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GENETIC DIVERSITY ANALYSIS IN NOVEL SELF-INCOMPATIBLE (SI) LINES OF CABBAGE BASED ON MORPHOLOGICAL TRAITS AND SSR MARKERS

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ABSTRACT
A comprehensive study on characterization and genetic diversity analysis was carried out in exclusively eight self-incompatible (SI) lines of cabbage using 13 morphological traits and 49 SSR markers, which can prove effective for selection of suitable SI parental lines for quality hybrid development in cabbage. Morphological characterization depicted considerable variations for both qualitative and quantitative traits studied. The genotypes, S-645 performed better for most of the quantitative traits. Further, plant height (0.786), head length (0.863) and head width (0.737) revealed significant positive correlation with net head weight. The principle component analysis (PCA) revealed that 95.28% of total variations were explained by first three components. Further, dendrogram divided eight SI lines into four distinct groups, showing considerable diversity. In molecular study, a total of 112 alleles were amplified by 49 simple sequence repeat (SSR) primers, averaging to 2.20 alleles in each locus. High mean values of Shannon's Information index (0.65), expected (0.46) and observed (0.38) heterozygosity, polymorphic information content (0.35), depicted substantial polymorphism. Dendrogram based on Jaccard's similarity coefficient constructed two major groups and five sub-groups, which revealed substantial diversity among different SI lines of cabbage. In conclusion, genotype S-645 and S-681 were found most divergent based on morphological and molecular studies, respectively. Hence, these genotypes have greater potential in future breeding programmes for the development of high yielding quality hybrids. Further, 49 SSR loci recorded high polymorphism and were found effective for differentiating different self-incompatible lines under study. Hence, SSR markers can be utilized for germplasm characterization and association mapping for future breeding programmes in SI lines of cabbage.

KEYWORDS: Cabbage; Dendrogram; PCA; Polymorphism; Simple sequence repeat (SSR).

INTRODUCTION

The genetic phenomenon of self-incompatibility (SI) has been widely used for commercialization of hybrid seed production in cabbage (Mohanty and Prusti, 2002). The major benefit of SI system is that we can produce hybrid seed by utilizing two self-incomptible lines as parents having dissimilar homozygous S alleles (Kucera *et al.*, 2006). But, in hybrid breeding of cabbage, selection of suitable SI parental lines for hybrid seed production is of utmost importance. In India, most of the public sector varieties and hybrids of vegetables have been replaced by private sector (Koundinya and Kumar, 2014) and their seed is sold at very high prices to the farmers. Therefore, there is an immense need to breed high yielding quality cabbage hybrids from public sector in the country, so that their seeds can be made available to the farmers at reasonable prices. The estimates of genetic diversity are useful for germplasm characterization and help to identify suitable parents for hybrid breeding in cabbage. The parental lines with sufficient diversity/polymorphism are selected based on degree of genetic diversity (Louarn *et al.*, 2007), which can be evaluated with the help of morphological traits and biochemical markers. In any plant breeding programme, morphological traits have immense value for the selection of parents with maximum variation (Zhang *et al.*, 2008). Nowadays, molecular markers are considered valuable than morphological traits as these are devoid from the perplexing effect of environment (Pejic *et al.*, 1998). Hence, marker assisted breeding aids to the selection of breeding material in conventional breeding programmes (Frey *et al.*, 2004; Liu *et al.*, 2004). In recent years, simple sequence repeats (SSRs) have become excellent tool for the plant breeders. These markers are highly valuable due to high reproducibility, polymorphism, co-dominance and transferability in allied plant species and genera (Rana *et al.*, 2015). They also resolve the variations that are caused due to widespread crossing and phenotypic plasticity imposed by environmental fluctuations (Nybom and Weising, 2010). Therefore, present investigation was aimed to characterize and estimate the extent of diversity in available SI lines of cabbage using morphological traits and SSR markers for selection of diverse and superior parental lines for quality hybrid seed production.

MATERIALS & METHODS

Experimental material and site

The present investigation was conducted at Experimental Research Farm and Molecular Laboratory of ICAR-IARI Regional Station, Katrain, Kullu, HP, India during the year 2014-16. Experimental materials for the present studies comprised of eight self incompatible lines of cabbage maintained at the Regional Station. For morphological characterization, seeds of all the genotypes were sown in the well prepared nursery beds during August, 2014 and 2015. After 30 days, seedlings were transplanted in the experimental field in Randomized Complete Block design (RCBD) at a spacing of 45 cm × 45 cm in the plots having size 3.0 m × 3.0 m during, September, 2014 and 2015 and replicated thrice. Data were recorded on different qualitative and quantitative traits periodically from arbitrarily selected 10 plants from all replication. All the standard cultural practices as necessary for raising the healthy crop stand of cabbage have been followed.

Isolation, purification and quantification of genomic DNA

For molecular characterization, seeds of each line were sown in pro-trays during July, 2016 and were kept in the polyhouse for further vegetative growth. Isolation and purification of genomic DNA was done from 100 mg fresh green leaves of 30 days old seedlings of each cabbage genotype by using CTAB (cetyltrimethylammonium bromide) methodology given by Doyle and Doyle (1990). For DNA quantification, 2 µl of each DNA sample along with the uncut lambda DNA (100 ng/µl) was run on 0.8% agarose gel (Sigma-Aldrich, USA). Dilution of samples (25-50 ng DNA/µl) was done in Tris-EDTA buffer and then they were stored at -80 ^{o}C for further analyses.

SSR markers and PCR amplification

A total of 106 primer pairs (Integrated DNA technologies, New Delhi, India) were tested for molecular diversity analysis in eight SI lines of cabbage. Out of which only 49 primer pairs showed polymorphic bands in the different lines under study (Table 1).

TABLE 1: List of SSR primers used in molecular characterization of different SI lines of cabbage

Sr. No.	LG	cM	Oligo Name	Sequence (5' to 3')
1.	C01	47.66	BoSF2345	F- GCTCCGATGATCACGATTCT
				R- CTTCATCCCCTCACCACACT
2.	C01	36.26	BoSF1207	F- CGACGCTAGACCAAGGTTTC
				R- GGAAAACCTTCTGCCAATGA
3.	C01	22.13	BoSF912	F- CTAGATCGCCTGCAAAGAGC
				R- AATACGGGGAGGTAACTCGG
4.	C01	0.00	BoSF1331	F- ACTGGGCTGGCTGCTAAATA
				R- AGAAATGCGCGTTTTTAAGG
5.	C01	74.46	CB10258	F- ATGATGCCTAGCATGTCC
				R- AAGCTAAAGCGAAAGAAGC
6.	C01	54.72	BoSF063	F- GAATGTTTCCTCTGCTTGGC
				R- TCAAAATCAGGAGAATCGGG
7.	C02	114.33	Na14H11	F- GGATGTTTTCACAGACCCTG
				R- CTTTGCAGGTATGAACACGC
8.	C02	77.19	BoSF1167	F- TTCGTTCCTTCGTTCATTCC
				R- AGGTAGTGGAGGAGGTCGGT
9.	C03	32.33	BoSF966	F- ATCCCATTGTCGTTATCCCA
				R- CGTCGTCTAGCGATGATGAA
10.	C03	20.89	BrBAC214	F- CGTATAATTTTCATAGGCGACG
				R- AGCATGCTTATGACTCTGGGA
11.	C03	9.90	BoSF1131	F- GAAGTTTCACTGCCTCTCGG
				R- CTTCGTTAACCTCGCGAAAG
12.	C03	61.81	BoSF042	F- CGGCTTGACAGAATTGGACT
				R- TCCTATTCCACACCAAAGCC
13.	C03	37.19	BoSF062	F- CTAGTGTTCGCCGAAGTGGT
				R- AAAAGGTGTCATGGAGTGCC
14.	C03	92.42	BoSF2985	F-GGTTTCATAAAACATCTGTAGTTCGTC
				R- TGCAAGACATCTTTATTTCTTCCTC
15.	C03	86.14	Na10E02	F- TCGCGCATGTAATCAAAATC
				R- TGTGACGCATCCGATCATAC
16.	C04	59.23	BoSF184	F- TTGCACGTACGTCTTTGAGG
				R- CTGCAACGAGGATGAAAACA
17.	C04	50.72	BoSF1957	F- TATGGACCACATGCCCCTAT
				R- ACTAGGGGCGGATTCAAAAA
18.	C04	62.62	BoE862	F- AGCAAAGGCGGGGGAATGATAC
				R- ATGACAAAGACCACCCACACCAAT
19.	C08	107.79	BoSF2612	F- CGTAGCCGTCTCTTACGCAT
				R- TTCAGTCCAGCGTTCAACAG
20.	C08	90.92	BoSF2680	F- AAAGGTTAGGTGGTTGGATAAAGA

				R- TGTCTTCTGATGCCTTGGTCT
21.	C02	49.35	BoSF2294a	F- CACCATCGTTTCTGTCCCTT
				R- TAACCACACCTTCCGTTTCC
22.	C07	95.67	Na12F03a	F- GGCGACATAGATTTGAACCG
				R- TCCACTTTCTCTCTCTTCCCC
23.	C07	121.17	BoSF2406	F- ATATGGCCCTGCAAACAGAG
				R- CAGCTTATGGAATGCCCCT
24.	C07	49.22	BoSF2033	F- CCACATTTACGCACTTGCAG
				R- TCCGTATAATGATTTAACAACCCA
25.	CO7	75.51	BoSF2313	F-AAGGAGGATCACGAGGAGGT
				R-CATGGTAGCATCGAAAGCCT
26.	C07	22.27	BoE7830	F- AATGGCGGTGGTGTTGG
				R- TTGGGCGACTAAAGAAAAAT
27.	C06	82.37	BoSF2054	F-GAAGGAACAAGAGGATGGCA
				R-TCATGTTGTCGAGAATCCCA
28.	C07	30.78	BoSF2860	F-CATGCTTGCCTGAAAAGACA
				R-CCTTGTACTGCTCCTCTGCC
29.	C06	67.96	BoSF1215	F-AGTATCAAACCCGCCTGTTG
				R-GGGTCGTATTAATCGCGTGT
30.	C03	136.35	Na12B09	F-ACGGAAGATCAAACAGCTCC
				R-TGAGCGACCCATTCTTTAGG
31.	C06	30.24	BoSF250	F-AAAAACACTTAGTTGTGGTGGGA
				R-TTTTTAATGCAGCCCGAAAC
32.	C06	58.70	BoSF2505	F-GGTTATTTCCACCATGCCAC
				R-CTTGCCGAGACTCATCATCA
33.	C05	70.88	BoSF317	F-CCAACTCCGGTCAATCATCT
				R-GCCCCTTTCTGTGTGACATT
34.	C05	27.01	BoSF2878	F-CCTTGCGTCTGAAACATCAA
				R-TTACCGGGAGTAAATGCAGC
35.	C09	92.51	BoSF1245	F-CTCCTGTCTTCTTCCATCGC
				R-ACCACAAGGTGTGTGAGCAA
36.	C09	73.74	BoSF2421	F-CACTCAGAGGAGGAGGTTGC
				R-GCCACGTGTAGGCATGTAGA
37.	C09	45.88	BoSF1640	F-AGCACAACTACCTGAACCTCT
				R-TTTATCCTCGGTCTTCTCTCT
38.	C09	35.57	BoGMS1498	F- TCAACAGAACACATCCACAG
				R- TAGTGCCATAGAAACCATCTT
39.	A10	78.65	BoGMS0206	F- TACTCCACGGTCTTCTACTTG
				R- GGGATAGTGATGTTGTTGATG
40.	C08	80.34	BoGMS1460	F- CGAGAGGTGAAGAACAAGAG
				R- AAATAAGAGAAGAGAAACCGTC
41.	C08	70.20	BoGMS0468	F- TGACAGCAACCAATGATG
				R- CTCTCTGGAACCTTTGAACT
42.	C06	3.00	BoGMS0952	F- CAGTGAGTAACATTTGGCTG
				R- CGAGAGAGAAAGTGATGAGAG
43.	C08	54.25	BoE615	F-TCTTCGTCTCCTCCCTCCTTCCT
				R-GGTGATTTTGACGGGGTTTGAT
44.	C02	49.35	BoSF2294a	F-CACCATCGTTTCTGTCCCTT
				R-TAACCACACCTTCCGTTTCC
45.	C02	129.76	BoSF376	F-CAACAGCGAGCATACCAAGA
				R-TTTGTCACTCGCCATCTCTG
46.	C02	137.23	cnu107	F-TGGACGTAACACCCATCTTGAA
				R-AGCTGAGGAAGTGGCTGAGG
47.	C04	26.11	BoSF1047	F- TTAAATATGTAAGCCGCCCG
				R- TTACCAGGGATAAAAGCTGAAG
48.	C04	66.73	BoE530	F-TGGCTCCACCGGTCACTATCAGA
				R-TCATGGACTTGGGAGGGTTTTG
49.	C07	39.06	BrSF567	F-AGGAAGAAAGCGGAGGAGAC
				R-AGTCGCGAGGATTTGAGAGA

PCR amplification was performed by the use of Eppendorf Mastercycler Nexus GSX1 in 10 µl reaction volume comprising: 5.00 µl pemixed ready to use Go Taq® green master mix [DNA polymerase, 2X reaction buffer (pH 8.5), dATP (400 µM), dGTP (400 µM), dCTP (400 µM), dTTP (400 µM) and MgCl2 (3mM)], primer pair (1.00 µl),

template DNA (1.00 µl) and nuclease free water (3.00 µl). DNA amplification was carried out for 35 cycles with denaturation at 94°C (1 min), annealing at 55°C (1 min) and extension at 72°C (2 min). The final extension was done at a temperature of 72°C for 10 min.

Electrophoresis and gel documentation of amplified DNA

The amplified DNA products along 1-kb DNA ladder (Fermentas, Lithuania) were separated by electrophoresis in agarose gel (2%) having ethidium bromide (10 mg/µl). The gel was run at 70 mA voltage for 2 hours in 1X TBE buffer (pH 8.0) and it was picturized on a gel documentation system (BioSpectrum® Imaging System™, UK).

Statistical analysis

Morphological data (pooled data of 2014 and 2015) were subjected to analysis of variance in OPSTAT software by following Gomez and Gomez (1984). The Pearson's correlation coefficient, PCA and dendrogram based on single linkage euclidean distance were calculated and constructed through SPSS 16.0 and Statistica software packages. Molecular data was analyzed only for 49 primers, which furnished scorable and polymorphic bands. Various genetic diversity estimates such as observed number of alleles (na), effective number of alleles (ne), expected heterozygosity (He), observed heterozygosity (Ho) and Shannon information index (I) were estimated through POPGENE software (version 1.32) by following Yeh et al. (1997). The polymorphism information content (PIC) was computed through Cervus version 3.0 software as per the formulae given by Botstein et al. (1980). UPGMA (unweighted pair group method of arithmetic mean) dendrogram and neighbor-joining (N-J) tree were constructed through NTSYSpc 2.0 (Rohlf, 1998) and DARwin (Perrier and Jacquemoud-Collet, 2006) software, respectively. Principal component analysis (PCA) was done with the help of NTSYSpc 2.0 software through the formulae given by Rohlf (1998).

RESULTS AND DISCUSSION

Morphological traits

Qualitatively assessed traits: Substantial variations were observed for different qualitative traits under study (Table 2). Leaf colour of two genotypes, S-645 and S-696 was observed as dark green, while rest of the genotypes exhibited green coloured leaves. Most of the genotypes had round shaped head except S-208 (flat). Head compactness varied from compact in six genotypes to very compact in three genotypes viz., S-602, S-681 and S-696. From, consumer's preference point of view round cabbage heads with dark green colour and high compacts are desirable. Hence, considerable variations observed for different qualitative traits in the available lines offers the chance for selection of suitable parental lines for quality hybrid development in cabbage. Earlier workers (Balkaya *et al*., 2005; Mohamed *et al*., 2012; Kibar *et al*., 2016) had correspondingly recorded wide variations in leaf colour, and head shape in cabbage. Quantitatively measured traits: The perusal of pooled data (2014 and 2015) in Table 2 revealed the considerable variations for different quantitative traits under study viz. plant height (15.83-25.83 cm), plant spread (39.27-62.83 cm), head length (11.90-16.60 cm), head width (14.30-18.97 cm), number of non-wrapper leaves (9.67-18.67), net head weight (0.92-1.55 kg), gross head weight (1.43-2.11 kg), stalk length (0.45-1.77 cm), compactness (0.30-0.39 kg/cm^3) and harvest index (50.05-73.38 %). Among all the genotypes, S-645 performed better for plant height (25.83 cm), plant spread (62.83 cm), head length (16.60 cm), net head weight (1.55 kg), gross head weight (2.11 kg), stalk length (1.77 cm), and harvest index (50.05-73.38 %). While, genotype S-681, S-602 and S-208 were found superior for number of non-wrapper leaves (18.67), compactness (0.39 kg/ cm^3) and head width (18.97 cm), respectively. Hence, these genotypes must be taken into consideration, while making the selection of parental lines for improvement in yield and its attributing traits in SI lines of cabbage. Similarly, Atter *et al.* (2009), Cervenski *et al*. (2012) and Chura et al. (2016) had reported wide variations for different quantitative traits using different lines of cabbage. Further, Pearson's correlation coefficient between different quantitative traits revealed significant positive correlation of plant height with plant spread (0.817), head length (0.753), net head weight (0.786) and stalk length (0.714); plant spread with gross head weight (0.761), head length with net head weight (0.863) and harvest index (0.739); head width with net head weight (0.737) (Table 3). Singh *et al.* (2010) and Kibar (2014) had also reported significant positive association of head weight with plant height & diameter and head diameter & length in cabbage. Significant negative correlation of compactness with head width (-0.921), net head weight (-0.935) and gross head weight (-0.753); number of non-wrapper leaves with harvest index (-0.881) was reported in eight SI lines of cabbage. Significant negative correlation of head compactness with gross plant weight in cabbage was also reported earlier by Singh et al. (2010). PCA is used to detect more important traits, which helps the plant breeders to carry out trait-specific breeding programmes (Yousuf *et al*., 2011). The outcome of PCA revealed that first three components having eigen values greater than one were retained in the analysis because of the substantial amount of the variations. They had the variance of 54.78, 25.50 and 15.00 per cent and aggregating to 95.28 per cent of total variations explained (Table 4). The first factor (PC1) had the highest positive values for plant height (0.883), plant spread (0.789), head length (0.812), head width (0.743), net head weight (0.972), gross head weight (0.752) and harvest Index (0.522). While second factor (PC2) was found superior for number of non-wrapper leaves (0.965) and third factor (PC3) recorded highest positive values for stalk length (0.840) and compactness (0.391). The positive values of different traits in three components indicated its importance in divergence among eight SI lines of cabbage, whereas negative values showed the lowest contribution to the divergence.

TABLE 2: Mean performance of eight SI lines of cabbage for different qualitative and quantitative traits (Pooled data of 2014 and 2015)

	Qualitative traits			Quantitative Traits									
Genotype	Colour	Shape	Compactness	Plant height (cm)	Plant spread (cm)	Head length (cm)	Head width (cm)	Number of non-wrapper leaves	Net head weight (kg)	Gross head weight (kg)	Stalk length (cm)	Compactness (kg/cm^3)	Harvest Index (%)
S-208	Green	Flat	Compact	17.17	54.50	11.90	18.97	17.00	1.19	2.08	0.45	0.32	57.03
S-602	Green	Round	Very Compact	18.17	51.00	12.17	14.30	17.33	0.92	1.83	1.53	0.39	50.05
S-621	Green	Round	Compact	20.00	59.17	13.00	17.27	15.00	1.18	1.97	0.95	0.34	59.89
S-624	Green	Round	Compact	16.50	39.83	14.40	16.17	11.00	1.23	1.81	0.93	0.34	67.94
S-645	Dark Green	Round	Compact	25.83	62.83	16.60	17.75	10.67	1.55	2.11	1.77	0.30	73.38
S-681	Green	Round	Very Compact	16.50	39.27	11.92	14.60	9.67	0.92	1.43	0.62	0.39	64.24
S-691	Green	Round	Compact	18.50	46.17	13.13	15.77	18.67	1.09	2.07	1.02	0.36	52.59
S-696	Dark Green	Round	Very Compact	15.83	40.00	13.20	14.77	11.33	1.03	1.62	1.00	0.37	63.32
C.D.$_{(0.05)}$	-	-	-	1.18	1.95	1.19	0.87	1.82	0.15	0.20	0.21	0.02	3.82
±SE(d)	-	-	-	0.55	0.90	0.55	0.40	0.84	0.07	0.09	0.10	0.01	1.76
C.V. (%)	-	-	-	3.59	2.25	5.07	3.04	7.42	7.57	6.05	11.22	3.52	3.54

TABLE 3: Pearson's correlation coefficients among quantitatively measured traits in eight SI lines of cabbage

Traits	Plant height (cm)	Plant spread (cm)	Head length (cm)	Head width (cm)	Number of non-wrapper leaves	Net head weight (kg)	Gross head weight (kg)	Stalk length (cm)	Compactness (kg/cm^3)	Harvest index (%)
Plant height (cm)	1.00	0.817*	0.753*	0.449	-0.076	0.786*	0.604	0.714*	-0.628	0.406
Plant spread (cm)		1.00	0.367	0.681	0.295	0.634	0.761*	0.443	-0.661	0.047
Head length (cm)			1.00	0.295	-0.436	0.863**	0.390	0.663	-0.638	0.739*
Head width (cm)				1.00	0.161	0.737*	0.743*	-0.155	-0.921**	0.249
Number of non-wrapper leaves					1.00	-0.229	0.575	-0.047	0.043	-0.881**
Net head weight (kg)						1.00	0.664	0.397	-0.935**	0.657
Gross head weight (kg)							1.00	0.298	-0.753*	-0.124
Stalk length (cm)								1.00	-0.117	0.172
Compactness (kg/cm^3)									1.00	-0.496
Harvest Index (%)										1.00

*Significant at p<0.05 **Significant at p<0.01

TABLE 4: Eigen vectors for first three principal components of quantitatively measured traits in eight SI lines of cabbage

Traits	Principle Component*		
	PC_1[#]	PC_2	PC_3
Plant height (cm)	0.883	-0.036	0.368
Plant spread (cm)	0.789	0.420	0.162
Head length (cm)	0.812	-0.468	0.208
Head width (cm)	0.743	0.353	-0.558
Number of non-wrapper leaves	-0.085	0.965	0.177
Net head weight (kg)	0.972	-0.148	-0.137
Gross head weight (kg)	0.752	0.617	0.021
Stalk length (cm)	0.495	-0.163	0.840
Compactness (kg/cm^3)	-0.910	-0.093	0.391
Harvest Index (%)	0.522	-0.812	-0.257
Eigen Value	5.48	2.55	1.50
Percentage of variance	54.78	25.50	15.00
Cumulative % of variance	54.78	80.28	95.28

[#]PC: Principal component

[*]Extracted through principle component analysis

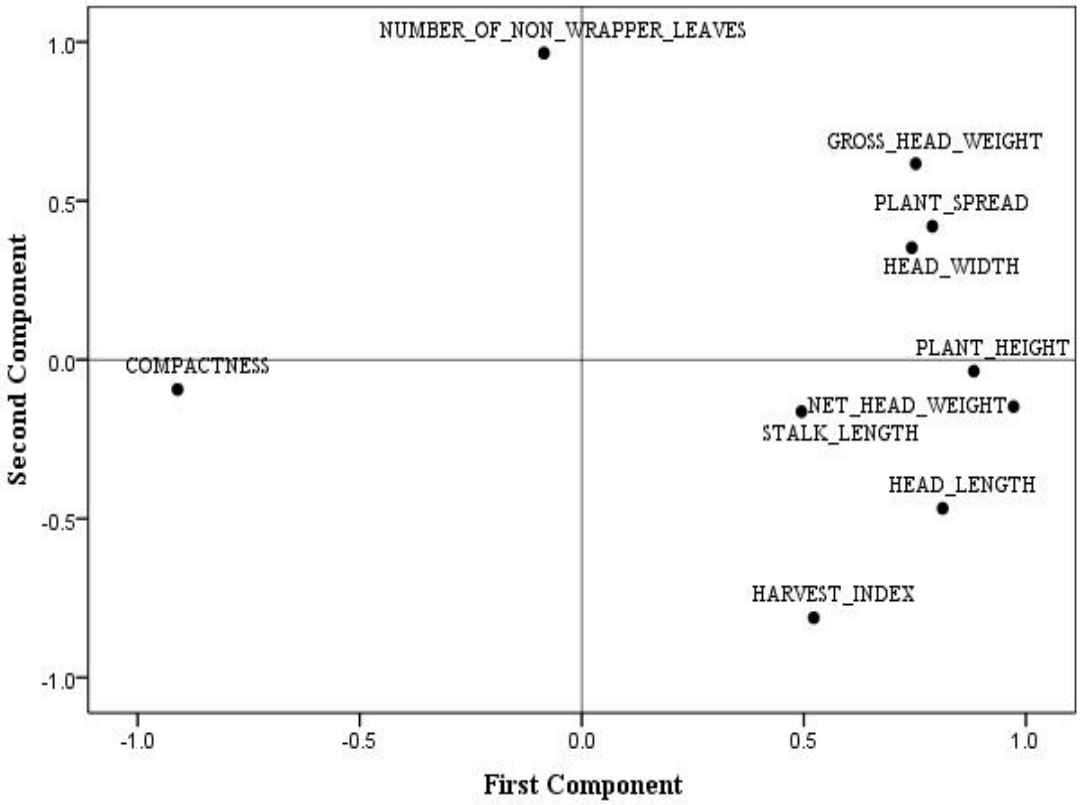

FIGURE 1: Loading of different traits based on first two principal components

Further, loading of different traits based on first two principle components indicates that gross head weight, plant spread, head width and plant height are the main components of divergence between eight SI lines of cabbage, whereas contribution of compactness was found least in divergence (Figure 1). Hence, main emphasis should be given on the gross head weight, plant spread, head width and plant height for yield improvement in cabbage. In a study on PCA for 15 morphological traits in cabbage, Cervenski *et al.* (2011) found that first three principal components having eigen values greater than one revealed 99.99 per cent of total variations among the cabbage cultivars for different traits under study. While, Kibar (2016) observed 45.34 per cent of total variations explained by first three principle components in different genotypes of cabbage. Dendrogram constructed using single linkage euclidean distance based on 10 morphological traits divided the eight SI lines of cabbage into four distinct groups viz., Group I (S-208 and S-624), Group II (S-621 and S-696), Group III (S-602, S-681 and S-691) and Group IV (S-645) as shown in Figure 2. The genotype S-645 was found most distinct from rest of the genotypes, while S-602, S-681 and S-691 were most similar among themselves. On the basis of observed clustering pattern in different genotypes, diverse parents can be selected for exploitation of heterosis breeding in cabbage. Cervenski (2010) and Kibar (2016) had also used hierarchical method of clustering to discriminate different cultivars of cabbage.

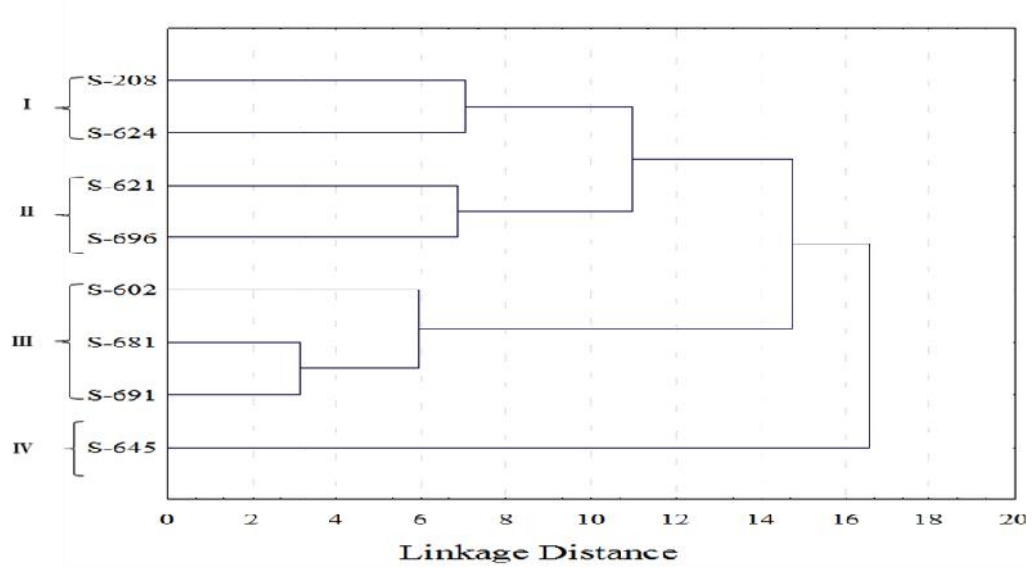

FIGURE 2: Dendrogram showing clustering pattern of eight SI lines based on 10 morphological traits constructed using single linkage Euclidean distance

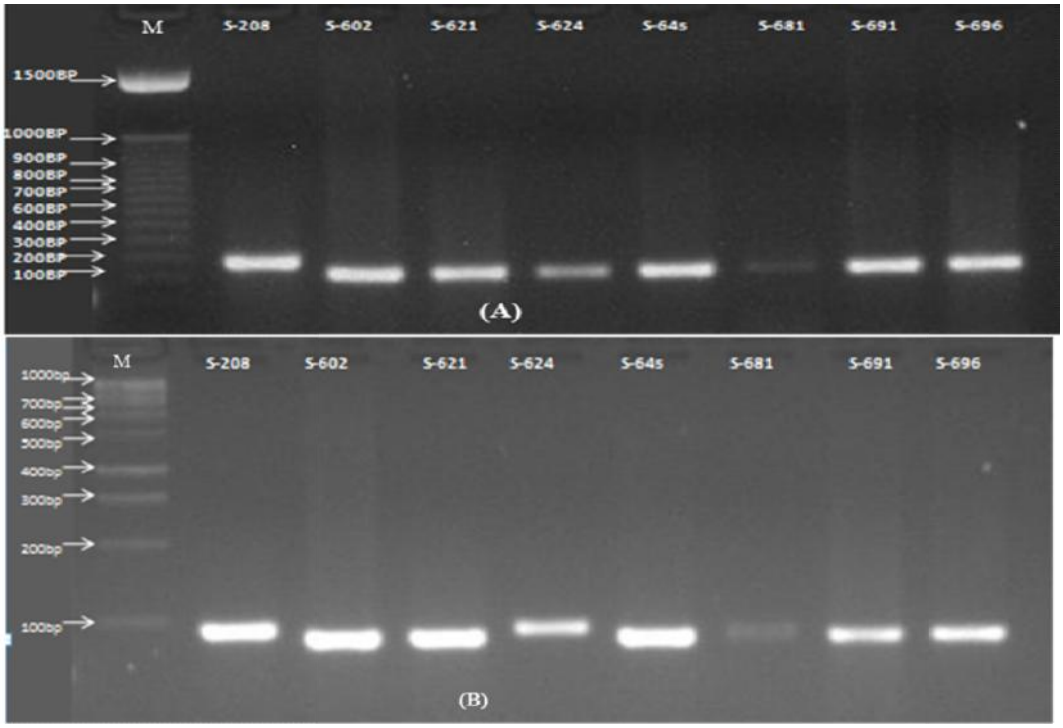

FIGURE 3: PCR amplification profile of eight SI lines of cabbage using SSR primer (A) BOSF184 and (B) BoGMS1460 where, M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on left

SSR polymorphism and diversity analysis

In this study, 106 primer pairs were tested to estimate molecular diversity in eight SI lines of cabbage. Out of them, only 49 primer pairs showed reproducible and polymorphic bands and were selected for further studies (Table 1). The 49 SSR primers were found highly polymorphic and useful to differentiate different genotypes under study (Figure 3). Raybould *et al*. (1999) and Louarn *et al.* (2007) had also revealed the usefulness of microsatellite markers to distinguish between different genotypes of cabbage. In overall 112 alleles were amplified through 49 SSR primers, averaging to 2.20 alleles in each locus. This average value is in agreement with the Cui *et al.* (2008) for Brassica rapa (2.91) suggesting appreciable allelic frequency among the genotypes studied, but lower than that as reported by Mohamed (2016) in different Brassica species (3.92). This might be due to the use of genotypes belonging to single species. Size of alleles among the amplified products

ranged from 70 to 700 bp. Among all the SSR markers each of three primer pairs viz. BoE615, BoSF1047 and BrSF567 amplified a maximum number of four alleles followed by BoSF2294a, BoSF376, cnu107 and BoE530 with three alleles each. Remaining SSR primers were able to amplify only two alleles per locus among the tested genotypes. The maximum value (1.32) of Shannon's Information Index (I) was exhibited by BoSF1047, while it was observed minimum (0.29) in the primer BoSF1957. In our study, mean value of 'I' was recorded as 0.65, which is greater than as observed earlier by Paulauskas et al. (2013) in Brassica napus (0.12). The expected heterozygosity (0.46) had higher mean values than observed heterozygosity (0.38). Prajapat *et al*. (2014) had also observed higher mean values of expected heterozygosity (0.56) than observed heterozygosity (0.30). Highest (1.00) observed heterozygosity (Ho) was reported in the seven primer pairs, while lowest value (0.00) was recorded for 21 SSR primers. The mean value of observed heterozygosity in this study was found greater than earlier reports by Pascher *et al.* (2010) in commercial varieties of Brassica napus (0.23) and Prajapat *et al.* (2014) in different Brassica species (0.30). In the meanwhile, expected heterozygosity (He) was recorded maximum (0.79) and minimum (0.17) with the primer pairs BoSF1047 and BoSF1957, respectively. In line with our study, Ofori and Becker (2008) had also reported similar mean value of expected heterozygosity in different cultivars of Brassica rapa. Polymorphic information content (PIC) was used to estimate allele frequency and diversity among different SI lines of cabbage (Table 5).

TABLE 5: Genetic diversity statistics for 49 SSR loci studied in eight SI lines of cabbage

Locus	Allel size range (bp)	n_a	n_e	I	H_o	H_e	PIC
BoSF2345	150-200	2	1.88	0.66	0.00	0.50	0.36
BoSF1207	150-210	2	1.38	0.45	0.00	0.30	0.24
BoSF912	250-300	2	1.85	0.65	0.71	0.50	0.35
BoSF1331	150-200	2	1.92	0.67	0.00	0.53	0.37
CB10258	150-190	2	1.88	0.66	0.00	0.50	0.36
BoSF063	150-220	2	1.75	0.62	0.63	0.46	0.34
Na14H11	110-150	2	1.95	0.68	0.83	0.53	0.37
BoSF1167	120-150	2	1.92	0.67	0.80	0.53	0.37
BoSF966	110-140	2	1.85	0.65	0.71	0.50	0.35
BrBAC214	100-120	2	1.96	0.68	0.00	0.53	0.37
BoSF1131	100-120	2	1.60	0.56	0.00	0.40	0.31
BoSF042	80-190	2	1.80	0.64	0.67	0.49	0.35
BoSF062	100-320	2	1.85	0.65	0.71	0.50	0.35
BoSF2985	100-200	2	1.28	0.38	0.00	0.23	0.20
Na10E02	80-120	2	1.69	0.60	0.00	0.44	0.33
BoSF184	100-200	2	1.28	0.38	0.00	0.23	0.20
BoSF1957	80-280	2	1.18	0.29	0.17	0.17	0.14
BoE862	170-220	2	1.75	0.62	0.63	0.46	0.34
BoSF2612	150-190	2	1.88	0.66	0.00	0.50	0.36
BoSF2680	150-190	2	1.47	0.50	0.00	0.36	0.27
BoSF2294a	110-140	2	1.60	0.56	0.00	0.40	0.31
Na12F03a	250-320	2	1.60	0.56	0.17	0.41	0.31
BoSF2406	230-290	2	1.47	0.50	0.00	0.36	0.27
BoSF2033	70-150	2	1.80	0.64	0.00	0.49	0.35
BoSF2313	180-250	2	1.60	0.56	0.00	0.40	0.31
BoE7830	210-280	2	1.80	0.64	0.67	0.49	0.35
BoSF2054	100-160	2	1.92	0.67	0.40	0.53	0.37
BoSF2860	260-300	2	1.80	0.64	0.67	0.49	0.35
BoSF1215	110-180	2	1.69	0.60	0.00	0.44	0.33
Na12B09	190-320	2	1.85	0.65	0.71	0.50	0.35
BoSF250	280-520	2	1.69	0.60	0.00	0.44	0.33
BoSF2505	100-150	2	1.38	0.45	0.00	0.30	0.24
BoSF317	150-260	2	1.51	0.52	0.43	0.36	0.28
BoSF2878	90-150	2	1.69	0.60	0.57	0.44	0.33
BoSF1245	100-160	2	1.51	0.52	0.14	0.36	0.28
BoSF2421	230-250	2	1.60	0.56	0.50	0.43	0.31
BoSF1640	150-200	2	1.60	0.56	0.50	0.43	0.31
BoGMS1498	250-280	2	1.60	0.56	0.50	0.41	0.31
BoGMS0206	220-290	2	1.60	0.56	0.50	0.41	0.31
BoGMS1460	80-110	2	1.88	0.66	0.00	0.50	0.36
BoGMS0468	100-130	2	1.32	0.41	0.00	0.26	0.22
BoGMS0952	100-150	2	2.00	0.69	0.00	0.53	0.38

BoE615	100-300	4	2.97	1.20	1.00	0.71	0.60
BoSF2294a	80-250	3	2.65	1.03	1.00	0.67	0.55
BoSF376	80-200	3	2.28	0.90	1.00	0.60	0.47
cnu107	80-300	3	2.28	0.90	1.00	0.60	0.47
BoSF1047	200-700	4	3.60	1.32	1.00	0.79	0.67
BoE530	200-280	3	2.51	0.99	1.00	0.65	0.52
BrSF567	80-400	4	2.72	1.14	1.00	0.68	0.57
Mean	-	2.20	1.83	0.65	0.38	0.46	0.35

Where, n_a= Observed number of alleles; n_e=Effective number of alleles; I= Shannon's Information index; H_o= Observed heterogygosity; H_e= Expected heterogygosity and PIC= polymorphic information content

PIC with a population mean of 0.35, was recorded highest in the primer BoSF1047 (0.67) and lowest value was observed for primer BoSF1957 (0.14). Naushad et al. (2012) had also reported varied values of PIC (0.17-0.75), with a mean value of 0.46 by using SSR markers in different Brassica species. In the present study, different parameters of diversity exhibited high mean values, signifying allelic abundance in the SI lines of cabbage. This allelic abundance might be attributed to wide cross-ability due to self-incompatible nature of cabbage genotypes under study (Franceschi *et al*., 2011). Because, mode of pollination significantly affects the abundance and diversity of alleles within and across different plant species (Rana et al., 2015).

Cluster and principal component analysis

Dendrogram constructed through Jaccard's similarity coefficient and UPGMA method exhibited the similarity coefficient of 0.46 and allocated the eight SI lines of cabbage into two major groups i.e., A & B (Figure 4).

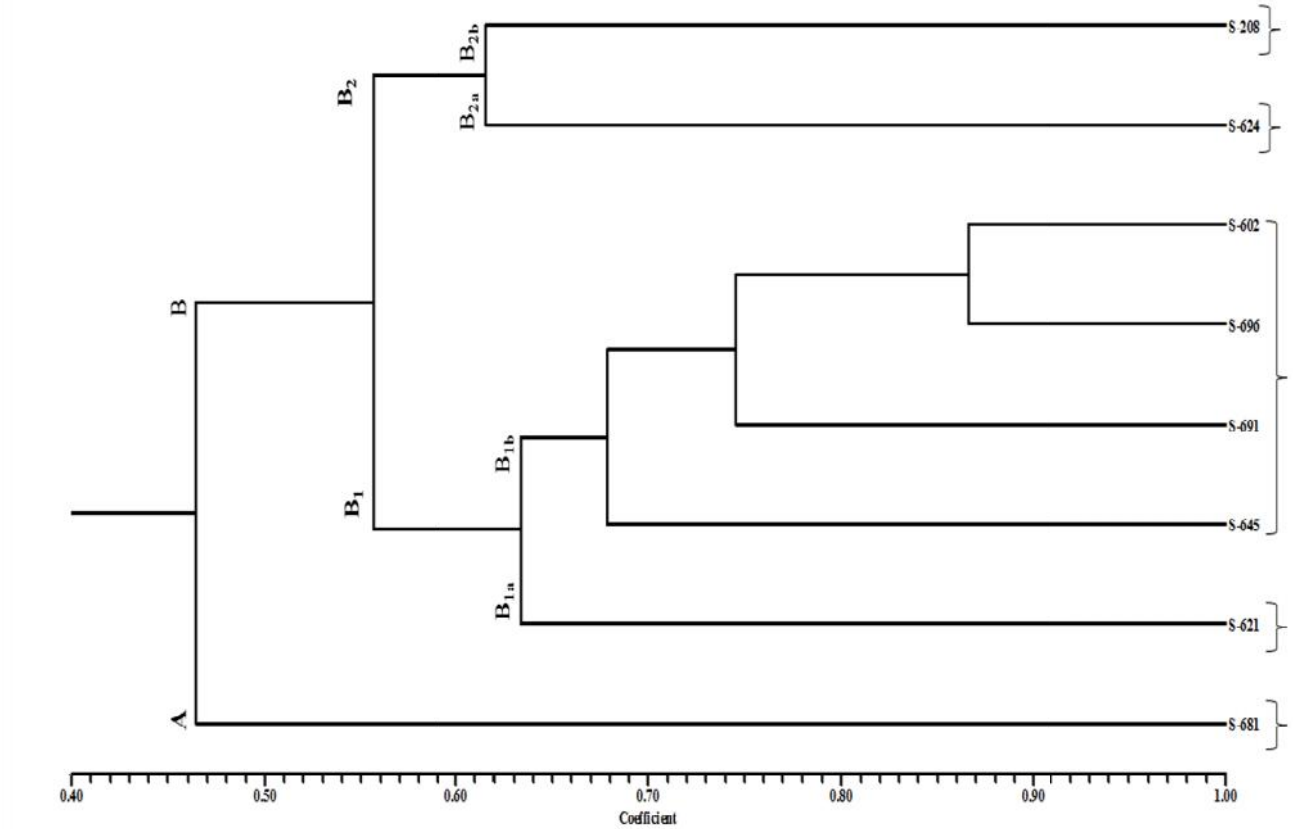

FIGURE 4: UPGMA dendrogram showing clustering pattern of eight SI lines based on 112 alleles constructed using Jaccard's similarity coefficient

Group A comprised of a single genotype i.e. S-681 with least similarity coefficient of 0.46, designating this to be most distant line among all the genotypes studied. This elite genotype can be used as one of the parent for making superior heterotic crosses with the genotypes of other groups. On the other hand, group B was further bifurcated into two sub-groups viz., B1 and B2. Further, B1 was alienated into two sub-groups (B1a and B1b). B1a comprised of only one genotype *i.e*., S-621, while B1b accommodated four genotypes *viz*., S-602, S-645, S-691 and S-696. In the mean while, sub-group B2 was further divided into B2a and B2b, which consisted of the genotype S-208 and S-624, respectively. In the present studies, two genotypes of group B2 *viz*., S-602 and S-696 due to highest similarity index (0.86), were found genetically most identical among all the tested SI lines of cabbage. Hence,

hybridization between these genotypes will not prove effective to yield superior hybrid combination. On the other hand, crossing between the genotypes of group A, B1 and B2, might have the opportunity to get superior heterotic combinations. Mohamed *et al*. (2016) based on SSR data, had also clustered different genotypes of Brassica oleracea into different groups, indicating considerable level of genetic variations among different Brassica spp. The results of principal component analyses (PCA) and neighbor-joining tree correspondingly revealed similar pattern of genetic diversity among different SI lines of cabbage. Again the genotype S-681 was found most distinct, while S-602 and S-696 were reported genetically most similar among all the genotypes (Figure 5 and 6). Saxena *et al*. (2011) using RAPD and SSR markers had also reported that UPGMA Dendgrogram and scattered plot diagram give similar pattern of genetic diversity among different cabbage cultivars.

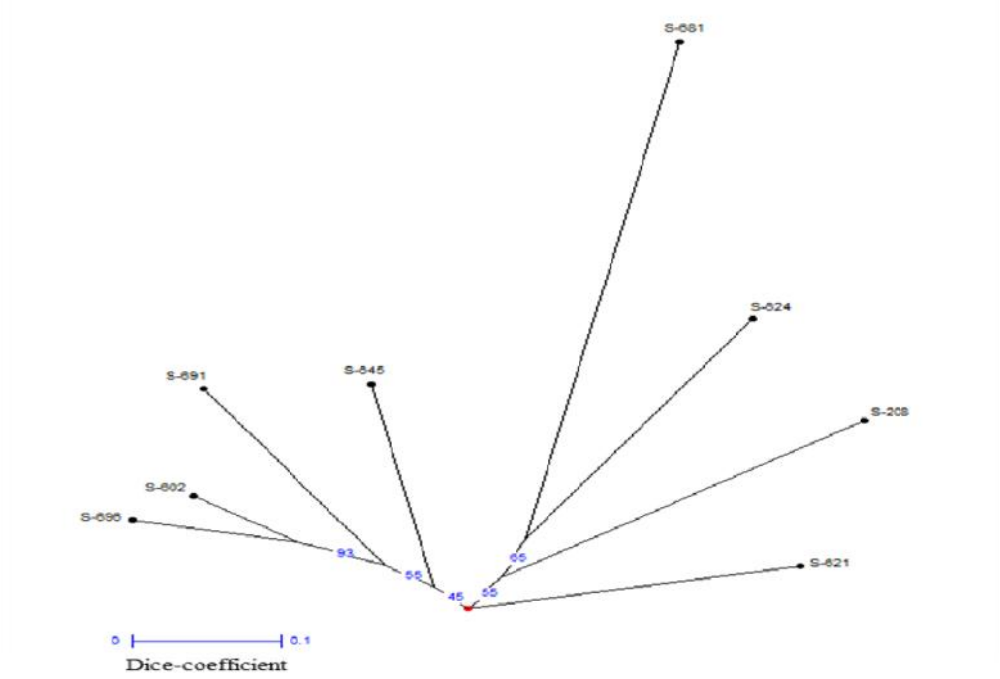

FIGURE 5: Radial neighbor-joining tree based on 112 alleles from 49 SSR loci among eight SI lines of cabbage

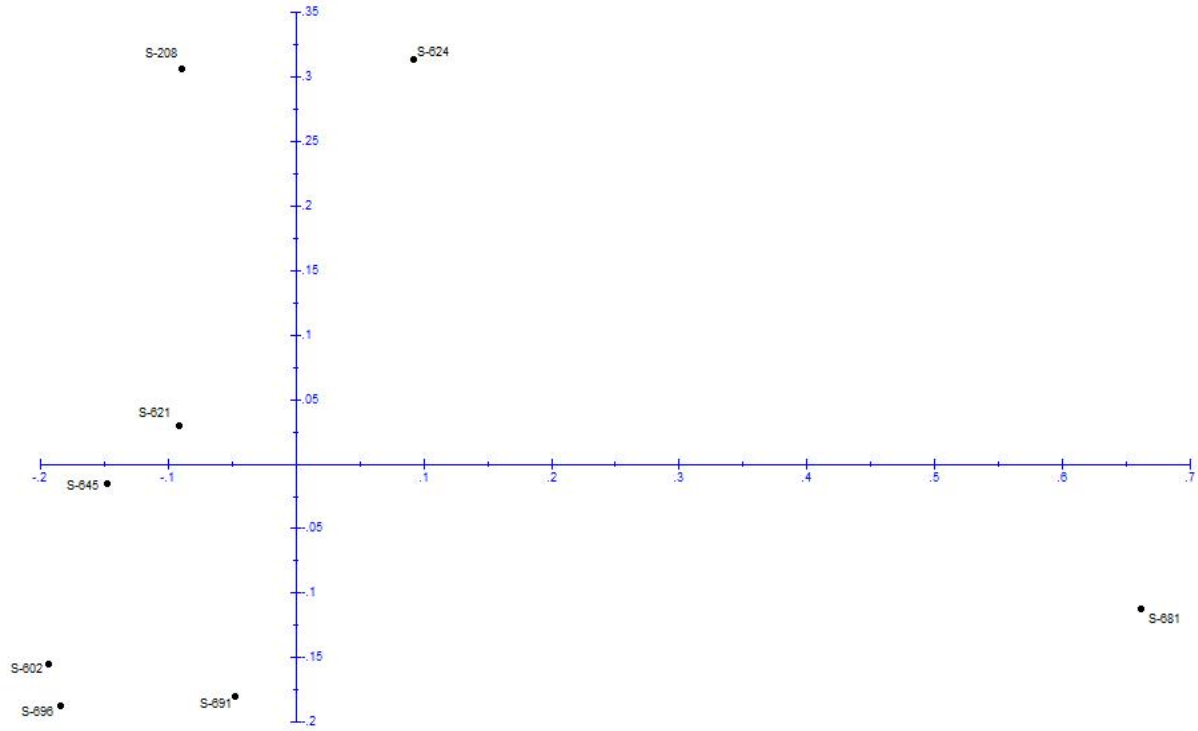

FIGURE 6: Genetic relationship among the eight SI lines of cabbage based on principle component analysis

CONCLUSION

The experimental results on morphogenetic characterization and diversity analysis conclude that eight SI lines of cabbage have appreciable genetic variations. Based on morphological and molecular studies, the genotype S-645 and S-681 were found most distinct and divergent. Hence, these genotypes have greater potential in future breeding programmes for the development of high yielding quality hybrids. Further, 49 SSR loci recorded high polymorphism and were found effective for differentiating the different self-incompatible lines under study. Hence, SSR markers can be utilized for germplasm characterization and association mapping for future breeding programmes in self-incompatible lines of cabbage.

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