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Analysis of genetic diversity among Indian garlic (*Allium sativum* L.) genotypes using SSR markers and morphological traits

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Abstract

Genetic diversity of seventeen garlic genotypes wwas evaluated using morphological characters and molecular markers. Twenty-three morphological characters related to the foliage and bulb traits of garlic were assessed and categorized into quantitative and qualitative parameters. Analysis on the basis of 16 SSR primers was used, out of which 7 produced clear polymorphic bands in garlic genotypes and conveniently distinguished all the genotypes under study. SSR markers used were capable of detecting 42 alleles with an average of 2.63 alleles per locus. The number of alleles per locus ranged from 1 to 7 and the allelic polymorphism information content (PIC) value ranged from 0.14 to 0.45 with an average of 0.26. Genetic divergence was calculated using the Pearson coefficient in which the genetic similarity varied from 0.04 to 1.0 and on the basis of morphological traits ranged from 1.65 to 6.12 with an average of 3.6 which was higher than molecular markers. Morphological traits are less valid to evaluate genetic diversity because these traits depend on the environmental and climatic factors like day length and temperature, as they are adapted for distinct climatic regions. The study revealed considerable amount of genetic variability among garlic genotypes and identified a correlation between molecular markers and morphological traits. The present study also indicates that though the microsatellite markers are fast and high throughput for DNA fingerprinting there is a need for more markers to be developed to assess genetic diversity.

Key words: Indian garlic, genetic diversity, molecular markers, morphological characterization, SSR

Introduction

Garlic (*Allium sativum* L.) is a diploid species (2n=2x=16) belonging to *Alliaceae* family and is one of the important crop grown all around the world. It is a bulbous perennial herb and is vegetatively propagated through cloves and believed to be native of Middle East Asia (Shaaf et al. 2014). Garlic has been known mainly for its culinary and medicinal properties, used as a spice or condiment throughout India. Regular use of garlic prevents various diseases such as diabetes, asthma, cardiovascular diseases and cancer (Cunha et al. 2012). For food purpose, it is used as green stalks, young leaves are also eaten fresh or cooked and furthermore, also a large quantity of garlic is used for pharmaceutical purposes (Khar et al. 2008). It consists of sulfur compounds namely allin, allicin, ajoene, allyl propyl disulfide, diallyl trisulfide, S-allyl cysteine, vinyldithiines, S-allyl mercaptocystein and some of the essential enzymes *viz.* allinase, peroxidases, myrosinase which are beneficial compounds for human health (Mikaili et al. 2013). Garlic production is reported to be highest in China (223.33 lakh tonnes) contributing to 78% of the world's production followed by India (17.21 lakh tonnes) with a productivity of 5.68 t/ha and total area is 3.03 lakh ha (FAOSTAT 2018). Several methods are available for genetic characterization of genotypes which includes morphological markers, biochemical markers and molecular markers depending on their ease of use, cost and reproducibility of results (Morales et al. 2013). Earlier morphological markers were primarily used to distinguish between garlic cultivars (Panthee et al. 2006). As morphological markers have limitations because they are environmentally influenced, most of these markers can only be evalu-ated in adult plants, which not only require time and space for plant growth, which often makes their evalua-tion unreliable. To overcome these barriers more precise techniques such as the use of DNA based molecular markers are widely used.

Recently molecular markers have been incorporated in *Allium* crop breeding for the use of selecting desirable genotypes, to study genetic diversity and cultivar stability, and to be used for cultivar identification. PCR based

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markers are used for identification of genetic diversity, DNA fingerprinting, phylogenetic analysis, characterization of genome organization thus, making advantage over morphological and biochemical markers (Kemper 2000). However, it has been seen that garlic displays wide range of phenotypic variations in characters such as number of leaves, plant height, leaf width, pseudostem length, colar thickness, bulb size, bulb number of cloves, weight of 5 bulbs, weight of 10 cloves and TSS while the qualitative characters *viz.*; density of leaves, foliage attitude, leaf intensity of green colour, leaf waxiness, bulb shape in longitudinal section and cross section, compactness of cloves in bulb, ground colour of dry external scales, anthocyanin stripes on dry external scales of bulb, skin adherence of dry external scale on bulbs and clove colour of flesh. Garlic displays various phenotypic variations therefore it is presumed that a wide range of genetic diversity is observed in cultivated garlic (Kesralikar et al. 2017). The study of ecological, morphological and genetic diversity is of great importance for plant improvement. Although garlic genotypes are vegetatively propagated by cloves, even though they exhibit greater variation between clones. Thus, the assessment of garlic genetic resources both by phenotypic traits and molecular markers will make us understand the distinction between garlic genotypes.

As the farmers are adopting the cultivation of new varieties of garlic therefore, there is a great need to develop productive combinations of traits in garlic cultivars. In the present investigation, morphological characterization and use of SSR molecular markers was employed. The molecular marker system is available to assess the genetic diversity study as they are unlimited in number, not affected by the environment and do not require much time. Though numbers of markers are used such as RFLP, RAPD, ISSR, CAPS and SNP but SSR markers are widely used to detect genetic diversity. SSR markers are simple, fast, cheap and easily automated and also co-dominant which can be successfully utilized to detect the genetic diversity and relationship in onion and garlic germplasm (Anandhan et al. 2014, Kumar et al. 2019 and Gupta et al. 2020). The objective of this study was to evaluate the genetic diversity of 17 garlic genotypes by combining morphological char-acters and SSR molecular marker data.

Materials and Methods

Planting materials: Present study was carried out during 2017-18 and 2018-19 at the Experimental Farm of ICAR-Directorate of Onion and Garlic Research, Rajugunagar, Pune (Maharashtra). Before planting of cloves all the essential nutrients were applied in the soil. Recommended cultural operations were carried out. Healthy and uniform sizes of cloves were planted in 2 cm depth at a spacing of 10×15 cm in a Randomized Block Design with three replications. All the remaining necessary fertilizers were applied in the field after planting of cloves. The crops were kept free of weeds by hand weeding. Seventeen Indian garlic genotypes *viz.*, Bhima Omkar, Bhima Purple, G-1, G-41, G-50, G-282, G-323, G-386, GG-2, GG-3, GG-4, Godavari, Phule Baswant, Rani Bennur Local, Ooty Local, Sikkim Local and Silkuei Local were used for morphological and molecular analysis.

Analysis of morphological parameters: Twenty-three morphological characteristics were assessed which includes 12 quantitative and 11 qualitative traits. Vegetative characteristic was evaluated 100 days after planting and non-vegetative characteristics were evaluated after harvesting of bulbs. Ten plants or ten bulbs of each replication were used for the analysis. Twelve quantitative traits *viz.*; number of leaves, plant height, leaf width, pseudostem length, collar thickness, days to harvest, bulb size, number of cloves per bulb, clove size diameter, weight of 5 bulbs, weight of 10 cloves and total soluble solids (TSS) wer assessed. The qualitative characters studied were density of leaves, foliage attitude, intensity of green colour in leaf, leaf waxiness, bulb shape in longitudinal section and cross section, compactness of cloves in bulb, ground colour of dry external scales of bulb, anthocyanin stripes on dry external scales of bulb, skin adherence of dry external scales on bulb and colour of colve flesh were analyzed after harvesting.

Genetic characterization using SSR markers: Genomic DNA was extracted from 17 garlic genotypes using the standard CTAB method (Dellaporta et al. 1983). About 0.1 g of young leaf tissue from each sample was homogenized in liquid nitrogen and incubated at 65°C for 60 minutes with 500 ìl of CTAB buffer (1.0 M pH 8.0 Tris-HCl, 3 M NaCl, 0.5 M EDTA, 10 µl â-mercaptoethanol). Then 500 ìl of chloroform: isoamyl alcohol mixture (24:1) was added and centrifuged thoroughly for 10 minutes at 12,000 rpm. After centrifugation, the supernatant layer was collected in a new eppendorf tube in which 600 ìl chilled isopropanol was added. After storage at -20°C for 30-60 minutes, precipitated DNA was centrifuged for 10 minutes at 10,000 rpm at 4°C. The pellet was washed twice with 70% chilled ethanol, air-dried for half-hour and finally dissolved in 100 ìl TE buffer, treated with 2 ìl RNase (10 mg/ml) solution at 37°C for 2 hours and finally stored at -20°C. DNA quantification was done on 1% agarose gel stained with ethidium bromide and visualized in the Gel Documentation System.

SSR analysis: PCR amplifications were performed using 16 SSR primers (Table 1). Amplifications were carried out in 10 µl reaction volume containing 1U of Taq DNA polymerase (Thermo scientific), 10X PCR buffer containing 15 mM $MgCl_2$ (Thermo scientific), 1 mM primer, 0.25 mM of dNTP (Thermo scientific) and 50 ng of template DNA. The conditions for PCR amplification were optimized for *Taq* DNA polymerase and DNA templates. Amplification conditions included a preliminary 5 minutes denaturation at 94ºC followed by 35 cycles of denaturation at 94 ºC for 1 minute where annealing temperature varied at 50-55ºC for 1 minute depending upon the specificity of primer and extension at 72 ºC for 2 minutes with final extension at 72ºC for 10 minutes and final hold at 4ºC. Final PCR products were resolved in 3% agarose gel stained with ethidium bromide and images were visualized under the gel documentation system (Vision View software).

Statistical analysis: Data obtained on morphological characterization were directly subjected to the statistical analysis. However, SSR data were scored as present (1) or absent (0) of the amplified products. Dendrogram and similarity matrices were generated according to the Pearson coefficient using UPGMA in MVSP software. The principal component analysis was carried out on the basis of morphology using ClustVis software (https://biit.cs.ut.ee/clustvis/). The dendrogram was generated on morphological traits by Euclidean coefficient using SAS JMP software.

Table 1: List of SSR markers used in genetic diversity analysis of Indian garlic

Marker	Forward primer 5'-3'	Reverse Primer 5'-3'	Tm	No. of alleles	Polymorphism status
ACM008	GCCGGAAGAGGAGAAGAAGT	CATAATTCCCATGGCTTTGC	50.3	2	P
ACM054	GAGTGAGAGGGGAAATGGAA	AAAGATGGTTTGTTGGTGGC	50.3	4	P
ACM066	CTCCCCGCAACCAGTAATAA	GCTTGGGTTTTGTTTCTCCA	50.3	2	M
ACM069	TTCTGCGCTCTTCCCAGTAT	CAAGCGGTTTTGTTTCTCCA	50.3	1	M
ACM080	GCATTATGCAGTAACGGGCT	GCAGCAGCATTTGATTGAAC	50.3	6	M
ACM154	CGATGAATACACCGATGACG	CTTGTTTTGGCAGTTGGGAT	50.3	3	P
ACM033	CCTTCTCCCCATTCTCTTCC	ATCATCGTCCTCGTCCTCAT	52.3	2	P
ACM038	ATGCCAGACTACGACAACGA	ACGCCTACCAACCTTCAATG	52.3	2	P
ACM047	CATTCATCTACTATCTTCTTCAGCC	GAGGTCATTGGTTTGGTTAGC	52.9	1	M
ACM077	AAATTATGGGCCACCTCCTC	CAAGATTGTCGACTCCCCAT	52.3	1	M
ACM081	CTGAAAAGAAACCCGCAGAG	TCAGGATGCACTTGCTTCAG	52.3	1	M
ACM180	CCTTCAGACCCTAAAAGGGC	CAAAGGACATTGGCAAGTGA	50.3	2	M
ACM078	CGCAGAATCTCGTCCTTTTT	AATGGTTTGGAGGTCAGTCG	50.3	7	P
ACM093	GCCAACAGTTTTCGTAAGTTGA	ATTCTCTTCGGCTTTCGTGA	50.3	2	M
ACM094	GATGATGGCGAAGACACAGA	AAAAACGGCTTAGGAATTTAACG	50.3	2	M
ACM151	TGTCAGACAAGCAACTCCTCC	AGGTGAGGCTTAGATGGGGT	54.4	4	P

Note: P- Polymorphic, M- Monomorphic, Tm- Annealing temperature; (Jayaswall et at. 2019)

Table 2: Mean performance of garlic (*Allium sativum* L.) genotypes for twelve quantitative morphological traits

Entries	NOL	Plant height (cm)	Leaf width (widest leaf) (cm)	PSL (cm)	CTh (mm)	Bulb size (diameter) (mm)	No. of cloves per bulb	Clove size (diameter) (mm)	Wt 5 bulb (g)	Wt. 10 cloves (g)	DTH	TSS (%)
Bhima Omkar	9.07	36.40	1.19	6.58	0.73	27.65	15.73	9.79	61.00	14.17	123.67	41.76
Bhima Purple	8.78	37.05	1.31	6.33	0.83	28.55	15.83	9.48	56.00	12.17	122.11	39.61
G-1	9.06	38.29	1.31	5.95	0.86	26.32	15.24	9.23	56.83	10.75	126.89	39.44
G-282	8.56	38.52	1.21	6.89	0.82	27.27	15.84	9.32	51.50	11.83	122.22	40.97
G-323	8.76	38.00	1.27	6.56	0.83	26.57	16.19	9.89	56.00	10.92	124.89	40.50
G-386	9.13	41.86	1.17	7.09	0.89	27.57	15.20	9.93	55.50	11.50	122.22	41.31
G-41	9.19	37.43	1.13	6.65	0.85	26.45	15.17	8.98	59.50	13.50	123.56	39.67
G-50	9.31	37.56	1.30	6.89	0.94	28.44	15.59	9.60	61.00	13.83	127.22	39.61
G-G-2	8.94	36.83	1.21	6.51	0.92	26.26	16.01	10.21	54.83	12.83	119.89	41.11
G-G-3	9.11	37.50	1.11	6.72	0.92	25.46	14.62	9.58	55.50	12.33	123.00	40.64
G-G-4	9.02	36.97	1.26	6.37	0.84	26.44	15.96	9.71	58.17	11.67	125.67	42.29
Godawari	8.93	41.74	1.18	6.96	0.79	27.37	15.22	9.59	53.67	13.50	123.89	40.09
Ooty Local	8.98	37.72	1.27	6.43	1.03	26.90	16.73	9.15	62.17	12.42	126.00	41.38
Phule Baswant	8.79	41.27	1.23	6.91	0.95	27.12	16.24	9.04	55.67	11.67	125.78	40.37
Rani Bennuar Local	7.76	38.72	1.14	6.48	0.76	26.01	15.10	7.92	53.50	12.33	120.22	41.77
Sikkim Local	8.02	39.34	1.06	6.32	0.94	26.71	17.90	8.93	56.17	10.83	117.56	39.54
Silkuei Local	8.83	38.54	1.18	6.62	0.67	26.67	11.43	7.90	54.33	10.30	136.67	38.59

NOL= Number of leaves, PSL= Pseudostem length, CTH= Collor thickness, DTH= Date to harvest, Wt.= Weight, TSS= Total soluble solids

Results and Discussion

Morphological diversity analysis: The observations were recorded in seventeen garlic genotypes at the successive stage of the plant development, which were analyzed statistically and presented in Table 2. Significant differences were recorded among the genotypes. With regard to the number of leaves, maximum leaves were recorded in G-50 (9.31) followed by G-41 (9.19), G-386 (9.13), GG-3 (9.11) and the minimum was recorded in Rani Bennuar Local (7.76). G-386 recorded maximum plant height (41.86 cm) followed by Godawari (41.74 cm), Phule Baswant (41.27 cm) and minimum plant height were recorded in Bhima Omkar (36.40 cm), respectively. The variation observed in plant height among the genotypes might be due to differences in genetic constituents as well as environmental effects. The maximum leaf width was recorded in two varieties G-1 and Bhima Purple (1.31 cm) and the minimum were recorded in Sikkim Local (1.06 cm). This variation in the leaf character may be due to genotypic or some environmental effects. Similar, investigations have been reported by Jogdande et al. (2004) and Panse et al. (2013). With the increase in the number of leaves, photosynthesis generally increases and thus plant can produce more food that is necessary for growth and development. So, the cultivars that can produce more leaves have more plant growth leading to higher yield. The investigation on pseudostem stem length indicated that all the lines differed significantly. The maximum pseudostem length was recorded in G-386 (7.09 cm) followed by Godawari (6.96 cm), Phule Baswant (6.91 cm) and the minimum was recorded in G-1 (5.95 cm). Similar findings were observed by Hasan et al. (2017) and Prajapati et al. (2018). Colar thickness was recorded maximum in Ooty Local (1.03 mm) followed by Phule Baswant (0.94 mm), Sikkim Local (0.94 mm) and the minimum was recorded in Silkuei Local (0.67 mm). The significant difference was recorded in bulb size diameter and maximum bulb size was recorded in Bhima Purple (28.55 mm) followed by G-50 (28.44 mm), Bhima Omkar (27.65 mm) and the minimum was recorded in GG-3 (25.46 mm). The investigation on a number of cloves per bulb was maximum in Sikkim Local (17.90) followed by Ooty Local (16.73), Phule Baswant (16.24) and the minimum was recorded in Silkuei Local (11.43). The maximum clove diameter was recorded in GG-2 (10.21 mm) and the lowest clove size was recorded in Silkuei Local (7.90 mm). The maximum average weight of 5 bulbs was recorded in Ooty Local (62.17 g) while the minimum average weight of 5 cloves was exhibited in G-282 (51.50 g). The maximum average weight of 10 cloves was recorded in Bhima Omkar (14.17 g) whereas, the minimum (10.30 g) was recorded in (Silkuei Local). Sikkim Local (117.56 days) was recorded as early maturing variety whereas, Silkuei Local (136.67 days) was late maturing genotype. High TSS was observed in Rani Bennuar Local (41.77%) and low TSS was seen in Silkuei Local (38.59%).

The data pertaining to qualitative parameters of different genotypes are presented in Table 3. The genotypes namely G-282, GG-3 and Rani Bennur Local have sparse

Table 3: Mean performance of garlic (*Allium sativum* L.) genotypes for eleven qualitative morphological traits

Entries	Density of leaves	Foliage attitude	Intensity of green colour in leaf	Leaf waxiness	Bulb shape in longitudinal section	Bulb shape in cross-section	Bulb compac-tness of cloves	Ground color of dry external scales of bulb	Anthocyanin stripes on dry external scales of bulb	Skin adherence of dry external scales on bulb	Clove: Colour of Flesh
Bhima Omkar	Medium	SE	D	Present	Elliptic	Elliptic	Compact	White	Absent	Medium	Whitish
Bhima Purple	Dense	E	D	Present	Ovate	Elliptic	Compact	Purple	Present	Medium	Whitish
G-1	Medium	E	D	Present	Ovate	Elliptic	Medium	White	Absent	Medium	Whitish
G-282	Sparse	SE	L	Present	Circular	Circular	Compact	White	Absent	Medium	Whitish
G-323	Medium	E	M	Absent	Ovate	Elliptic	Medium	White	Absent	Medium	Yellowish
G-386	Dense	SE	D	Absent	Ovate	Elliptic	Compact	Purple	Present	Strong	Yellowish
G-41	Dense	SE	D	Present	Ovate	Elliptic	Medium	White	Absent	Medium	Yellowish
G-50	Dense	SE	D	Present	Ovate	Elliptic	Medium	White	Absent	Strong	Whitish
G-G-2	Medium	E	D	Present	Ovate	Elliptic	Medium	White	Absent	Medium	Yellowish
G-G-3	Sparse	SE	L	Absent	Circular	Circular	Compact	White	Absent	Strong	Whitish
G-G-4	Medium	SE	M	Present	Ovate	Elliptic	Medium	White	Present	Medium	Whitish
Godawari	Medium	E	D	Present	Elliptic	Elliptic	Medium	Purple	Present	Medium	Yellowish
Ooty Local	Medium	SE	M	Absent	Ovate	Elliptic	Compact	White	Absent	Medium	Whitish
Phule Baswant	Dense	E	D	Present	Elliptic	Elliptic	Medium	Purple	Present	Medium	Yellowish
Rani Bennuar Local	Sparse	SE	L	Absent	Circular	Circular	Medium	Purple	Present	Weak	Yellowish
Sikkim Local	Medium	E	L	Absent	Circular	Circular	Compact	White	Present	Strong	Whitish
Silkuei Local	Medium	SE	M	Present	Elliptic	Elliptic	Medium	Purple	Present	Medium	Yellowish

SE= Semi Erect , E= Erect, D= Dark, L= Light, M= Medium

leaf density in canopy having semi-erect foliage attitude with light green color intensity of their leaves while genotypes Bhima Purple, G-386, G-41, G-50 and Phule Baswant were having dense leaves with dark green color of leaves. However, the genotypes comprising medium leaves density in the canopy have an erect or semi-erect type of foliage attitude and generally have medium or dark green color intensity of leaves. The genotypes comprising sparse leaf density leaves have circular shape bulbs while other genotypes have ovate or elliptic shape bulbs. Maximum of genotypes have a waxy layer on their leaf surface except for G-323, G-386, GG-3, Ooty Local, Rani Bennur Local and Sikkim Local. The waxy layer protects plants from biotic as well as abiotic stress by avoiding water loss during transpiration and restricting penetration of fungal spores by thrips.. The genotypes Bhima Purple, G-386, Godawari, Phule Baswant, Rani Bennur Local and Silkuei Local have purple colour bulbs and purple anthocyanin strips present on their dry external scales. The flesh colour of the clove ranges from whitish to yellowish among the different genotypes. Similar results were reported by Nandini et al. (2018).

Principal component analysis: During the present investigation, principal component analysis (PCA) analysis represented a similar grouping of genotypes which helps in the assessment of diversity in multivariate scales. PCA indicates the morphological variation of the germplasm under study. It measures the key characters which have a higher impact on the total variables where each coefficient of suitable vectors indicated the degree of contribution of every original variable with which each principal component is associated. The principal component analysis was carried out with 17 genotypes of garlic. According to this scaling PC1 accounted for 25.9 % of variation and PC2 accounted for 20.8% variation (Fig. 1). It was seen that Silkuei Local was diverse and grouped separately. The diverse nature in Silkuei Local is due to morphological difference in collar thickness, number of cloves in bulb, clove size diameter, weight of 10 cloves and TSS which is lower as compared to other genotypes and require maximum days to harvest. The rest of the other genotypes were clustered together. Also, Sikkim Local and Rani Bennuar Local were grouped separately.

Molecular marker analysis by SSR: Out of 16 SSR markers screened, 7 markers clearly showed the polymorphic bands in all the garlic genotypes evaluated in this study. The unambiguous and clear bands were only used for the scoring. The PIC value ranged from 0.14 (ACM033) to 0.45 (ACM054) with an average of 0.26. However, the marker index ranged from 0.07

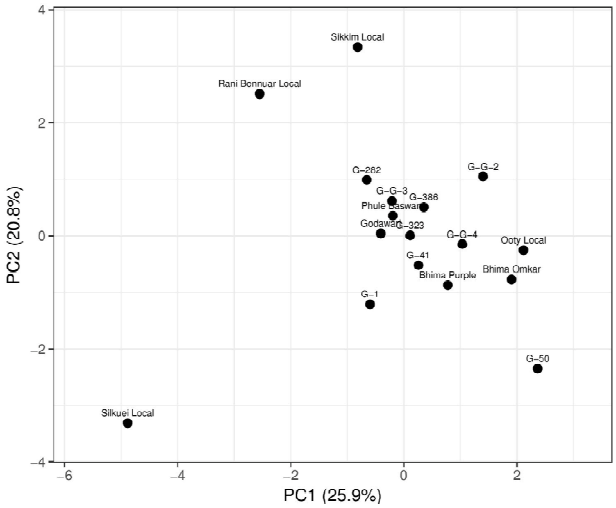

Fig. 1: Principal component analysis (PCA) showing diversity based on morphological traits of different garlic genotypes

(ACM033) to 0.75 (ACM154) with an average of 0.28. The effective multiplex ratio (EMR) value was reported highest in ACM154 and ACM038 (2) and the lowest in ACM151, ACM033, ACM078 and ACM054 (0.5) (Table 4). The amplicons size generated by 7 SSR primers ranged from 75 to 500 bp (Fig. 2). Pearson's original measurement of genetic identity analysis of 17 garlic genotypes showed the genetic similarity values ranging from 0.04 to 0.1 with the mean value of 0.69 (Table 5) among garlic genotypes from different parts of India. Whereas, the lower degree of similarity was found in the genotype Sikkim local ranging from 0.05 to 0.44 with all genotypes included in this study. Khar et al. (2008) reported 47 to 97% similarity between different onion and garlic cultivars. Whereas, 32 to 98% similarity were reported in 53 garlic accessions by Kumar (2015).

Cluster Analysis: The UPGMA dendrogram constructed using Pearson's similarity matrix of SSR data discriminated all the genotypes into four clusters (Fig 3). The genetic relatedness amongst individuals in the tested populations was analyzed. The genetic similarity coefficient of *Allium sativum* ranged from 0.04 to 1.00. Similar results were obtained using random amplified polymorphic DNA (RAPD) markers by Buso et al. (2008) and Zahim et al. (1997) which reported genetic variation in 27 garlic accessions. Khar et al. (2008) investigated genetic diversity among Indian cultivars and the dendrogram showed the genetic diversity ranging from 0.12 to 0.97 among Indian garlic cultivars.

Cluster I comprised of Sikkim Local, obtained from Sikkim (North-Eastern hills) which is characterized by compact bulbs with sparse plant density, narrow leaf,

Table 4: Details of SSR primers used for genotyping in 17 garlic genotypes.

Marker	PIC	Total number of bands	Polymorphic bands	Effective multiplex ratio (EMR)	Marker Index (MI)
ACM151	0.27	4	1	0.5	0.14
ACM154	0.38	3	2	2.0	0.75
ACM078	0.19	7	3	0.5	0.10
ACM054	0.45	3	1	0.5	0.22
ACM038	0.25	2	2	2.0	0.49
ACM033	0.14	2	1	0.5	0.07
ACM008	0.20	2	1	1.0	0.20
Average	0.26	3.28	1.5	-	0.28

12-16 cloves per bulb, requiring 118 days to mature. It requires a short day condition for its cultivation. Cluster II consisted of two genotypes GG-4 and G-386 with the genetic similarity of 0.44%. Both genotypes showed compact bulbs, but were dissimilar in colour. GG-4 had white color however, G-386 had a purple colour of bulb. Cluster III was the biggest cluster and comprised of 12 garlic genotypes at the similarity of about 33%. Phule Baswant and GG-2, Rani Bennuar and GG-2 showed the highest similarity value (GS=81%). Other genotypes namely, Godawari, G-50, G-41, G-282, Ooty Local, GG-3, Silkuei Local, G-323 and G-1 were included in this cluster. All genotypes developed through clonal selection are clustered in this group. Godawari, Phule Baswant and Rani Bennuar Local has purple colour bulb while the rest of the genotypes had white colour bulbs. G-1, GG-2, GG-3 and G-50 are commercially cultivated in the northern Indian plains of Haryana whereas, G-323 is temperate type grouped in this cluster. Genotypes G-41 and G-282 are commercially grown in tropical regions. It is known that the genotypes which are grown in temperate regions have big size bulbs, with more average bulb weight (50-70 g) and have less number of cloves (10-15) whereas, the tropical types are small in size, with average bulb weight (10-15 g) and have more number of cloves per bulb (15-25). Hence, grouping based on molecular basis doesn't differentiate the genotypes neither according to their colour nor according to their climatic adaptation.

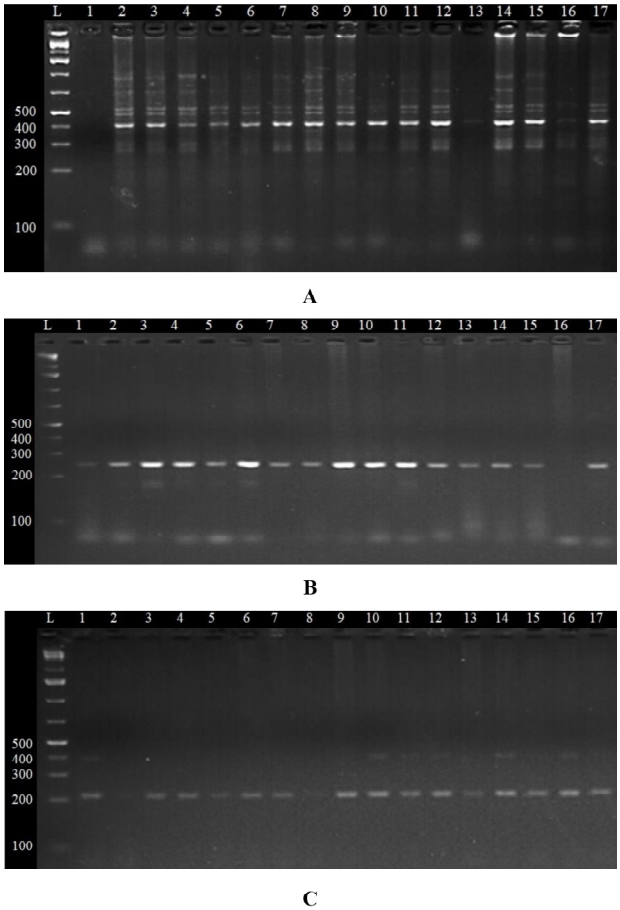

Fig. 2: Amplification products obtained from DNA extracted from garlic genotypes using primer A) ACM078 B) ACM033 C) ACM008. L-Ladder (Left), 1-17: Garlic genotypes (Bhima Omkar, Bhima Purple, G-1, G-282, G-323, G-386, G-41, G-50, GG-2, GG-3, GG-4, Godawari, Ooty Local, Phule Baswant, Rani Bennuar Local, Sikkim Local, Silkuei Local)

Cluster IV comprised of two genotypes Bhima Omkar and Bhima Purple with the genetic similarity of 40%. As both the varieties have originated from the same location of Maharashtra but having different characteristics such as Bhima Omkar is characterized by dense leaves with erect foliage and dark green colour, bulbs are white in colour, 12-14 cloves per bulb, requiring 124 days to mature while Bhima Purple has compact cloves, with dense and wide leaves, bulb has purple color, 15-20 cloves per bulb and require 122 days to

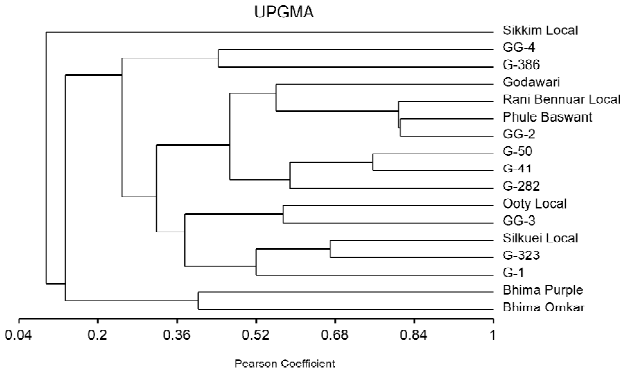

Fig. 3: Dendrogram depicting the genetic relationship among 17 garlic genotypes based on the SSR data using UPGMA

Table 5: Similarity matrix computed with Pearson's coefficient of 17 garlic genotypes

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Bhima Omkar	1.00	0.40	0.26	0.01	0.40	-0.26	0.16	0.12	-0.08	0.20	0.01	0.16	0.03	-0.12	-0.12	-0.03	0.30
2 Bhima Purple		1.00	0.32	0.35	0.19	-0.16	0.25	0.47	0.50	-0.16	0.09	0.25	-0.32	0.37	0.37	0.08	0.16
3 G-1			1.00	0.39	0.48	0.20	0.63	0.48	0.32	0.20	0.06	0.00	0.10	0.09	0.37	-0.10	0.56
4 G-282				1.00	0.27	0.19	0.61	0.56	0.35	0.19	0.17	0.35	0.06	0.21	0.44	0.44	0.27
5 G-323					1.00	0.06	0.47	0.36	0.19	0.33	-0.33	-0.09	0.48	0.03	0.28	-0.21	0.67
6 G-386						1.00	0.32	0.33	0.32	0.33	0.44	0.08	0.13	0.26	0.47	0.10	0.43
7 G-41							1.00	0.76	0.50	0.55	0.35	0.25	0.40	0.37	0.59	0.08	0.65
8 G-50								1.00	0.76	0.33	0.27	0.47	0.21	0.53	0.78	0.06	0.67
9 GG-2									1.00	0.32	0.35	0.50	0.16	0.81	0.81	0.32	0.65
10 GG-3										1.00	0.44	0.32	0.58	0.47	0.47	0.10	0.66
11 GG-4											1.00	0.35	0.06	0.44	0.44	0.19	0.27
12 Godawari												1.00	-0.08	0.59	0.59	0.32	0.40
13 Ooty Local													1.00	-0.05	0.16	-0.10	0.48
14 Phule Baswant														1.00	0.80	0.26	0.52
15 Rani Bennuar Local															1.00	0.05	0.74
16 Sikkim Local																1.00	-0.03
17 Silkuei Local																	1.00

mature. Both the varieties are short-day types and grown in tropical regions of India. The present study revealed that SSR are reliable markers that differentiated the genotypes and the out-groups but there is a need to develop more markers. Similar results were reported by Hernandez et al. (2008) using the RAPD marker. India holds a huge resource of garlic cultivars that are of great significance for breeders as well as for the farmers. The result of the present study proved the utility of SSR markers in genetic diversity at random regions of the genome of garlic cultivars. Similar findings were earlier reported by Sharma et al. (2018) by screening as many as 131 garlic genotypes using 4 ISSR markers and Shaaf et al. (2014) evaluated 31 garlic genotypes using 6 ISSR markers and Chen et al. (2014) screened 39 genotypes using17 ISSR primers and 8 SSR primer combination.

Morphological character analysis: Distance estimates based on 12 morphological characters ranged from 1.65 to 6.12 with an average of 3.6 (Fig 4). Cluster analysis based on morphological data assigned the genotype into three clusters. Among them cluster I was found to have large number of genotypes with the morphological data it was found that genotypes in cluster IA (Bhima Omkar, Bhima Purple, G-50) were tropical types and commercially grown in northern Indian plains of Haryana, genotypes in cluster IB (G-1, G-323, GG-4 Ooty local, G-1, GG-2 and GG-3) included both tropical and temperate which is similar to the cluster III B in SSR marker analysis. While the second cluster was divided into two sub-groups, the first one included G-282, G-386, Phule Baswant and Godavari cultivated in north and central regions of India which require tropical type of climate. On the other hand the second branch included Rani Bennuar Local and Sikkim Local which were temperate types. Third cluster comprised of single

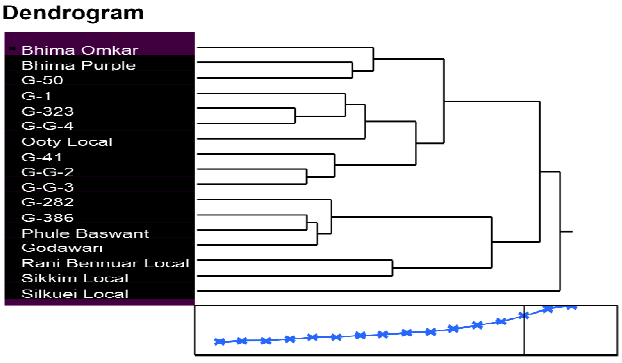

Fig. 4: Dendrogram of seventeen garlic genotypes based on 12 morphological characters

genotype Silkuei local which was characterized by minimum collar thickness, and requiring 136.67 days to harvest, having 11.43 number of cloves per bulb which is the long day type.

The knowledge of genetic relationships of genotypes provides useful information to address germplasm resource management (Salem et al. 2008). In this study morphological data analysis of the garlic genotypes was coupled with molecular analysis to investigate genetic relationships among 17 garlic genotypes and showed diverse morphological traits and distinct SSR marker patterns (Fig 3 and Fig. 4). However, from the molecular analysis some of the genotypes were included in the same group even when they showed no similarities for morphological traits. Thus, there was inconsistencies among the outcomes of the analysis of molecular and morphological characters. These inconsistencies were also observed in garlic (Chen et al. 2014) and cucumber (Yang et al. 2015). Further studies are required to fully explain the relationships between molecular markers and morphological traits. The range of markers based on morphological traits was higher than SSR markers,

which reflect the influence of the environment on the performances of the genotypes. Therefore, the DNA markers and morphological traits will not necessarily gain close similarity results (Salem et al. 2008 and Devos and Gale 1992)

Conclusion

This study revealed considerable amount of genetic variability among genotypes of garlic and identified a correlation between molecular markers and morphological traits. These diverse genotypes would be utilized for genotypic improvement of *Allium sativum*. When compared with DNA fingerprinting techniques, morphological traits are less valid and not efficient for precise discrimination of closely related genotypes and analysis of their genetic similarities. However, morphological traits are useful for preliminary, fast, simple and inexpensive varietal identifications and can be used as general approach for evaluating genetic diversity among phenotypically distinguishable genotypes although they are inefficient on account of the time and cost involved. The results obtained showed the potential of SSR markers for characterizing the genetic diversity in garlic. These results will be useful for future programs to develop new garlic genotypes and will facilitate further studies into garlic genetics and development.

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सारांश

लहसुन की कुल 17 प्रभेदों की आनुवांशिक विविधता का मूल्यांकन बाह्यदृश्य लक्षणों और आण्विक मार्करों को उपयोग करके किया गया। लहसुन की पत्तियों और शल्क कंद लक्षणों से सम्बन्धित 23 बाह्यदृश्य लक्षणों का मूल्यांकन किया गया और उन्हें मात्रात्मक और गुणात्मक मापदंडों में वर्गीकृत किया गया। कुल 16 एस.एस.आर. प्राइमरों के आधार पर विश्लेषण किया जिसमें से 7 ने लहसुन के प्रभेदों में स्पष्ट पॉलीमॉर्फिक बैंड प्रदर्शित किए और अध्ययन के तहत सभी प्रभेद को आसानी से अंतर (विभेदित) किया गया। प्रयोग किए गए एस.एस.आर. मार्कर ने 42 एलील का पता लगाने में सक्षम रहे जिनका औसत 2.63 एलील प्रति लोकस था। एलील की संख्या प्रति लोकस 1–7 तक और एलील पॉलीमॉर्फिज्म सूचना सामग्री (पी.आई.सी.) मान 0.14–0.45 के साथ औसत 0.26 तक थी। आनुवांशिक विचलन की गणना पियर्सन गुणांक का उपयोग करके की गयी जिसमें आनुवांशिक समानता 0.04–1.0 तक प्राप्त हुई और बाह्यदृश्य लक्षणों के आधार पर 1.65–6.12 के साथ औसत 3.6 थी जो आण्विक मार्करों से अधिक थी। आनुवंशिक विविधता का मूल्यांकन करने के लिए रूपात्मक लक्षण कम मान्य हैं क्योंकि ये लक्षण दिन की लम्बाई और तापमान जैसे–पर्यावरणीय और जलवायु कारकों पर निर्भर करते हैं क्योंकि वे अलग–अलग जलवायु क्षेत्रों के लिए अनुकूलित होते हैं। अध्ययन में लहसुन के प्रभेदों के मध्यम काफी मात्रा में आनुवंशिक विविधता पायी गयी और आण्विक मार्करों और बाह्यदृश्य लक्षणों के बीच एक सह–संबंध की पहचान की गयी। वर्तमान अध्ययन यह भी स्पष्ट होता है कि माइक्रोसेटेलाइट मार्कर डी.एन.ए. फिंगर प्रिंटिग के लिए तेज और उच्च थ्रूपूट है फिर भी आनुवंशिक विविधता का आंकलन करने के लिये और अधिक मार्कर विकसित करने की आवश्यकता है।

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