about genetic relationships among genotypes for effective selection and use in hybridization process (Xu et al., 2014; Wu et al., 2017, Wang et al., 2018). There are very limited genomic resources developed for bottle gourd, which limits the breeding efforts to develop potent genotypes for enhanced production and productivity (Contreras-Soto et al., 2021). The potency of selection depends upon the nature and magnitude of genetic variation found within the breeding material. The larger the variability, a lot scope for improvement by selection procedure is expected. In present investigation, huge genetic variability for agro-morphological traits was obtained among the genotypes, this is may be due to diverse source of materials, still as environment influences the phenotypes. Early picking of crop is crucial for increasing income through catching the early marketplace for higher remuneration. The earliness in bottle gourd is ensured through appearance of pistillate flower at lower node and a smaller number of days taken to 50% flowering that favours early fruit picking. BRBG 21 – 2 in our investigation has taken a smaller number of days to first fruit harvest. Similarly, significant variation for different characters was also reported by Afangideh and Uyoh (2007), Kumar (2008) and Chikezie et al. (2016) in cucumber and Gupta et al. (2015) in bitter gourd. The variation in fruit weight and vine length may be because of the genetic makeup and vigour of different genotypes. Genotypes show significant inter-population variation for quantitative morphological descriptors (Gangopadhyay et al. 2008). Significant phenotypic differences amongst agro-morphological traits of 36 bottle gourd landraces were also reported from South Africa (Mashilo et al. 2016). There is a huge scope for improvement within the crop through the characters like vine length, number of primary branches per vine, node number to first pistillate flower, number of fruits per vine, fruit weight, fruit circumference, fruit length and yield of marketable fruits vine (Yadav et al. 2008). The high genotypic coefficients of variation (GCV) and phenotypic coefficient of variation (PCV) was recorded for fruit circumference, fruit length, yield of marketable fruits per vine, number of seeds per fruit and yield per hectare with maximum in yield of marketable fruits per vine. The phenotypic coefficient of variation (PCV) was higher than GCV for all traits indicating a significant influence of the environment on their expression (Kumar et al. 2011). The estimates of GCV and PCV for all traits indicated that the variability was mainly because of their genetic makeup. Thus, selections are effective on the basis of phenotypic traits alone (Mandal et al. 2015). Heritability estimate is an informative parameter for the breeders for choosing the genotypes for future use. Transmissible variation provides accurate estimates if heritability is studied in conjunction with genetic advance. Almost all morphological traits provided high heritability and genetic advance may offer good response to selection due to additive gene action (Quamruzzaman and Ahmad, 2010). The correlation coefficient between yield and its component traits indicated that all the traits were positively correlated with yield per hectare at both genotypic and phenotypic level. Positive and significant correlation with yield per hectare was determined for the traits like fruit

weight, number of fruits per vine and yield of marketable fruits per vine. Similar results regarding fruit weight were also obtained by Tamang et al. (2018), in cucumber. Yield is a complex trait; these results clearly indicate the influence of these component traits to increase the yield (Singh et al. 2014). Negative correlation of fruit yield with days to 50% flowering and days to first fruit harvest indicates the existence of earliness (Dalamu et al. 2012). Path analysis helps in partitioning the genotypic correlation coefficients into direct and indirect effects of component characters in yield. Number of fruits per vine exhibited highest positive direct effect via positive indirect effect of vine length and negative indirect effect of yield of marketable fruits per vine at genotypic level. Fruit weight exhibited high positive direct impact via positive indirect effect of days to first fruit harvest and number of fruits per vine at genotypic level. Residual effect of path analysis was very low at both genotypic and phenotypic level suggesting inclusion of maximum yield influencing traits in the analysis. These findings are found in agreement with Singh et al. (2014) and Hossain et al. (2010).

Through principal component analysis the agro-morphological traits were grouped under six principal components accounted for 79.33% of the variation. Most of the traits like peduncle length, fruit length, 100 seed weight, fruit weight, yield of marketable fruits per plant are positively correlated to first two components and accounted for maximum variation. In the similar findings first two components representing 52.57% of the total variation for thirteen quantitative traits among nineteen inbred lines of the pumpkin (Ahmed et al. 2016). The first four axes (69.50%) accounted for the overall variation between the thirteen characters of fifty-five snake gourd genotypes (Islam et al. 2020). Results observed in this study are in agreement with the work by several other authors that bottle gourd exhibits significant variation with respect to fruit characteristics (Morimoto et al. 2005; Achigan-Dako et al. 2008; Xu et al. 2014). Quamruzzaman et al. (2008b), also reported that fruit diameter, fruit weight and fruits per plant contributing major portion of the total genetic diversity in ridge gourd. The variation in fruit characteristics among the bottle gourd genotypes in the study could be due to the result of long-term selection by growers, effect of cropping system and environmental condition and the reproduction system of the crop (Koffi et al. 2009; Mashilo et al. 2016).

Genetic diversity of bottle gourd genotypes was assessed and total of 48 reproducible alleles were generated by seven ISSR primers and number of amplified fragments per locus varied from 5 to 10 with an average around 6.85 amplified fragments per locus which was higher than the findings of Xu et al. (2014), reported 2 to 8 amplified fragments (mean = 3.64) per marker in 44 bottle gourd entries using 14 polymorphic SSR loci. Bhawna et al. (2015b) also reported the smaller number of amplified fragments in bottle gourd than our finding. Similar results were also reported by Mahapatra et al. (2022), while assessing the genetic diversity of bottle gourd genotypes from agroclimatic zones of India using SSR markers. ISSR marker assessment is more effective (i.e., more banding morphotypes identified) than other marker analysis. Similar results were obtained when RAPD and ISSR marker analysis was applied to diversity analyses in bitter gourd by Behera et al. (2008). Saxena et al. (2014), also recorded 68 alleles using 51 SSR markers, and the number of alleles per locus ranged from 2 to 5 (on average, 2.80 alleles per locus). The number of loci and their genome coverage are critical in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al., 1996). In our study, number of polymorphic loci were ranged from 19 to 24, with a mean of 21.28. Percentage of polymorphic loci also varied accordingly 79.17%-10 % with mean of percentage of polymorphic loci as 88.69% for all the locus. Similar findings were also reported by several other workers like Levi et al. (2004) and Behera et al. (2008) in assessing cultivar variation in watermelon and bitter gourd respectively using ISSR primers. Observed number of alleles per locus was ranged between 1.79 and 2.00 with mean value of 1.88 per locus. Effective number of alleles also varied between 1.33 and 1.79, with mean value of 1.59 for all locus. In the current study, these values were in agreement with the report of Bhawna et al. (2015b) with 1.46–3.29 (mean = 2.08) and Mashilo (2016) with 1.58–6.14 (mean = 3.1) and Mashilo et al. (2016), with 1.99–6.72 (mean = 3.75) by using SSR markers. This indicates a high level of genetic diversity among bottle gourd genotypes useful for future breeding and conservation. The major allele frequency was ranged from 0.50 to 0.80 with an average of 0.59. Similar results were reported by Alhariri et al. (2021), in bitter gourd and Mahapatra et al. (2022), in bottle gourd using SSR markers. Nei's gene diversity was ranged from 0.22 to 0.43 with an average value of 0.33. The Nei's gene diversity in the study was consistently in agreement with the mean value reported by Gurcan et al. (2015). Shannon's information index also varied from 0.36 to 0.62 with the mean value of 0.50. These results indicate that bottle gourd population expressed moderate interpopulation diversity, thereby enhance their survival capacity to adverse climatic condition (Bhawna et al., 2014) and also equally useful for further breeding. In the present study, the average polymorphic information content value was found to be 0.56 and ranged from 0.29 to 0.76. Almost each primer in the study exhibited higher PIC value. The increased level of discrimination of ISSR markers witnessed in the study may be due to comparatively higher values of average polymorphic information content (0.56) as the diverse nature of the accessions examined (Behera et al., 2008). These PIC values were higher than those found by Saxena et al. (2014), in 54 accessions of bitter gourd, with a polymorphic information content of 0.139–0.775 (average 0.369), whereas Dalamu et al. (2012), found 0.17 PIC values using 11 ISSR markers in 50 accessions of bitter gourd. These values are also comparatively higher than those reported by Sarao et al. (2013) and Xu et al. (2011). Yildiz et al. (2015), also reported PIC values of 0.12 to 0.52 in bottle gourd, whereas Bhawna et al. (2015b), found PIC values of 0.05 to 0.54 using 19 SSR markers. The PIC defines a discriminatory power of a molecular marker, which depends on the number of alleles and relative frequency of an allele in the population (Gaikward et al., 2008). In our study, a large percentage of the markers exhibited a fair discriminatory power. Generally, most of the markers exhibited PIC values > 0.5, suggesting a high discriminating ability of the ISSR markers for analysing the bottle gourd genotypes (Mashilo, 2016). The high levels of polymorphism observed in the current study could be due to the inherent genetic variability of the studied bottle gourd genotypes (Mashilo et al., 2016). The UPGMA dendrogram generated from the Jaccard's similarity values showed association among different genotypes in the form of dendrogram prepared. Based upon UPGMA dendrogram, the genotypes were grouped into two major clusters I and II. The cluster I was the largest, comprising of 18 genotypes. Cluster II was the small cluster comprising of 5 genotypes. In cluster II, BRBG 61 – 1 was presented an outgroup or unique genotype. The genotype BRBG 65 was presented as a unique genotype or outgroup outside of these clusters. The dendrogram didn't show any correlation with geographical location. Jaccard's similarity coefficient was ranged between 0.37 to 0.91. Maximum similarity was found between genotype BRBG 52 and BRBG 11 – 1 with similarity coefficient of 0.91. The smallest similarity value (0.37) suggested maximum genetic divergence between BRBG 65 and BRBG 11 – 1. The genotype BRBG 65 was separated genetically from the other 23 genotypes studied, which may be due to its unique morphological features compared with other genotypes (Alhariri et al., 2021). Similar findings were also reported by Bhawna et al. (2014), in which dendrogram revealed two major clusters with one unique genotype also while assessing 42 bottle gourd genotypes for genetic diversity analysis using ISSR marker. This proves the high discrimination ability of ISSR marker in bottle gourd. The finding is also in agreement with the report of Xu et al. 2011; Sarao et al. 2013 and Mashilo, 2016.

Conclusion

Results obtained in the study concluded that set of materials used in the study were in wide range of genetic variation for all the attributes. Most of the genetic diversity was observed with respect to fruit characteristics. Important fruit traits that represented the diversity of the landraces were: node number to first female flower, number of fruits per vine, average weight of fruit, fruit length, fruit circumference and yield of marketable fruits per vine. The PCV exhibited higher value than GCV the traits under investigation indicating a considerable influence of the environmental factors on their gene expression. The high variability and heritability along with high value for genetic advance for these traits indicated that the genotypes might be evaluated in multiplication trials and may be chosen as donors for mentioned traits in hybridization programme. Quantitative traits related to fruit characteristics have shown strong character association with yield per hectare. Principal component analysis on quantitative traits depicted six principal components, accounted for great degree of the total variation. Based on ISSR markers analysis, the UPGMA dendrogram classified twenty-four genotypes into two major clusters indicating the existence of moderate variation at the genetic level. This ISSR marker-based study has also identified the existence of a broad genetic base in the available germplasm lines of bottle gourd. The analysis of genetic variability among the bottle gourd germplasm evaluated herein defines a complex marker array with ISSR analysis for genetically improved genotype curation, for the generation of standard accession for genetic analysis in future. It provides an ample scope for the selection of desirable parents for crossing programme and breeding populations for traits of significance and also other breeding analysis, such as association mapping, and trait-specific breeding for bottle gourd.

Declarations

Acknowledgements The authors thank Department of Horticulture (Vegetable & Floriculture) and Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India for providing the facility to conduct the experiment.

Authors contribution Conceptualization:R.K.[1], R.K.[2]; investigation: R.K.[1]; formal analysis: R.K.[1], J.K.; data analysis: BSB; writing original draft: R.K.[1]; writing review and editing: R.K.[2], B.D.P.; supervision: R.K.[2], B.D.P.

Conflict of interest All the authors of this manuscript declare that they have no conflict of interest.

Human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Al-Jibouri HA, Miller PA, Robinson HF (1958) Genotypic and environmental variance in an upland cotton cross of interspecific origin. Agron J 50:633-637.
2. Afangideh U, Uyoh EA (2007) Genetic variability and correlation studies in some varieties of cucumber (*Cucumis sativus* L.). Jordan J Agric Sci 3(4):376-384.
3. Achigan-Dako EG, Fuchs J, Ahanchede A, Blattner FR (2008) Flow cytometric analysis in Lagenaria siceraria (Cucurbitaceae) indicates correlation of genome size with usage types and growing elevation. Plant Syst Evol 276: 9-19.
4. Ahmed B, Masud MAT, Zakaria M, Hossain MM and Mian MAK (2016) Genetic divergence among inbred lines of pumpkin (*Cucurbita moschata* Duch Ex Poir). Bang J Pl Breed Genet 29(2): 25-31.
5. Alhariri A, Behera TK, Jat GS, Devi MB, Boopalakrishnan G, Hemeda NF, Teleb AA, Ismail E, Elkordy A (2021) Analysis of genetic diversity and population structure in bitter Gourd (*Momordica charantia* L.) Using Morphological and SSR Markers. Plants 10: 1860.
6. Anonymous (2022) Data base area, production and productivity. National Horticulture Board, Gurgaon, Haryana, India.
7. Burton GW, Devane EH (1953) Estimation of heritability in tall fescue from replicated clonal material. Agron J 45:478-487.
8. Bornet B, Branchard M (2001) Non-anchored inter-simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Rep 22:427-432.
9. Behera TK, Singh AK, Staub JE (2008) Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies. Sci Horti 115:209-217.
10. Bhawna M, Abdin Z, Arya L, Saha D, Sureja AK, Pandey C, Verma M (2014) Population structure and genetic diversity in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] germplasm from India assessed by ISSR markers. Plant Syst Evol 300:767-773. https://doi.org/10.1007/s00606-014-1000-5.
11. Bhawna AMZ, Arya L, Verma M (2015b) Transferability of cucumber microsatellite markers used for phylogenetic analysis and population structure study in bottle gourd (*Lagenaria siceraria* (Mol.) Standl.). Appl Biochem Biotech 175:2206-2223.
12. Bamaniya BS, Shree S, Verma RB, Kumar R, Verma RK (2018) Polygenic variations and character association studies in garlic. Curr J Appl Sci Tech 31(3):1-8.
13. Cutler HC, Whitaker TW (1967) *Cucurbits from the Tehuacan caves, In: Prehistory of the Tehucan Valley*, (Eds. D.S. Byers), University of Texas Press, Austin, Texas, USA. 212-219 p.
14. Chowdhury MA, Vanderberg B, Warkentin T (2002) Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica 127:317-325.
15. Chikezie OE, Peter EO, Christian UA, Uche PC. (2016) Studies of phenotypic and genotypic variation in sixteen cucumber genotypes. Chile J Agric Res 76(1): 307-313.
16. Contreras-Soto R, Salvatierra A, Maldonado C, Mashilo J (2021) The genetic diversity and population structure of different geographical populations of bottle gourd (*Lagenaria siceraria*) accessions based on genotyping-by-sequencing. Agron 11: 1677. https://doi.org/10.3390/agronomy11081677.
17. Dewey DR, Lu KH (1959) A Correlation and Path-Coefficient Analysis of Components of Crested Wheatgrass Seed Production. Agron J 51(9):515-518.

18. Decker-Walters DS, Wilkins-Ellert M, Chung SM, Staub JE (2004) Discovery and genetic assessment of wild bottle Gourd [*Lagenaria siceraria* (Mol.) Standley; Cucurbitaceae] from Zimbabwe. Econ Bot 58(4):501-508.
19. Dalamu TK, Behera TK, Gaikwad AB, Saxena S, Bharadwaj C, Munshi, AD (2012) Morphological and molecular analyses define the genetic diversity of Asian bitter gourd (*Momordica charantia* L.). Aust J Crop Sci 6(2):261-267.
20. Debnath A, Kumar R, Prasad SK, Sharma N, Kushwah JK (2020) Multivariate analysis for genetic diversity estimation among tomato (*Solanum lycopersicum* L.) genotypes. Eco Env Cons 26(3): 1208-1211.
21. Debnath A, Kumar R, Prasad SK, Sharma N (2021) Genetic variation and character association study among morphological and biochemical traits of tomato (*Solanum lycopersicum* L.) genotypes. The Biosc 16(1):69-75.
22. Ferdous M, Nath UK and Islam A (2011) Genetic divergence and genetic gain in bread wheat through selection practices. J Bangla Agric Univ 9(1): 1–4.
23. Gonçalves LSA, Rodrigues R, Amaral ATJR, Karasawa M, Sudre CP (2008) Comparison of multivariate statistical algorithms to cluster tomato heirloom accessions. Genet Mol Res 7:1289-1297.
24. Gaikward AB, Singh AK, Chandel D, Karihaloo JL, Staub JE (2008) Amplified fragment length polymorphism analysis provides strategies of bitter gourd (*Momordica charantia* L.). Hort Sci 43:127-133.
25. Gangopadhyay KK, Kumar G, Meena BL, Bishat IS (2008) Genetic Diversity Studies in Ash Gourd (*Benincasa hispida)* from Northern India. National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. 21(3):206- 212.
26. Gupta N, Bhardwaj ML, Singh SP, Sood S (2015) Genetic Diversity for Growth and Yield Traits in Bitter Gourd. Brazil Archiv Biol Techn: 480-489.
27. Gurcan K, Say A, Yesitir H (2015) A study of genetic diversity in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] population, and implication for the historical origins on bottle gourds in Turkey. Genet Resour Crop Evol 62:321-333.
28. Hossain MF, Rabbani MG, Hakim MA, Ahsanullah, ASM (2010) Study on variability character association and yield performance of cucumber (*Cucumis sativus* L.) Bangla Res Publ J 4(3):297-311.
29. Hanson CH, Robinson HF, Comstock RE (1956) Biometrical studies of yield in segregating population of Korean Lespedeza. Agron J 48:268-272.
30. Hailu F (2011) Genetic diversity and grain protein composition of tetraploid wheat (*Triticum durum* Desf.) germplasm from Ethiopia. Doctoral thesis Swedish University of Agricultural Sciences: SLU Service/Repro, Alnarp.
31. Islam MR, Hossain MS, Bhuiyan MSR, Hasan GN, Syed MA (2010) Multivariate analysis of bitter gourd (*Momordica charantia* L.). Middle-East J Scient Res 5(2):86-90.
32. Islam MR, Rahman MM, Zakaria M, Hoque MA, Hasan M (2020) Genetic diversity in snake gourd (*Trichosanthes cucumerina* var. *anguina* L.). Bang J Agril Res 45(2): 99-111.
33. Johnson HW, Robinson HF, Comstock RE (1955) Estimate of genetic and environmental variability in soybean. Agron J 46:314-318.
34. Jackson ML (1973). Soil chemical analysis. Prentice hall of India Pvt. Ltd., New Delhi, 498.
35. Kumar A, Kumar S, Pal AK (2008) Genetic variability and characters association for fruit yield and yield traits in cucumber. Ind J Hortic 65(4):423-428.
36. Koffi KK, Anzara GK, Malice M, Djè Y, Bertin P, Baudoin J-P, Zoro Bi IA (2009) Morphological and allozyme variation in a collection of Lagenaria siceraria (Molina) Standl. from Côte d'Ivoire. Biotech Agron Société et Environ 13: 257-270.
37. Kumar A, Singh B, Kumar M, Naresh, RK (2011) Genetic variability, heritability and genetic advance for yield and its components in bottle gourd (*Lagenaria siceraria* (Mol.) Standl.). Annals Horticulture 4(1):101-103.
38. Kundu BC, Hossain MM, Mian KMA, Mian IH (2012) Genetic divergence in bitter gourd (*Momordica charantia*L.). J Asia Soci Bangla Sci 38(2):125-134.
39. Kumar R, Chhetri S, Bamaniya BS, Kushwah JK, Verma RK (2021) Molecular tools for Bacterial wilt resistance in Tomato. In: Biotic stress management in Tomato (Biotechnological Approaches) in the series- Innovation in Horticultural Science. Apple Academic Press, CRC Press (Taylor & Francis Group), USA. Pp 25-42.
40. Lush JL (1949) Heritability of quantitative characters in farm animals. *Proceedings of 8th International Congress*, Genet Suppl Vol Hereditas: 356-395.
41. Loarce Y, Gallego R, Ferrer E (1996) A comparative analysis of genetic relationships between rye cultivars using RFLP and RAPD markers. Euphyt 88:107-115.
42. Lynch M and Walsh JB (1998) *Genetics and analysis of quantitative traits*. Sinaure Assocs. Inc., Sunderland, Massachusetts, USA, 980 p.
43. Levi A, Thomas CE, Newman M, Reddy OUK, Zhang X, Xu Y (2004) ISSR and AFLP markers differ among American watermelon cultivars with limited genetic diversity. J Am Soc Horti Sci 129:553-558.
44. Morimoto Y, Maundu P, Fujimaki H, Morishima H (2005) Diversity of landraces of the white-flowered gourd (Lagenaria siceraria) and its wild relatives in Kenya: fruit and seed morphology. Genet Res Crop Evol 52: 737-747.
45. Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol 147: 969–977.
46. Munhoz REF, Prioli AJ, Amaral ATJR, Scapim CA (2009) Genetic distances between popcorn populations based on molecular markers and correlations with heterosis estimates made by diallel analysis of hybrids. Genet Mol Res 8:951-962.
47. Mandal J, Tirumalesh M, Dhangrah VK (2015) Studies on genetic variability and trait inter-relationship in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] Hortic Flora Res Spectr 4(1):34-38.
48. Mashilo J, Shimelis H, Odindo A (2016) Genetic diversity of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces of South Africa assessed by morphological traits and simple sequence repeat markers. South Afri J Plant Soil 33(2):113–124.
49. Mashilo J (2016) Genetic diversity of south African bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces revealed by simple sequence repeat markers. Hort Sci 51(2):120-126.

50. Mashilo J, Shimelis HA, Odindo AO, Amelework BA (2016) Genetic differentiation of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces assessed by fruit qualitative traits and simple sequence repeat markers. Sci Horti 216(2017):1-11.
51. Mahapatra S, Sureja AK, Behera TK, Verma M (2022) Assessment of genetic diversity of ninety-one bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] genotypes from fourteen diferent agro-climatic zones of India using agro-morphological traits and SSR markers. Mol Biol Rep 49: 6367-6383.
52. Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J (2012) PIC calc: an online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet 50:670–672.
53. Olsen SR, Cole CV, Watanabe FS (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circular No. 939, US Government Printing Office, Washington DC.
54. Panse VG, Sukhatme PV (1984) *Statistical methods for agricultural workers*. Third Edition, Indian Council of Agricultural Research, New Delhi. 347
55. Peng X, Liu JJ, Xiang Y, Huang S (2006) *A practical handbook of plant molecular biotechnology*. Chemical Industry Press, Beijing.
56. Peakell, R. and P.E. Smouse. 2007. GENALEX 6: Genetic analysis in Excel. Population software for teaching and research. Mol Ecol Notes 6:288–295.
57. Quamruzzaman AKM, Rahman MH, Nazrul Islam Md, Rahman SML and Sarker BC, (2008b) Genetic diversity in land races of ridge gourd. Bangl Res Pub J 1(1): 5-8.
58. Quamruzzaman AKM, Ahmad S (2010) Genetic analysis of some yield components of bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]. J Agric 8(1):1-9.
59. Subbiah BV, Asija GL (1956) A Rapid Procedure for the Estimation of Available Nitrogen in Soils. Curr Sci 25:259-260.
60. Searle SR (1961) Phenotypic, genotypic and environmental correlation. Biometric 17(3):474-480.
61. Sarao NK, Neha MP Kaur K (2013) Microsatellite-based DNA fingerprinting and genetic diversity of bottle gourd genotypes. Plant Genet Resour Charact Util (First View Article):1-4.
62. Saxena S, Singh A, Archak S, Behera TK, John JK, Meshram SU, Gaikwad AB (2014) Development of novel simple sequence repeat markers in Bitter Gourd (*Momordica charantia* L.) through enriched genomic libraries and their utilization in analysis of genetic diversity and cross-species transferability. Appl Biochem Biotech 175:93-118.
63. Singh HK, Singh VB, Kumar R, Barnawal DK, Ray PK (2014) Assessment of genetic diversity based on cluster and principal component analyses for yield and its contributing characters in bitter gourd (*Momordica charantia* L.). Ind J Hortic 71: 55-60.
64. Sidhu GK, Pathak M (2016) Genetic diversity analysis in bitter gourd (*Momordica charantia* L.) using morphological traits. Int J Agric Innov Res 5(1):2319-1473.
65. Thormann CE, Camargo MEA, Osborn TC (1994) Comparison of RFLP and RAPD markers to estimating genetic relationships within and among Cruciferous species. TheorAppl Genet80:973-980.
66. Tamang B, Bhutia KD, Kumar R, Sharma L, Bamaniya BS (2018) Genetic variation and character association study in local cucumber (*Cucumis sativus* L.) genotypes of Sikkim. Curr J Appl Sci Tech 31(1):1-9.
67. Wright S (1921) On the nature of size factors. Genet 3:367-374.
68. Wu S, Shamimuzzaman M, Sun H, Salse J, Sui X, Wilder A, Fei Z (2017) The bottle gourd genome provides insights into Cucurbitaceae evolution and facilitates mapping of a Papaya ring-spot virus resistance locus. Plant J 92:963–975.
69. Walkley AJ, Black IA (1934) Estimation of soil organic carbon by the chromic acid titration method. Soil Sci 37:29-38.
70. Wang Y, Xu P, Wu X, Wu X, Wang B, Huang Y, Li G (2018) Gourd Base: A genome-centered multi-omics database for the bottle gourd (*Lagenaria siceraria*), an economically important cucurbit crop. Sci Rep 8:3604.
71. Xu P, Wu X, Luo J, Wang B, Liu Y, Ehlers JD, Lu Z, Li G (2011) Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genomi 12:467.
72. Xu P, Xu S, Wu X, Tao Y, Wang B, Wang S, Li G (2014) Population genomic analyses from low-coverage RAD-Seq data: A case study on the non-model cucurbit bottle gourd. Plant J 77:430-442.
73. Yadav M, Singh DB, Chaudhary R, Singh D (2008) Genetic variability in bitter gourd (*Momordica charantia* L.). J Hortic Sci 3(1):35-38.
74. Yildiz M, Cuevas HE, Sensoy S, Erdinc C, Baloch FS (2015) Transferability of Cucurbita SSR markers for genetic diversity assessment of Turkish bottle gourd (*Lagenaria siceraria*) genetic resources. Biochem Syst Ecol 59:45-53.
75. Zietkiewick E, Rafalski A and Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomic 20:176-183.

Figures

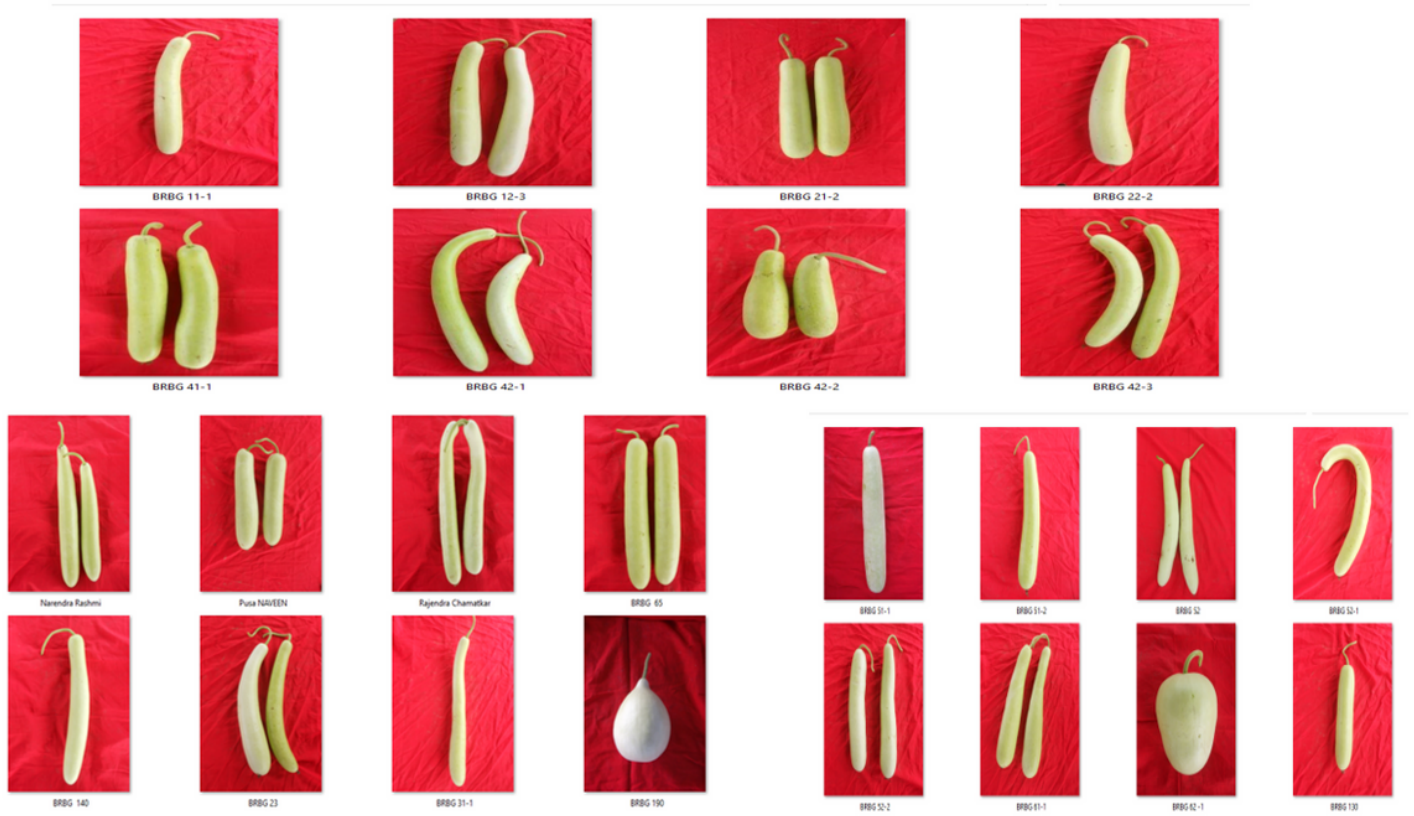


Figure 1

Genetic variability among different genotypes of Bottle Gourd

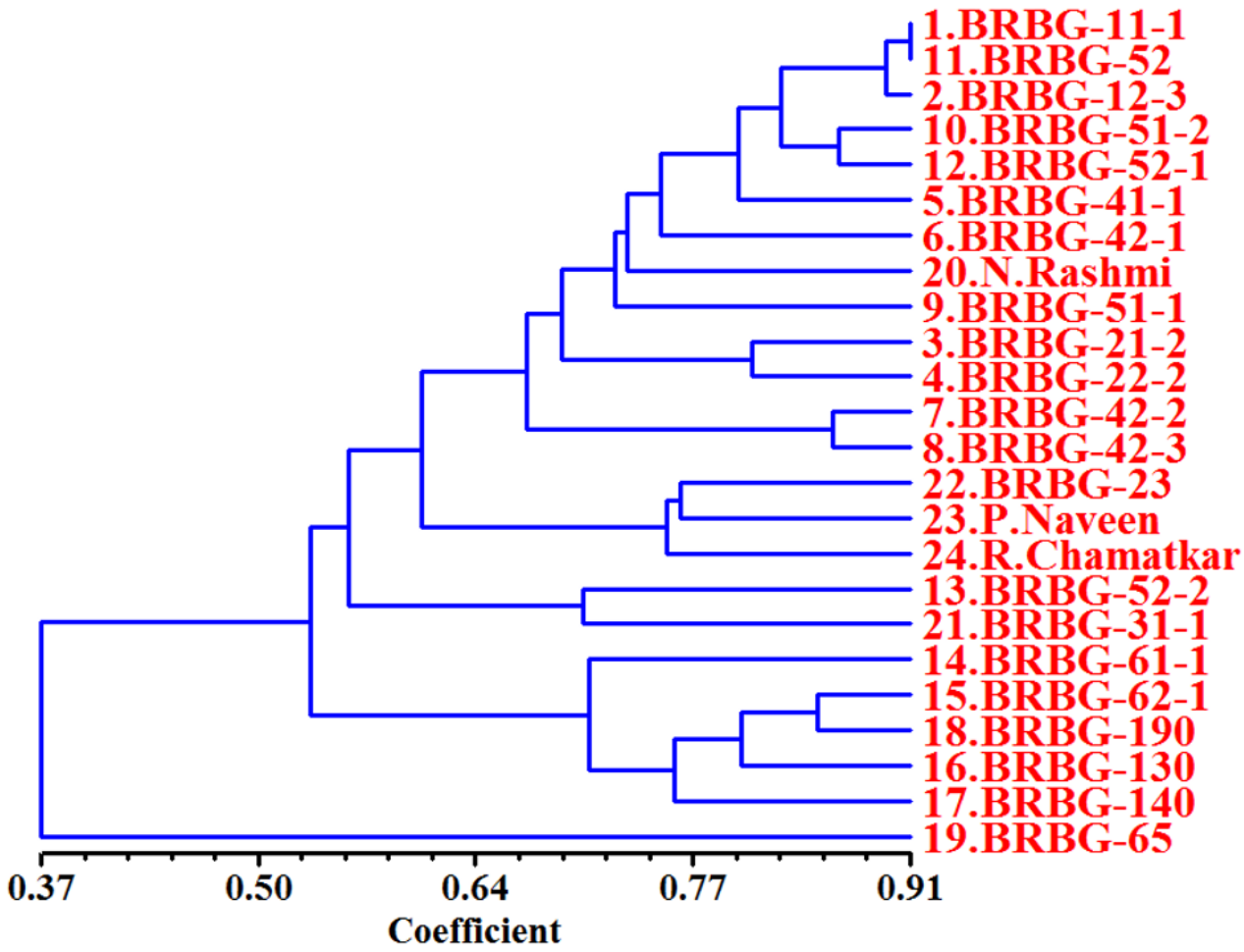


Figure 2

UPGMA dendrogram based on ISSR marker using Jaccard's similarity coefficient