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RESEARCH ARTICLE

RANDOM AMPLIFIED POLYMORPHIC DNA AND INTER SIMPLE SEQUENCE REPEAT FINGERPRINTS FOR ASSESSING GENETIC DIVERSITY OF INDIAN GARLIC

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ABSTRACT

96 garlic accessions assembled at Pune were evaluated using RAPD and ISSR markers to determine the genetic relationship among accessions exhibiting morphological variations. A total of 160 decamer RAPD and 100 ISSR primers were used, of these 20 RAPD and 14 ISSR primers showed good amplification and polymorphism. RAPD Primers generated total 189 bands out of which 65 were polymorphic, showing 34.39% polymorphism. OPM 09 exhibited the maximum polymorphism (63.63%) while OPE03 shown the least polymorphism (12.5%). A total of 123 ISSR bands were produced with 14 primers out of which 45 were polymorphic (36.58%), with average of 3.2 polymorphic bands per primer. Based on present studies, the molecular markers analyses detected extremely low genetic diversity in the present accessions. No relationship was observed between the patterns generated by the primers and the geographic origin of the clones. No specific association between morphologic diversity and genetic diversity due to lack of sexual reproduction. Possibly, the strong selection pressure for agronomic traits in many cases, did not affect molecular markers which are generally neutral, but contributed to phenotypic and genetic divergence. Sixteen genetically distinct types having favorable agronomic traits were identified from the 96 accessions.

INTRODUCTION

The latest global review of area and production shows that among the major vegetables, garlic ranks fifth in area and seventh in production. In India, garlic is grown in the Northern parts especially in Gujarat, Haryana, Punjab, central Madhya Pradesh, Maharashtra and in a few southern localities, especially the cold hilly tracts of Ooty and Kodaikanal, Tamil Nadu. A total of 1,264 MT garlic is been produced in year 2010-11. Gujarat is the largest producer of garlic in India with production of 275 MT, followed by Madhya Pradesh with 228 MT production (NHRDF, 2011). Garlic enjoys a wide distribution worldwide and has long been known as a cultivated species. Its native home and existence as a truly wild species is still not clear and has been discussed by Regel (1875, 1887) and Candolle (1885). According to Vavilov (1951) and Vvedensky (1946), *Allium longicuspis* Rgl. is an endemic species of central Asia and wild ancestor of garlic. Garlic bulbs are rich sources of carbohydrates and proteins. Analysis of garlic indicates that it contains 61 to 64% moisture, 31% carbohydrate, 5 to 6% protein and only 0.2% fat.

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Significant levels of phosphorous (3.9 to 4.6 mg/g), potassium (1.0 to 1.2 mg/g) and calcium (0.5 to 0.9 mg/g) are present (Kaufmann *et al.*, 1999). All *Allium* species produce volatile chemicals which act as repellants to many insects. Garlic has some other chemicals as additional protection for those insects and animals not deterred by its volatile metabolites. Sequestered in vacuoles within the plant's cells is an odorless, sulfur based compound, (+) S-allyl-1 cysteine sulfoxide or alliin.

MATERIALS AND METHODS

The present investigation was carried out at the Directorate of Onion and Garlic Research, (DOGR) Rajgurunagar, Pune (ICAR).

PLANT MATERIAL:

A total of 96 garlic accessions were used in the present study. The list of plant material with their origin is given below:

Genetic Diversity Studies

For genetic diversity analysis RAPD and ISSR primers were used.

RAPD Primers: RAPD primers were acquired from Operon Technologies, USA.

ISSR primers: ISSR primers were procured from UBC (University of British Columbia) Canada.

Reagents

DNA Extraction Protocol (CTAB Method):

1. Young leaves of garlic approximately 1 g were grinded in liquid Nitrogen.
2. The fine powdered leaves were taken into 5 ml tubes and then dissolved in preheated CTAB buffer.
3. These tubes were placed in water bath for 30 min at 60 °C.
4. After incubation tubes were removed and allowed to cool at room temperature.
5. Equal volume of Chloroform: Isoamyl alcohol (24:1) was added to tubes.
6. Tubes were centrifuged at 10,000 rpm, 15-25^{o} C for 10 min.
7. After centrifugation aqueous layer was taken out in another tube.
8. Equal volume of ice cold iso propanol was added in this tube. Tube was then inverted gently until two phases are no longer evident. DNA collected as white stringy mass. Tubes containing DNA was stored overnight at minus 20°C .
9. Next day tubes were centrifuged for 2 min at 2000 rpm.
10. Supernatant was discarded; DNA was settled in the bottom of the tube.
11. DNA was washed using wash buffer (76% ETANOL +10mM amonium acetate). DNA was kept in wash buffer for at least 20 minutes.
12. Tubes were again centrifuged for 2 minutes at 2000 rpm and supernatant was discarded.
13. DNA samples were dried until odor of ethanol is no longer evident.
14. DNA was dissolved in TE buffer.
15. 1ul RNase (10mg/ml) was added to each 1 ml TE/ DNA (1 ml DNA/ 1 µl RNase).
16. After adding RNase DNA is kept for incubation at 37 °C For 60 minutes.
17. After incubation again equal volume of Chloroform: Isoamyl alcohol (24:1) was added.
18. Centrifuged at 10,000 rpm for 10 minutes.
19. Aqueous layer was taken in a new tube.
20. 2.5 M Ammonium acetate (7.5 M ammonium acetate pH 7.7) and 2.5 volume of 100% ice cold ethanol or (1/10 Sodium acetate pH 5.2) and add 2 volume of absolute ethanol) were added.
21. Refrigerated for 30 minutes to get precipitate.
22. After refrigeration the tubes were centrifuged at 10,000 rpm for 10 min at 4°C to pellet DNA.
23. Supernatant was discarded and DNA pellet were washed with 70% ethanol.
24. Centrifuged at 10,000 rpm for 10 min to pellet the DNA Ethanol was discarded.
25. DNA was kept for drying until the odor of ethanol is not evident.
26. DNA was dissolved in 10 mM TE BUFFER.

Quantification of DNA

DNA obtained from the above procedure were quantified by analyzing the samples in 0.8% Agarose gel (dissolved in 1 x TBE). Quantification was done visually by comparing the DNA with another standard gel loaded with different known concentrations of DNA (12.5 to 200 ng). For confirmation, quantification was carried out using Nano Photometer (IMPLEN).

PCR Analysis

RAPD PCR

The PCR amplification for RAPD analysis was performed according to Williams *et al*. (1990) with certain modifications. RAPD assay was carried out in 20 µl reaction mixture containing 2.0 µl 10x amplification buffer (50 mM KCl, 100 mM Tris HCl, 1.0% Triton x-100 and 15 mM $MgCl_2$), 2 µl dNTPs mix (dATP, dTTP, dGTP and dCTP), 1.0 U Red Taq DNA polymerase (Bangalore Genei), 10µM of 10-mer primers (OPP) series, Operon Technologies, USA (Table 4) and 50 ng of genomic DNA. Amplification was performed in a Peltier Thermal Cycler (PTC 100). The sequential steps involved were:

ISSR PCR

ISSR assay was carried out in 20 µl reaction mixture containing 2.0 µl 10x amplification buffer (50 mM KCl, 100 mM Tris HCl, 1.0% Triton x-100 and 15 mM $MgCl_2$), 1.6 µl of 0.25mM dNTPs mix (dATP, dTTP, dGTP and dCTP) (Sigma), 0.8 µl of 50 mM $MgCl_2$, 1.0 U Red Taq DNA polymerase (Bangalore Genei), 1.0 µl of 10µM of ISSR Primers of UBC(University of British Columbia) Canada, (Table 5) and 50 ng of genomic DNA. Amplification was performed in a Peltier Thermal Cycler (PTC 100). The PCR amplification for ISSR analysis was performed with certain modifications.

The optimum conditions for DNA amplification are given in Table 5.

Agarose Gel Electrophoresis and visualization of amplified products

The amplified products were resolved on 1.2 % agarose gel (Sigma Aldrich) for RAPD and 1.5% for ISSR (dissolved in 1x TBE). The amplified sample was loaded into the wells along with 100 bp Plus and 1 Kb DNA ladder (Fermentas, USA) and electrophoresis was carried out at 200 V supplied from a power pack in a horizontal gel electrophoresis unit (C.B.S, SCIENTIFIC COMPANY, CALIFORNIA, USA).

Data Analysis

The products of electrophoresis were detected and documented using BIO RAD gel documentation unit (USA). Amplified products that were reproducible and consistent alone were used for scoring. Only reproducible bands were considered to evaluate the results. Each band was considered as an independent attribute and was accounted for qualitatively by its

presence (1) or absence (0) generating a binary matrix. The genetic similarity between genotype pairs was estimated using the Jaccard's similarity coefficient with the SIMQQUAL option. The similarity matrix was calculated by means of sequential, agglomerative, hierarchical and nested clustering (SAHN) (Sneath and Sokal, 1973). The dendrogram was generated by the hierarchical method and the UPGMA grouping algorhythm. The COPH option was used to generate a matrix of cophenetic values. The MXCOMP option of this matrix was used to calculate the correlation between the cophenetic correlation matrix and the original grouping matrix (SIMQUAL).

This analysis measures if the original data are represented in the grouping analysis with 1000 permutations, which generated a cophenetic correlation value (r), It is considered that a correlation value of r = 0.9 shows a good adjustment. These analyses were made using the NTSYSpc 2.1 program (Rohlf, 2000). Marker index for RAPD and ISSR markers was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula PIC = $1\text{-}\Sigma pi^2$where *pi* is the frequency of the *i*th allele (Smith *et al.*, 1997). Resolving power (Rp) is the sum of Ib values of all the bands amplified by a primer. Band in formativeness (Ib) and resolving power (Rp) were calculated as given by Prevost and Wilkinson (1999).

The formulae used for the above-mentioned parameters are

(i) Band informative ness of a given band: $Ib = 1 - (2 \times |0.5 - p|)$, where p is the proportion of the total genotypes containing the band;

(ii) Resolving power of a primer is the sum of band informative ness: $Rp = \Sigma Ib$.

RESULTS

The results obtained in the present investigation with respect to genetic diversity assessment is described below:

RAPD Data

A total of 160 RAPD primers (Operon Technologies USA) OPA1-20, OPB1-20, OPC1-20, OPD1-20, OPE1-20, OPG1-20, OPJ1-20 and OPM1-20 were used for screening of suitable primers giving amplification. PCR protocols were standardized as required. Eight genotypes were used for primary screening of primers. A total of 40 primers showed amplification out of which 20 primers gave good amplification and hence were used for further amplification on all the genotypes. Only clear and unambiguous bands were taken for scoring. The number of bands for each primer varied from six to thirteen, within average of 9.4 bands and the size of amplicons generated from these primers ranged from 200 bp to 3 Kb. A total of 189 RAPD bands were generated of which 65 bands (34.39%) were polymorphic, with an average of 3.2 polymorphic bands per primer. The maximum numbers of polymorphic bands were 7 generated from OPM 9 followed by OPA 09 which generated 5 polymorphic bands. OPJ 16 and OPE 3 showed minimum polymorphism by generating only 1 polymorphic band. Different primers showed variation in their ability to detect polymorphism. The polymorphic primers exhibited variation with regard to resolving power (Rp), PIC (Polymorphism Information Content) and MI (Marker Index) values. Rp, PIC and MI values of these polymorphic primers have been depicted in Table 13.

Analysis of the RAPD data using Nei's original measures of genetic identity and genetic distance showed that the genetic similarity (GS) value ranged from 1.0 to 0.74 with a mean value of 0.87 among garlic germplasm The UPGMA (Unweighed Pair Group Method with Arithmetic mean) dendrogram constructed using Jaccard's similarity matrix of RAPD data discriminated all the genotypes in two major groups (I & II). Group I consisted of four sub clusters Ia, Ib, Ic and Id showing around 88% similarity between them. Cluster I a consisted of 22 germplasm (ACC 518, ACC 432, ACC 371, G1, ACC 323, ACC 336, ACC 401, ACC 050, ACC 321, ACC 416, ACC 074, ACC 114, G41, ACC 282, ACC 312, ACC 365, ACC 044, ACC 516, ACC 175, ACC015BR, ACC 316BR1 and ACC 514).Cluster I^{st} a showed a similarity of 100% except ACC 514 and ACC 015 BR which showed genetic similarity around 98%. ACC 316 BR1 showed 95% similarity with rest of the germplasm. Among these accessions, G1, G41 and Godavari tropical types are commercially grown in the northern Indian plains of Haryana. Cluster Ib consisted of 34 accessions viz., ACC183, ACC471, ACC 463, ACC521, ACC066, ACC 376, ACC061, ACC 056, ACC 367, ACC 543, ACC 059, ACC 330, ACC 038, ACC 321BR5, GODAVARI, ACC 520, ACC 476, ACC 201 BR5, ACC 335, ACC 155, ACC 343, ACC 279, ACC 453, ACC 015, ACC389, IC 338528, ACC 323, ACC 310, ACC 321BR4, ACC 356, ACC 338, ACC 316 BR2, ACC 147 BR, ACC 275). Cluster Ib is showing 100% similarity within them. Cluster Ic consisted of 14 germplasm (IC372924, ACC 321BR2, ACC 508, ACC 378, ACC 293, G 41 MPR, ACC 464, ACC 079, ACC 482, ACC 321DR3, ACC 477, ACC 074 BR, ACC 452, and ACC 237.Cluster Ic showing similarity of 98% within them except ACC 237 which is showing 96% similarity with rest of the germplasm. Cluster Id consisted of 13 germplasm (ACC 112, BHIMA OMKAR, ACC 219 BR, ACC 416, ACC 339, ACC 012, ACC 316BR, ACC 409, ACC 287, ACC 221 BR, ACC 321BR1, ACC 410 and ACC 593). In cluster Id ACC 012 and ACC 316BR are showing Genetic similarity of 100%. While ACC 409 and ACC 287 showing 90% similarity between them. ACC 593 and ACC 410 are having 86% genetic similarity between them.

Group II consisted of only one sub-cluster, with 13 germplasm in it and showing the genetic similarity around 89%. The germplasm in group II are ACC 624, ACC 522, ACC 629, ACC 623, ACC 507, ACC 545, ACC 632, ACC 546, ACC 544, ACC 625, ACC 523, ACC 628 and ACC 627. Group II is showing 87% of genetic similarity. Group I and Group II were having 74% genetic similarity within them. However, there was no association of banding patterns with geographic locations. This non-association with geographical location to the fact that garlic cultivars could have been passed between

Table 1.Garlic Germplasm

Sr. No.	Name	Accession Code	Origin Code	Origin
1	514	ACC514	WB1	West Bengal
2	518	ACC518	WB2	West Bengal
3	432	ACC432	AP1	Andhra Pradesh
4	371	ACC371	AP2	Andhra Pradesh
5	G1	G1	MAH1	NHRDF
6	323	ACC323	OR1	Odisha
7	409	ACC409	AP3	Andhra Pradesh
8	183	ACC183	UNK	Unknown
9	WG-471	ACC471	AP4	Andhra Pradesh
10	521	ACC521	WB3	West Bengal
11	RG-66	ACC066	GJ1	Gujarat
12	RG-56	ACC056	UNK	Unknown
13	477	ACC477	MAH2	Maharashtra
14	624	ACC624	RAJ1	Rajasthan
15	RG-336	ACC336	MAH3	Maharashtra
16	RG-401	ACC401	AP5	Andhra Pradesh
17	M-112	ACC112	UNK	Unknown
18	RG-50	ACC050	GJ2	Gujarat
19	WG-452	ACC452	AP6	Andhra Pradesh
20	543	ACC543	AP	Andhra Pradesh
21	625	ACC625	HR1	Haryana
22	RG-59	ACC059	GJ3	Gujarat
23	M-330	ACC330	OR2	Odisha
24	RG-321-14	ACC321BR2	OR6	Odisha
25	M-321	ACC321	OR3	Odisha
26	520	ACC520	WB4	West Bengal
27	RG-416-8-1	ACC416BR	AP7	Andhra Pradesh
28	476	ACC476	RAJ2	Rajasthan
29	629	ACC629	RAJ3	Rajasthan
30	WG-378	ACC378	AP8	Andhra Pradesh
31	628	ACC628	HR2	Haryana
32	WG-74-7	ACC074BR	UNK	Unknown
33	M-12	ACC012	UNK	Unknown
34	RG-410	ACC410	AP8	Andhra Pradesh
35	627	ACC627	RAJ4	Rajasthan
36	WG-316-11-1	ACC316BR	UNK	Unknown
37	WG-221-4-1	ACC221BR	UNK	Unknown
38	RG-321	ACC321BR1	OR4	Orissa
39	RG-74	ACC074	GJ4	Gujarat
40	RG-114	ACC114	GJ5	Gujarat
41	G-41	G-41	MAH4	NHRDF,Maharashtra
42	RG-282	ACC282	GJ6	Gujarat
43	WG-365	ACC365	AP9	Andhra Pradesh
44	WG-316-12-3	ACC316BR1	UNK	Unknown
45	M-287	ACC287	AS1	Tezpur, Assam
46	RG-44-5	ACC044BR	UNK	Unknown
47	516	ACC516	WB5	West Bengal
48	M-175	ACC175	GJ7	Gujarat
49	522	ACC522	WB6	West Bengal
50	RG-338	ACC338	MAH5	Maharashtra
51	RG-356	ACC356	MAH6	Maharashtra
52	RG-321-2-1	ACC321BR4	OR8	Odisha
53	RG-339	ACC339	MAH7	Maharashtra
54	623	ACC623	UP1	Uttar Pradesh
55	IC-338528	IC338528	UNK	Unknown
56	WG-389	ACC389	AP10	Andhra Pradesh
57	M-15	ACC015	UNK	Unknown
58	WG-453	ACC453	AP11	Andhra Pradesh
59	WG-323	ACC323	UP2	Uttar Pradesh
60	M-279	ACC279	GJ8	Gujarat
61	RG-343	ACC343	MAH8	Maharashtra
62	RG-464	ACC464	AP12	Andhra Pradesh
63	523	ACC523	WB7	West Bengal
64	RG-275	ACC275	GJ9	Gujarat
65	RG-335	ACC335	MAH9	Maharashtra
66	RG-174-11-1	ACC174BR	UNK	Unknown
67	WG-201-7-1	ACC201B5	UNK	Unknown
68	RG-316-8-3	ACC316BR2	UNK	Unknown
69	M-237	ACC237	UNK	Unknown
70	508	ACC508	SING 1	Singapore
71	593	ACC593	TW1	Taiwan
72	RG-310	ACC310	MAH10	Maharashtra
73	Godavari	Godavari	UNK	Unknown
74	RG-312	ACC312	OR5	Odisha

75	WG-321-4-2	ACC321BR5	OR9	Odisha
76	507	ACC507	US1	United States
77	WG-219-7-1	ACC219BR	UNK	Unknown
78	RG-155	ACC155	MAH11	Maharashtra
79	AC-200	Bhima Omkar	Bhr1	BIHAR
80	545	ACC545	AP	Andhra Pradesh
81	IC-372924	IC-372924	UNK	Unknown
82	RG-38	ACC038	GJ10	Gujarat
83	WG-416	ACC416	AP13	Andhra Pradesh
84	RG-482	ACC482	MAH12	Maharashtra
85	M-293	ACC293	GJ11	Gujarat
86	632	ACC632	MP1	Madhya Pradesh
87	RG-61	ACC061	GJ12	Gujarat
88	WG-367	ACC367	AP14	Andhra Pradesh
89	M-NO-15	ACC015BR	UNK	Unknown
90	WG-376	ACC376	AP15	Andhra Pradesh
91	546	ACC546	AP	Andhra Pradesh
92	BIG BULB	G41MPR	UNK	Unknown
93	544	ACC544	AP	Andhra Pradesh
94	RG-321-9	ACC321BR3	OR7	Odisha
95	M-79	ACC079	GJ13	Gujarat
96	RG-463	ACC463	AP16	Andhra Pradesh

Table 2. RAPD primers and sequences

Sr. No.	Primer code	Sequence
1	OPA-01	CAGGCCCTTC
2	OPA-07	GAAACGGGTG
3	OPA-09	GGGTAACGCC
4	OPA-10	GTGATCGCAG
5	OPA-11	CAATCGCCGT
6	OPA-17	GACCGCTTGT
7	OPB-16	TTTGCCCGGA
8	OPC-07	GTCCCGACGA
9	OPC-11	AAAGCTGCGG
10	OPD-15	CATCCGTGCT
11	OPD-20	ACCCGGTCAC
12	OPE-03	CCAGATGCAC
13	OPE-20	AACGGTGACC
14	OPG-04	AGCGTGTCTG
15	OPG-08	TCACGTCCAC
16	OPG-16	AGCGTCCTCC
17	OPJ-16	CTGCTTAGGG
18	OPM-02	ACAACGCCTC
19	OPM-09	GTCTTGCGGA
20	OPM-16	GTAACCAGCC

Table 3. ISSR primers and sequences

Sr. No.	Primer code	Sequence
1	808	AGA GAG AGA GAG AGA GC
2	810	GAG AGA GAG AGA GAG AT
3	811	GAG AGA GAG AGA GAG AC
4	817	CAC ACA CAC ACA CAC AA
5	825	ACA CAC ACA CAC ACA CT
6	834	AGA GAG AGA GAG AGA GYT
7	841	GAG AGA GAG AGA GAG AYC
8	857	ACA CAC ACA CAC ACA CYG
9	860	TGT GTG TGT GTG TGT GRA
10	864	ATG ATG ATG ATG ATG ATG
11	866	CTCCTCCTCCTCCTCCTC
12	873	GACAGACAGACAGACA
13	891	HVH TGT GTG TGT GTG TG
14	900	ACT TCC CCA CAG GTT AAC ACA

Table 4. The optimum conditions for RAPD

Sr. No.	Step	Temperature (°C)	Duration (min)	Number of cycles
1	Denaturation	95	5	1
2	Denaturation	94	1	35
3	Annealing	36	1	35
4	Extension	72	2	35
5	Final extension	72	10	1
6	Hold	4	-	-

Table 5. The optimum conditions for ISSR

Sr.No.	Step	Temperature (°C)	Duration (min)	Number of cycles
1	Denaturation	94	5	1
2	Denaturation	94	1	35
3	Annealing	53	1	35
4	Extension	72	2	35
5	Final extension	72	7	1
6	Hold	4	-	-

Table 6. RAPD data

Sr. No.	Primer code	Primer Sequence	Total No. of bands	No. of polymorphic bands	Percent polymorphism
1	OPA-01	CAGGCCCTTC	13	4	30.76
2	OPA-07	GAAACGGGTG	7	4	57.14
3	OPA-09	GGGTAACGCC	12	5	41.66
4	OPA-10	GTGATCGCAG	8	2	25
5	OPA-11	CAATCGCCGT	7	3	42.85
6	OPA-17	GACCGCTTGT	9	3	33.33
7	OPB-16	TTTGCCCGGA	7	4	57.14
8	OPC-07	GTCCCGACGA	8	4	50
9	OPC-11	AAAGCTGCGG	12	4	33.33
10	OPD-15	CATCCGTGCT	9	2	22.22
11	OPD-20	ACCCGGTCAC	9	2	22.22
12	OPE-03	CCAGATGCAC	8	1	12.5
13	OPE-20	AACGGTGACC	10	2	20
14	OPG-04	AGCGTGTCTG	9	4	44.44
15	OPG-08	TCACGTCCAC	9	3	33.33
16	OPG-16	AGCGTCCTCC	12	3	25
17	OPJ-16	CTGCTTAGGG	7	1	14.28
18	OPM-02	ACAACGCCTC	13	4	30.76
19	OPM-09	GTCTTGCGGA	11	7	63.63
20	OPM-16	GTAACCAGCC	9	3	33.33
TOTAL			189	65	34.39

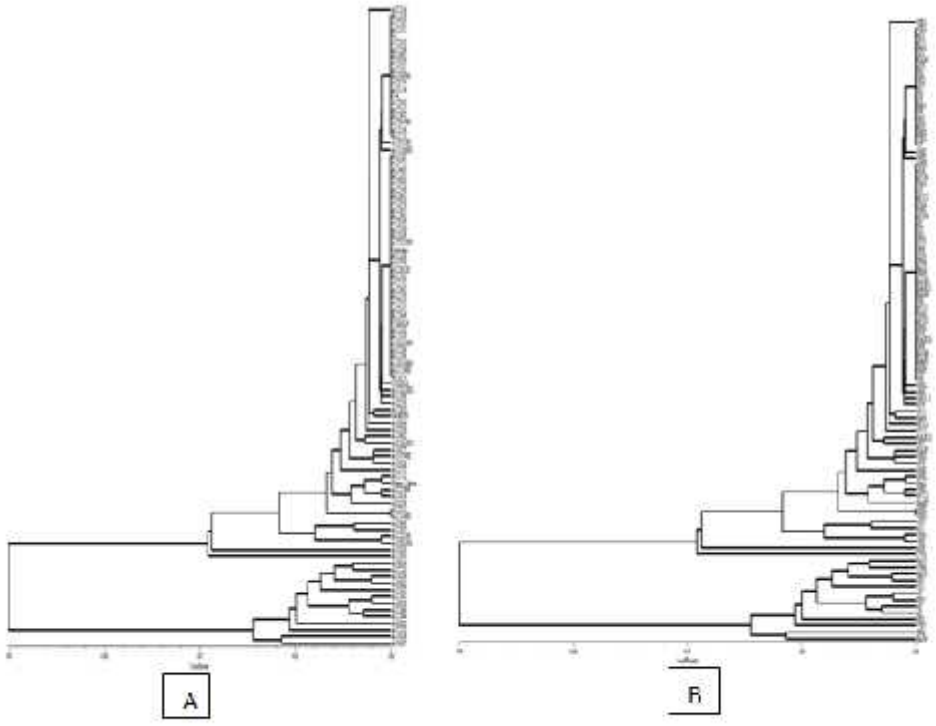


Fig.1.A Jaccard's Similarity matrix Dendrogram for RAPD data. (B) Jaccard's Similarity matrix Dendrogram for RAPD data of Origin

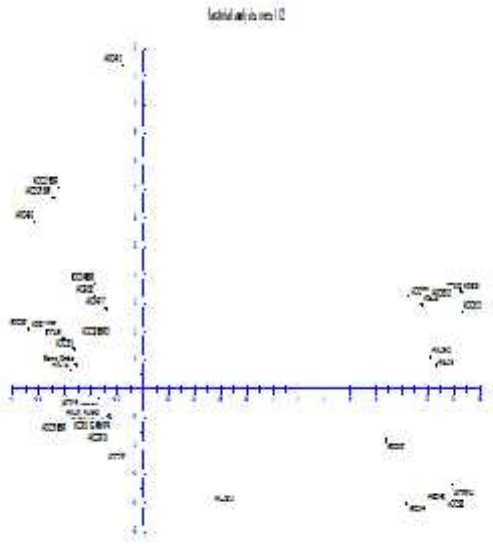


Figure 2.DARwin's factorial analysis for RAPD data

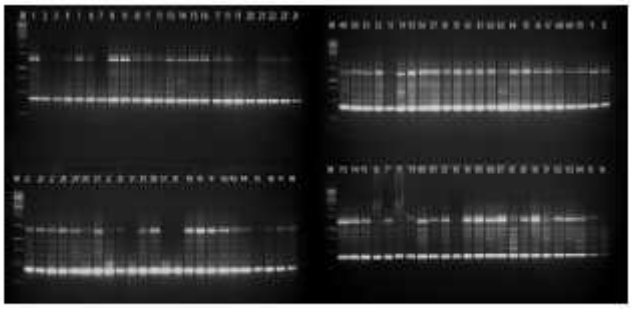


Figure3. RAPD- OPM 16

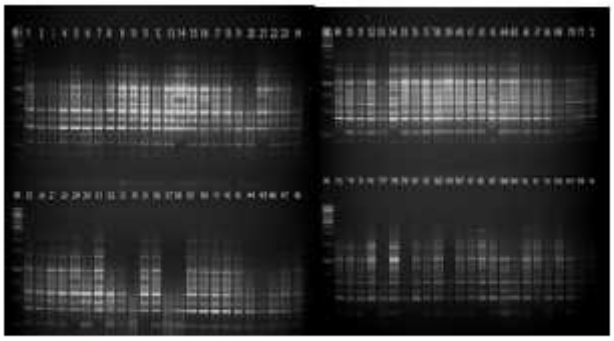


Figure 4 RAPD- OPC 11

many regions and their true geographical origin were consequently lost. To check the fit of dendrogram with the original similarity matrix, a cophenetic correlation coefficient was computed as described in materials and methods. The correlation coefficient was 0.98 which is described as a good fit. A total of 100 ISSR primers 801-900 (UBC, Canada) were screened and out of these 27 gave amplification out of which 14 primers gave good amplification and hence were used for further amplification on all the genotypes. A total of 123 ISSR bands were produced with 14 primers out of which 45 were polymorphic (36.58%), with average of 3.2 polymorphic bands per primer. The number of polymorphic bands varied from 1 to 4. Primer 808 generated maximum number of polymorphic bands 6, followed by 825 which generated 5 polymorphic bands. The size of amplicons generated from these primers ranged from 200 bp to 2.5 Kb. Analysis of ISSR data revealed that genetic similarity ranged from 1.0 TO 0.79 with a mean value of 0.89 among the Indian garlic. Different primers showed variation in their ability to detect polymorphism. The maximum resolving power was exhibited by primer 857(20.94) while the minimum was in 834(9.73) with the average resolving power of these primers was 15.01. The UPGMA dendrogram was constructed using Jaccard's similarity matrix. The UPGMA (Unweighed Pair Group Method with Arithmetic mean) dendrogram constructed using Jaccard's similarity matrix of ISSR data discriminated all the genotypes in two major groups (I & II).

Group I consisted of five sub clusters (Ia, Ib, Ic, Id and Ie) showing 93% of genetic similarity between them. ACC102 showing 98% genetic similarity. Sub-cluster Ia consisted of 46 accessions (ACC 514, ACC 518, ACC 432, G1, ACC 323, ACC 471, ACC 521, ACC 066, ACC 056, ACC 482, ACC 477, ACC 401, ACC 112, ACC 050, ACC 330, ACC 310, ACC 061, ACC 508, ACC 201BR5, ACC 321, ACC 520, ACC 279, ACC 416 BR, ACC 476, ACC 378, ACC 356, ACC 074BR, ACC 516, ACC 015BR, ACC 316 BR1, ACC 365, G41, ACC 316 BR, ACC 336, ACC038, ACC335, ACC 155, ACC343, ACC 312, ACC 174 BR, ACC059, ACC 464, ACC 175, ACC 338, ACC 074, ACC 282) showing 100% genetic similarity between them. Sub-cluster Ib consisted of 14 germplasm (ACC 543, ACC 321BR4, G41 MPR, ACC 376, ACC 367, ACC 323, ACC 316BR2, ACC 275, ACC 389, ACC453, ACC 015, ACC 114, GODAVARI and IC338528). ACC 321BR4, G41 MPR, ACC 376, ACC 367, ACC 323, ACC 316BR2, ACC 275, ACC 389, ACC453, 114, GODAVARI and IC338528 are showing 100% similarity between them while ACC 543 and ACC 015 showed 98% similarity between them. Sub-cluster Ic consisted of 5 germplasm (ACC 287, ACC 463, ACC 012, ACC 293, ACC 321 BR3). ACC 287 and ACC 463 are showing 99% genetic similarity. ACC 293 and ACC 321 BR3 having 98% similarity between them. ACC102 showing 98% genetic similarity. Sub-cluster Id consisted of 11 germplasm (ACC 371, ACC 452, ACC 321BR2, ACC 079, ACC044BR, ACC 237, ACC 321BR5, IC372924, ACC 339, BHIMA OMKAR and ACC 219BR) showing 100% of similarity between them. Sub-cluster Ie consisted of 6 germplasm (ACC 416, ACC 183, ACC 410, ACC 221BR, ACC 321BR1 and ACC 409). ACC 410 and ACC 221BR showing 100% similarity between them, rest of the germplasm in cluster showing 98% similarity in between them. Group II consisted of 14 germplasm, ACC 624, ACC 629, ACC 507, ACC 623, ACC 627, ACC 545, ACC 625, ACC 523, ACC 632, ACC 546, ACC 544, ACC 522, ACC 628 and ACC 593. Group II showing 90% genetic similarity. Group I and II are showing 79% genetic similarity between them. The composition of clusters obtained using independently RAPD and ISSR markers have revealed similar groupings in maximum clusters. The performance of these markers was evaluated using various parameters such as percentage of polymorphism, average band informative ness, resolving power, and clusters formed in the dendrogram. Percentage of polymorphic markers: the fourteen ISSR primers yielded average 8.78 bands per primer, while the twenty RAPD primers amplified average 9.45

Table 7. ISSR Data

Sl. No.	Primer Code	Primer Sequence	Total No. of bands	No. of polymorphic bands	Percent polymorphism
1	808	AGA GAG AGA GAG AGA GC	10	6	60
2	810	GAG AGA GAG AGA GAG AT	9	5	55.55
3	811	GAG AGA GAG AGA GAG AC	9	3	33.33
4	817	CAC ACA CAC ACA CAC AA	9	3	33.33
5	825	ACA CAC ACA CAC ACA CT	8	5	62.5
6	834	AGA GAG AGA GAG AGA GYT	5	1	20
7	841	GAG AGA GAG AGA GAG AYC	9	4	44.44
8	857	ACA CAC ACA CAC ACA CYG	13	4	30.76
9	860	TGT GTG TGT GTG TGT GRA	9	2	22.22
10	864	ATG ATG ATG ATG ATG ATG	9	2	22.22
11	866	CTCCTCCTCCTCCTCCTC	9	2	22.22
12	873	GACAGACAGACAGACA	9	3	33.33
13	891	HVH TGT GTG TGT GTG TG	6	2	33.33
14	900	ACT TCC CCA CAG GTT AAC ACA	9	3	33.33
TOTAL			123	45	36.58

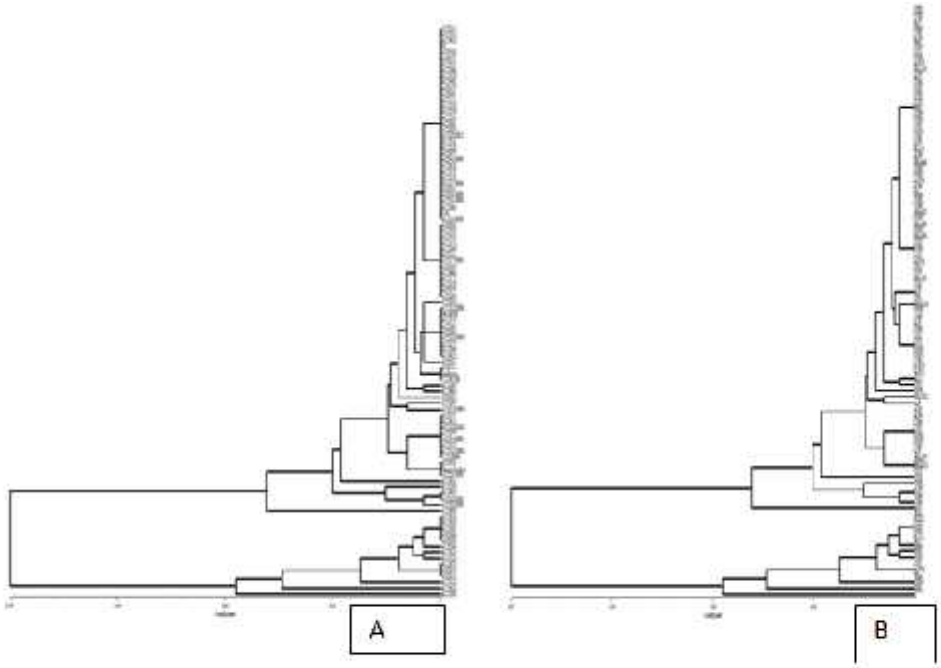
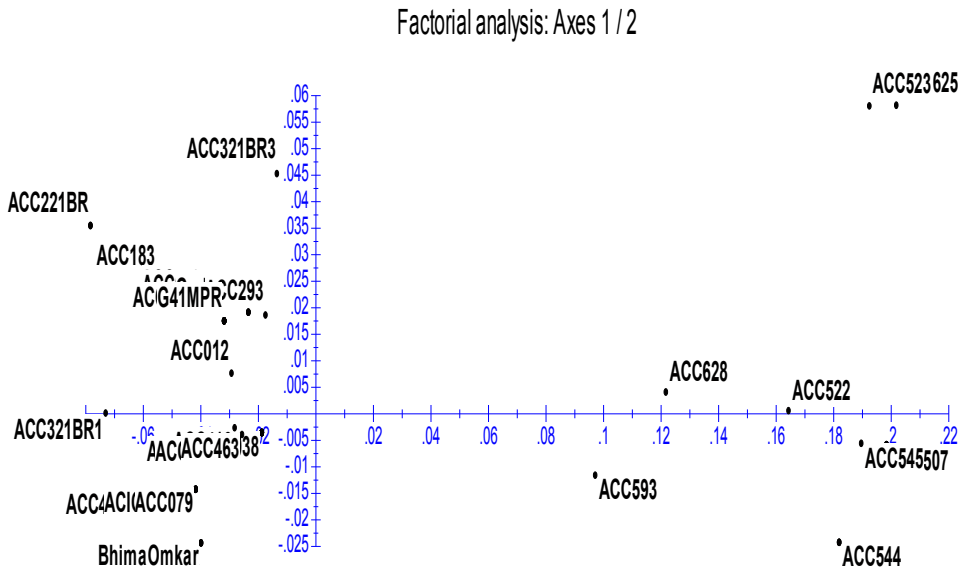


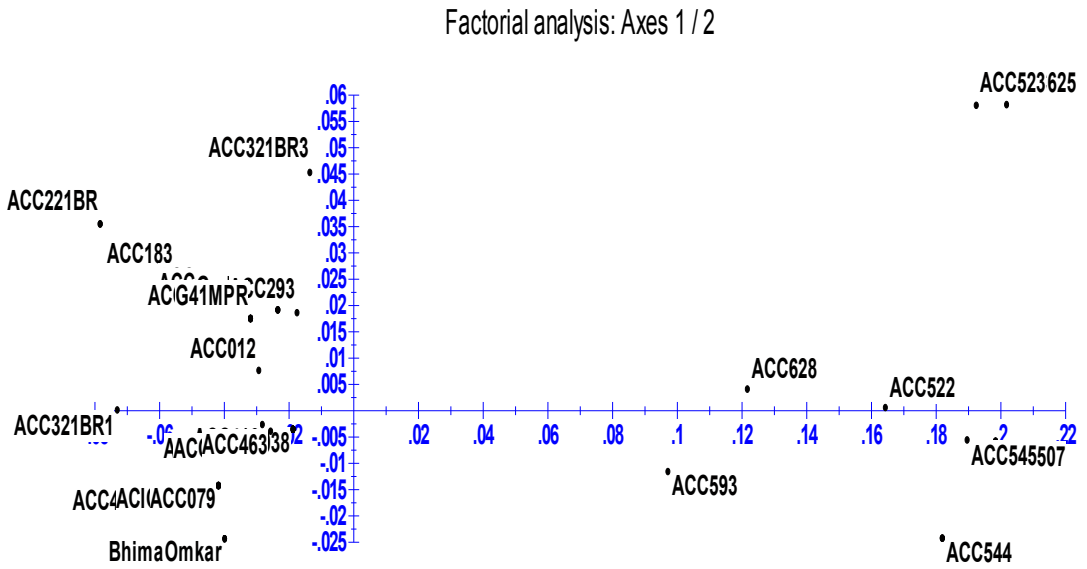
Figure 5. (A)Jaccard's Similarity Matrix Dendrogram for ISSR data (B)Jaccard's Similarity matrix Dendrogram for ISSR data for Origin

bands per primer. The average number of polymorphic bands per primer was higher in case of RAPDs (3.25) as compared to that in ISSRs (3.21). A strict consensus dendrogram based on RAPD and ISSR data as well as based on origin of the germplasm were constructed. Both the dendrograms shows similarity, Jaccard's coefficient for the consensus data matrices ranged from 0.67 to 0.33. The dendrogram was divided into two groups. The matrices of RAPD and ISSR markers were also compared using Mantel's test (Mantel, 1967) for matrix correspondence. The correlation between the matrices of cophenatic correlation values for the dendrogram based on RAPD and ISSR data was very good (0.98).

DISCUSSION

DNA content of garlic is very high and has the largest genome amongst the cultivated crops (Ipek *et al*., 2005). Despite being propagated asexually, garlic displays wide variations in morphologic and agronomic traits. The studies characterizing garlic germplasm on phenotypic characters alone added to the complexity of characterization of garlic. Some workers have shown relationship between morphological traits and molecular markers with respect to floral phenotype (Pooler *et al*., 1993), as well as bolting vs. non-bolting plants. In a recent study Fernandez *et al*. (2003) succeeded in differentiating bolting vs non-bolting and timing of senescence. In fact these workers compared the polygenetic trees constructed with 48 AFLP polymorphic bands and phylogenetic tree of same number of accessions constructed with 2 mtDNA and 24 RAPD polymorphic bands. Distinct heterogeneity in the segregation of non-bolting group B as well as clones U094-4 was exhibited. The late senescing groups showed moderate similarities in segregation of groups C, D and E when compared with groups 3 and 4. Interestingly, high correlation





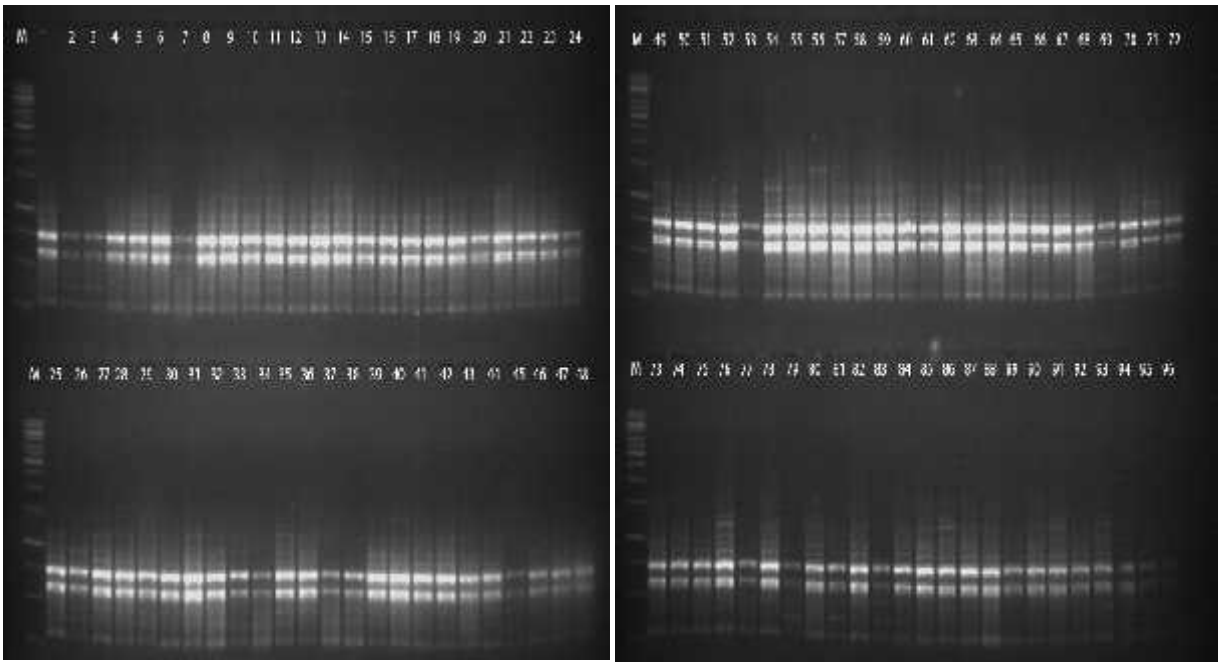


Figure 7.ISSR-841

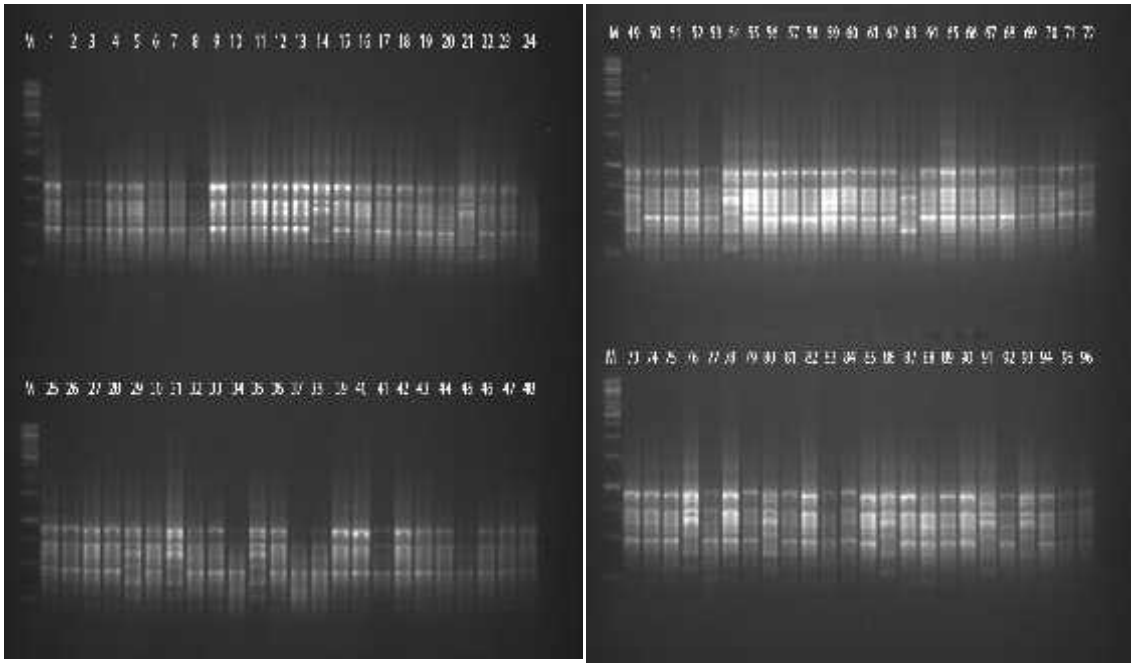


Figure 8. ISSR-808

Table 8. Comparison between RAPD and ISSR primers

Primer	Number of Primers	Total Bands	No. of Polymorphic bands	Per cent Polymorophism	PIC Content	Rp Value
RAPD	20	189	65	34.39	0.08	16.29
ISSR	14	123	45	36.58	.06	15.01

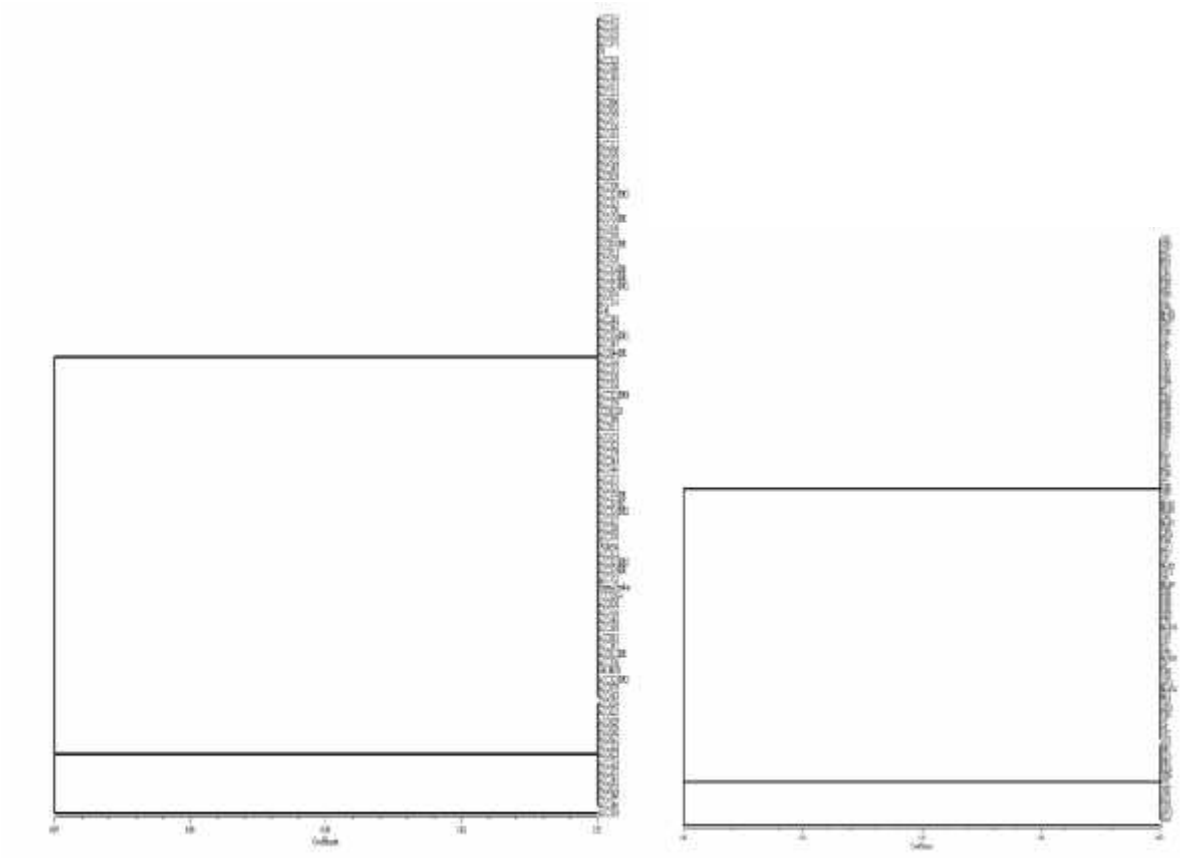


Figure9.(A)Consensus Dendrogram of ISSR and RAPD data (B)Consensus Dendrogram of RAPD and ISSR data with Origin

was made out between group A and group1, early senescing groups. Specific factors may have helped in the heterogeneity as observed between the two trees in their studies. It is of special mention that these authors used very restricted number of primers i.e. five only and consequently number of bands was restricted, inconsistent and some of the bands were faint and difficult to distinguish. Panes *et al*. (2004) described genetic diversity of Philippine *Allium sativum* L. (Alliaceae) Using Random Amplified Polymorphic DNA (RAPD) analysis. RAPD yielded a total of 69 bands from eight RAPD primers, ranging from 6 to 15 bands, corresponding to a mean of 8.6 bands per primer. The total polymorphic loci for all the sixteen *A. sativum* accessions are 78.3% which is fairly high. The genetic variation ranged from 0.1107 to 0.4983 with a mean of 0.2532. These values are also fairly high. Dendrograms constructed using the NTSYS-pc program produced 6 clusters. Similarity coefficients are between 24%-75% only, which means that *A. sativum* from the ten sampling sites is genetically diverse. *Allium ascalonicum* which is an out-group and diverged from all the *A. sativum* accessions in the dendrogram had confirmed that it is indeed an out-group (Panes *et al.,* 2004). This study has proven that RAPD is a useful DNA marker technology in determining genetic diversity among the various accessions of *A. sativum* collected from 10 sampling sites in the Philippines. In our studies the bands were consistent, clear and well marked. We used as many as 160 decamer primers.

Interestingly, Fernandez *et al*. (2003) demonstrated very high resolution of the AFLP dendrogram since they succeeded in scoring large number of bands, these authors scored as many as 183 bands. It may mentioned that in the present investigation for RAPD analysis, a total of 160 decamer primers (OPA1-20, OPB1-20, OPC1-20, OPD1-20, OPE1-20, OPG1-20, OPM1-20 and OPJ1-20) were screened out of which forty primers gave good amplification. Upon further selection a set of twenty primers were used based on the intensity of their amplification in all the accessions. A total of 189 RAPD bands were generated, with an average of 9.45 bands per primer and the number of bands for each primer ranged from six to thirteen. Sixty five bands were polymorphic showing 34.4 % of polymorphism and the size of amplified fragment ranged from 200 bp to 3 Kb. Analysis of RAPD data using Nei's (1972) original measure of genetic identity and genetic distance showed that the genetic similarity (GS) value ranged from 1.0 to 0.74 with a mean value of 0.87. Khar *et al.* (2008) obtained genetic similarity from 0.97 to 0.47 with a mean value of 0.72 in twenty three breeding lines of garlic cultivars based on RAPD data whereas Etoh *et al.* (2001) reported the genetic similarity (GS) within the garlic clones from the center of origin and the westernmost area of distribution around having 0.98 which is accordance with the present results of investigation.. Fernandez *et al*. (2003) failed to demonstrate geographical relations in the two trees. In another study Lallemand *et al*. (1997) correlated isozyme patterning to their point of origin. In a study conducted by Pooler *et al*. (1993) they failed to report such correlations. In our studies such attempts to correlate with the geographical relations were not successful. One reason could be pitiable documentation of distribution. We believe that such type of research may not yield or serve any useful purpose. One may hasten to add that such like correlations in another species have resulted in interesting data.

Garlic is an extraordinary crop being totally propagated asexually over several decades; it displays diverse phenotype amongst various clones and tempts one to undertake its heritage and diversity. Initially this was one of the prime objectives of the present study. The number of morpho types used for various studies varied with different authors. Ma *et al*. (2009) screened as many as 75 accessions and a total of 16 SSR loci. Singh *et al.* (2011) screened 41 accessions maintained at Central Inst of Temperate Horticulture, Srinagar. Buso *et al.* (2008) studied genetic diversity in 17 most planted garlic cultivars. Jabbes *et al.* (2011) assessed genetic diversity in 31 Tunisian garlic accessions with 4 French classified clones using as control.

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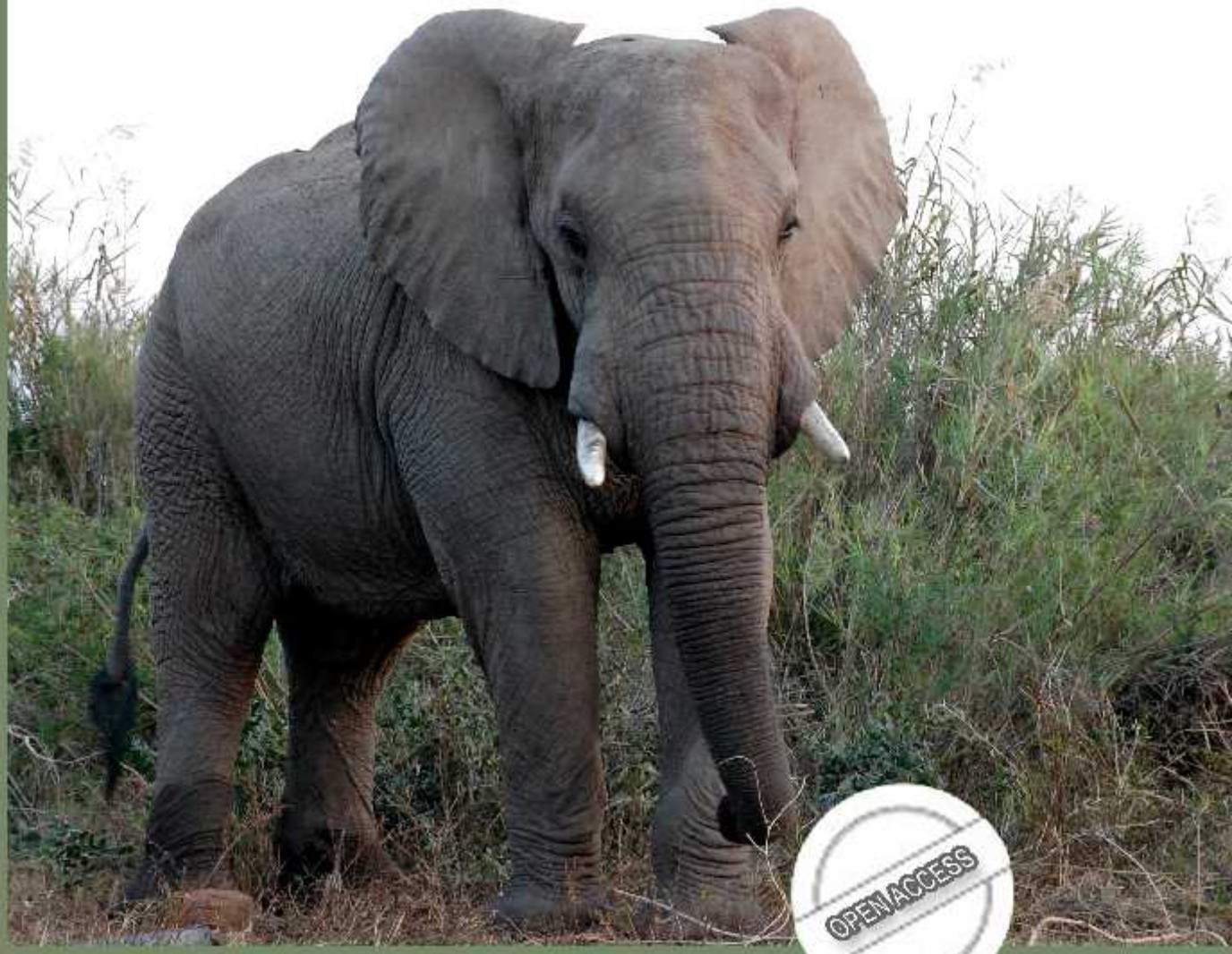

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