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Restriction fragment length polymorphism and random amplified polymorphic DNA analysis of chickpea accessions

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Abstract

Genetic diversity analysis was carried out in chickpea accessions using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) techniques. RFLP analysis using 26 *Pst* I sub-genomic clones on ten chickpea accessions in 130 probe-enzyme combinations detected polymorphism with only two clones. *Pst* I clones, CG 141 detected polymorphism in ICC 4918 and Pusa 209 while CG 500 detected polymorphism in Pusa 261, ILC 26 and in ILC 13326. These clones detected very few polymorphic markers. Analysis using 10 *Eco* RI clones on twelve chickpea accessions have shown better hybridisation signal and one clone detected polymorphism in Pusa 256. RFLP analysis of both cultivated and wild *Cicer* species using heterologous DNA probe *Cab3C* revealed polymorphism only in wild *Cicer* species (*Cicer reticulatum* L., JM 2100). RAPD analysis of 13 chickpea accessions which includes mutants of C 235 and E100Y showed greater degree of polymorphism with 1 - 5 unique DNA bands for all the accessions. Phylogenetic analysis of the RAPD data helped to group the accessions. C 235 and its mutants were found to be closely grouped while E100Y and its mutant E100Ym grouped apart. *Desi* and *kabuli* chickpea accessions however, could not be separately grouped.

Additional key words: *Cicer arietinum*, *Cicer reticulatum*, DNA polymorphism, genetic diversity, phylogeny.

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Abbreviations: RAPD - random amplified polymorphic DNA; RFLP - restriction fragment length polymorphism.

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Introduction

Cultivated chickpea has been subdivided into *macrosperma* and *microsperma* types based on 23 characters (Moreno and Cubero 1979). *Macrosperma* or *kabuli* types are characterised by upright growth, big pods and white or pinkish seeds, large leaves and leaflets, white flowers, cold tolerance and they are predominantly found in the Mediterranean region, while *microsperma* or *desi* types are characterised by bushier habit, small pods and dark brown seeds, small leaves and leaflets, coloured flowers, resistance to drought and salinity and they are found throughout the geographic range of the species (Gowen *et al.* 1989). Low level of genetic variability in the cultivated accessions has resulted in only marginal success in attempts of horizontal gene transfer through hybridisation (Gowen *et al.* 1989). Cultivated chickpea has shown low genetic polymorphism based on morphological, seed protein profile and isozyme studies (Muehlbauer and Singh 1987, Foolad *et al.* 1993, Gaur and Slinkard 1990a, Ahmed *et al.* 1992, Kazan *et al.* 1993, Gaur and Slinkard 1990b, Gaur and Slinkard 1991). Search for economically important genes in the wild relatives revealed that a few wild species are a treasure house of resistance genes to a variety of biotic and abiotic stress factors which affect chickpea yield and production. Genetic relationships among the wild annual and few perennial species of *Cicer* was examined using isozymes (Ladizinsky and Adler 1976, Ahmed *et al.* 1992, Kazan and Muehlbauer 1991, Kazan *et al.* 1993, Tayyar and Waines 1996). In recent years, the molecular marker techniques like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and microsatellites have helped to identify useful polymorphic loci in various crop species (Helentjaris *et al.* 1985, Tanksley *et al.* 1989, Miller and Tanksley 1990, Winter and Kahl 1995, Sharma *et al.* 1995). RFLP analysis in chickpea has detected very low polymorphism and confirmed its narrow genetic base (van Rheenen 1992, Udupa *et al.* 1993). RAPD analysis have proved useful in cases where RFLP analysis failed and RAPD technique detected sufficient number of polymorphic DNA markers (Winter and Kahl 1995).

In this paper we report the effectiveness of the RFLP and RAPD analysis for identifying molecular diversity in chickpea cultivars and deciphering the phylogenetic relationships.

Materials and methods

Plants: Seeds of the chickpea accessions and wild species used for analysis were obtained from various locations (Table 1). Plants were grown in pots in a glasshouse. The cultivated accessions and wild species were chosen on the basis of their importance in breeding, morphological diversity and the genetic traits they represent.

DNA extraction: Plant DNA was extracted from the young leaves (5 g) using the modified CTAB method (Doyle and Doyle 1987).

Table 1. List of chickpea cultivars used for molecular analysis (IARI - Indian Agricultural Research Institute, HAU - Haryana Agricultural University, ICRISAT - International Crops Research Institute for Semi-Arid and Tropics, ICARDA - International Centre for Agricultural Research in Dry Areas, D - *desi*, K - *kabuli*, W - wild).

S. No.	Species	Accession	Description	Origin[a]
1.	*Cicer arietinum*	Pusa 209	D, susceptible to *Ascochyta* blight	IARI, India
2.	*C. arietinum*	Pusa 256	D, high yield	IARI, India
3.	*C. arietinum*	Pusa 261	D, resistant to *Ascochyta* blight	IARI, India
4.	*C. arietinum*	E100Y	D, resistant to *Ascochyta* blight	IARI, India
5.	*C. arietinum*	E100Ym	D, drought resistant, poor yield	HAU, Hissar, India
6.	*C. arietinum*	ICC 4918	D, high yield	ICRISAT, India
7.	*C. arietinum*	GL 769	D, high yield	ICRISAT, India
8.	*C. arietinum*	C 235	D, high yield	IARI, India
9.	*C. arietinum*	161-3-C	D, mutant of C 235, big size pods, bold seeded	IARI, India
10.	*C. arietinum*	88-9-B	D, mutant of C 235, tall plants, weak stem	IARI, India
11.	*C. arietinum*	38-8-B	D, mutant of C235,dwarf, anthocyanins in leaves	IARI, India
12.	*C. arietinum*	175-2	D, mutant of C 235, dwarf, spreading habit, round seed shape	IARI, India
13.	*C. arietinum*	ILC 26	K, leaf miner tolerant	ICARDA, Syria
14.	*C. arietinum*	ILC 2506	K, resistant to *Ascochyta* blight, cold tolerant	ICARDA, Syria
15.	*C. arietinum*	ILC 3748	K, high yield, cold tolerant	ICARDA, Syria
16.	*C. arietinum*	ILC 6158	K, high yield	ICARDA, Syria
17.	*C. arietinum*	ILC 11001	K	ICARDA, Syria
18.	*C. arietinum*	ILC 12636	K	ICARDA, Syria
19.	*C. arietinum*	ILC 12921	K	ICARDA, Syria
20.	*C. arietinum*	ILC 12924	K	ICARDA, Syria
21.	*C. arietinum*	ILC 13326	K	ICARDA, Syria
22.	*C. arietinum*	P 9847	K, tall plants, low yield	Russia
23.	*C. reticulatum*	JM 2100	W, drought resistant	ICRISAT, India
24.	*C. reticulatum*	JM 2105	W, drought resistant	ICRISAT, India
25.	*C. reticulatum*	JM 2106	W, drought resistant	ICRISAT, India
26.	*C. echinospermum*	ICCW 44	W, drought resistant, tolerant to *Botrytis* grey mold	ICRISAT, India

***Eco* RI sub-genomic library construction:** Total plant DNA isolated from the etiolated seedlings of chickpea accession ICC 4918 was digested with restriction enzyme *Eco* RI and size fractionated in 0.8 % low melting agarose gel. DNA fragments of 2 kb to 3 kb size were eluted from the gel and ligated to plasmid vector pUC18. *Escherichia coli* (DH5α) bacterial cells were then transformed with the ligated plasmid DNA. Colonies of cells containing the plasmid with chickpea DNA insert were selected based on X-gal/IPTG screening procedure (Davis *et al.* 1986). Individual colonies were isolated and plasmid alkaline mini pre-prepared (Birnboim and Doly 1979) and

random genomic clones were denoted with consecutive numbers (CGE #) as they were isolated.

DNA probes: Twenty six genomic clones isolated from the *Pst* I sub-genomic library (CG clones) (Udupa *et al.* 1993); ten genomic clones isolated from the *Eco* RI sub-genomic library (CGE clones) and one heterologous nuclear DNA probe, *Cab*3C, from tomato (gift from E. Pichersky, Univ. of Michigan, USA) were used as probes in RFLP analysis.

Restriction digest, electrophoresis and Southern analysis: Five microgram of DNA from each individual accession was digested with restriction enzymes *Eco* RV, *Xba* I, *Hind* III, *Msp* I, *Hpa* II, *Hinf* I, *Dra* I and *Pvu* II (*Stratagene*, La Jolla, USA and *Amersham*, Buckinghamshire, UK) at conditions specified for each enzyme. Electrophoresis of the digested plant DNA, Southern analysis were generally similar to the methods outlined by Udupa *et al.* (1993).

Primers and RAPD analysis: Random decamer primers (*OPERON Tech. Inc.*, Alameda, USA) were dissolved in sterile distilled water at a concentration of 0.015 mg cm^{-3}. Forty primers belonging to *OPERON* kits - OPA (A-4, 7, 8, 9, 11, 13, 14), OPB (B-1, 4, 5, 19, 20), OPC (C-4, 19), OPD (D-1, 2, 3, 5, 7, 8, 11, 13, 15), OPF (F-1, 3, 4, 6, 7, 9, 11, 16) and OPI (I-1, 3, 4, 5, 6, 7, 13, 16, 20) were used for RAPD analysis. PCR amplification was performed according to Williams *et al.* (1990) with slight modification. Amplification reaction (in reaction volume of 0.025 cm^3) were performed in 0.2 cm^3 thin walled PCR tubes (*Micro-Amp* reaction tubes, *Perkin Elmer*, Norwalk, USA) containing 1 X reaction buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01 % gelatin (m/v)], 120 µM of each dNTP from a 10 mM stock (*Perkin Elmer*, Norwalk, USA), 15 ng of primer, 25 ng of the template DNA and 2 units of *Taq* DNA polymerase (*Stratagene*, La Jolla, USA). Amplification was carried out in a thermalcycler (*GeneAmp PCR System 9600*, *Perkin Elmer*, Norwalk, USA) for a first denaturation step of 2 min at 94 °C followed by 38 cycles of 94 °C for 1 min, 40 °C for 1 min and 72 °C for 2 min using the fastest available temperature transitions. The 38th cycle was followed by the last cycle of 94 °C for 1 min, 40 °C for 1 min and 72 °C for 5 min to ensure a complete primer extension. The PCR products were electrophoresed in a 1.2 % agarose gel in 1X TAE buffer (Tris-acetate 0.04 M, EDTA 0.001 M, pH 7.8) at 40 V for 5 h, stained with ethidium bromide and visualised by illumination against UV light and recorded on a *Polaroid* type 667 film (*Polaroid*, Cambridge, USA).

Data analysis: Each fragment of DNA produced during PCR amplification across all the accessions was assigned a number (1,2,3...) in order of decreasing base pair size. Each fragment was treated as an unit character, and scored as 1 (presence) or 0 (absence) for each sample. Data was analysed by both phenetic (cluster and principal co-ordinate analysis) and cladistic (neighbour-joining method) approaches. Phenogram based on similarities was constructed via the UPGMA using NTSYS-pc

1.60 microcomputer program (Rohlf 1993). Dendrograms based on neighbour-joining (NBJ) method were constructed (Saitou and Nei 1987).

Results and discussion

RFLP analysis: Isolated DNA from ten chickpea cultivars (Pusa 209, Pusa 261, E100Y, E100Ym, ICC 4918, GL-769, ILC 26, ILC 11001, ILC 12921 and ILC 13326) were digested with restriction enzymes *Eco* RV, *Hind* III, *Xba* I, *Pvu* II and *Hinf* I and hybridised with twenty six low to medium copy CG clones. Only two of the CG clones were able to detect polymorphism (Table 2). CG 141 showed polymorphism between accessions ICC 4918 (3.2 kb fragment) and Pusa 209 (4.3 kb and 3.2 kb fragment) in their *Xba* I digest while CG 500 showed polymorphism between Pusa 261 (1.8 kb fragment), ILC 26 (4.1 kb and 3.18 kb fragment) and in ILC 13326 (4.1 kb fragment) (Fig 1*A*). The remaining twenty four CG clones either showed weak hybridisation signal or anomalous hybridisation (hybridising to only a few of the accessions). Modifications tried to ensure good hybridisation by increasing the DNA concentration to 10 µg and washing the blots at a lower stringency have failed to improve the results. Earlier studies using *Pst* I library derived low copy genomic clones have revealed very little polymorphism in chickpea accessions (Udupa *et al.* 1993).

Table 2. Details of polymorphic band pattern obtained with different probe-enzyme combinations used.

S. No.	Probe	Restriction enzyme	Number of hybridising bands	Size of hybridising bands [kb]	Polymorphic bands [kb] in accessions
1.	CG 141	*Xba* I	2	3.2, 4.3	3.2, 4.3 in Pusa 209
2.	CG 141	*Eco* RV	5	3.23, 3.48, 4.3, 6.5, 9.4	4.3 in JM 2105, JM 2106; 6.5, 9.4 in JM 2106, ICC 4918
3.	CG 141	*Hind* III	2	3.48, 2.33	2.33 in JM 2106; 3.48 in JM 2105
4.	CG 500	*Pvu* II	6	1.8, 2.8, 3.18, 4.1, 5, 8.5	1.8 in Pusa 261; 4.1, 3.18 in ILC 26; 4.1 in ILC 13326
5.	Cab 3 C	*Xba* I	3	2.68, 4.8,5.1	4.8 in JM 2100
6.	CGE 12	*Msp* I	4	0.5, 1, 1.2, 1.3	1.2 in Pusa 256
7.	CGE 12	*Hpa* II	4	0.5, 1, 1.2, 1.3	1.2 in Pusa 256

Studies were also carried out on five chickpea accessions (ICC 4918, ILC 26, Pusa 256, ILC 11001 and ILC 2506) DNA digested with restriction enzymes *Eco* RV, *Xba* I, *Msp* I and *Hpa* II and hybridised with five CGE clones. A second set of ten chickpea accessions (Pusa 261, Pusa 209, E100Y, E100Ym, ICC 4918, GL-769, ILC 26, ILC 11001, ILC 12921 and ILC 13326) DNA samples were restriction digested with *Eco* RV, *Xba* I, *Dra* I, *Pvu* II and *Hinf* I and hybridised with another

five CGE clones. All CGE clones have shown better hybridisation compared to CG clones. However only CGE 12 detected polymorphism among the *Msp* I and *Hpa* II digest of Pusa 256 DNA (1.2 kb fragment) (Fig. 1*B*, Table 2). The remaining CGE clones tested have revealed a monomorphic pattern. Ease of hybridisation using CGE

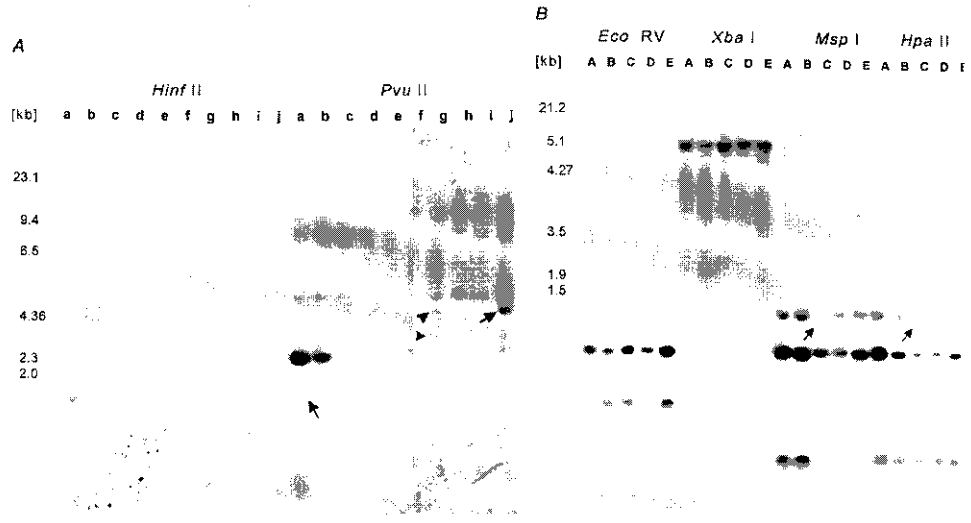

Fig. 1*A*. RFLP pattern after hybridisation of *Hinf* I and *Pvu* II digested chickpea DNA probed with CG 500. *Lanes a-j*: Pusa 261, Pusa 209, E 100Y, E 100Ym, ICC 4918, GL 769, ILC 26, ILC 11001, ILC 12921, ILC 13326. *Arrows* indicate polymorphic bands in Pusa 261, ILC 26 and ILC 13326. Molecular mass marker used: λ *Hind* III digest.

Fig. 1*B*. RFLP pattern after hybridisation of *Eco R*V, *Xba* I, *Msp* I and *Hpa* II digested chickpea DNA probed with CGE 12. *Lanes A-E*: ICC 4918, ILC 26, Pusa 256, ILC 11001, ILC 2506. *Arrows* indicate polymorphic band in *Msp* I and *Hpa* II digest of Pusa 256 DNA. Molecular mass marker used: λ *Eco* RI and *Hind* III digest.

clones compared to CG clones may help to reveal more variations if tested in larger numbers. Analysis of polymorphism using *Cab*3C probe by hybridising to nuclear DNA was carried out on five cultivated accessions (ICC 4918, Pusa 261, ILC 6158, E100Y and E100Ym), three accessions of *C. reticulatum* (JM 2100, JM 2105 and JM 2106) and one accession of *C. echinospermum* (ICCW 44). JM 2100 showed polymorphism for only one fragment (4.8 kb) with the *Xba* I digest (Table 2). Studies carried out by Udupa *et al.* (1993) have also shown low variability of organellar genome in chickpea.

RFLP analysis in chickpea is hampered by the lack of sufficient variability being detected at the molecular level (Van Rheenen 1992, Udupa *et al.* 1993). A similar study in *Phaseolus* had also revealed lack of DNA variation initially when analysed with clones from the *Eco* RI-*Bam* HI sub-genomic library. Later studies using *Pst* I low copy genomic clones revealed more polymorphism and was further confirmed following the discovery of two distinct centres of origin of cultivated beans in South America (Nodari *et al.* 1992). In chickpea too, it is worthwhile to test genomic

libraries generated with different restriction enzymes and heterologous clones from other legumes to detect higher frequency of polymorphism.

RAPD analysis: RAPD-PCR based analysis of plant genome is sensitive to changes in various experimental parameters (Devos and Gale 1992). Six variables were taken into consideration when optimising the conditions for RAPD amplification. These include, the amount of template DNA (15, 25, 50, 75, and 100 ng); concentration of dNTPs (100, 120, 150, and 200 μM); $MgCl_2$ concentration (1, 1.5, 2, and 2.5 mM); amount of primer (15 and 30 ng); amount of enzyme per reaction (1, 1.5, 2, and 2.5 U) and the annealing temperature of the PCR reaction (36 and 40 °C). Low concentration of template DNA resulted in weak amplification of DNA while a smear was observed at 100 ng of template DNA; at very low and very high dNTP concentration the amplification was inhibited at both annealing temperatures tested; $MgCl_2$ concentration is critical for the activity of the *Taq* DNA polymerase and a consistent amplification was obtained at 1.5 mM $MgCl_2$ concentration; 2 U of the enzyme per reaction helped to amplify maximum number of reproducible DNA bands; at higher amount (30 ng) of primer many DNA bands were not amplified while a few of them were strongly amplified when compared to amplification obtained with lower (15 ng) amount of primer. After analysing all these variables, the 25 ng of template DNA, 1.5 mM of $MgCl_2$, 120 μM of dNTP, 15 ng of primer, 2 U of *Taq* DNA polymerase and an annealing temperature of 40 °C were chosen and found optimal for the purpose of producing a reproducible and clear DNA profile among the samples analysed.

Chickpea accessions identified by RAPD markers: A total of 235 DNA bands were amplified across all the accessions with 38 primers revealing an average of 5.78 - 5.94 bands per primer in each accession. Thirteen primers were found to reveal polymorphic variations among 13 accessions (Fig. 2*A,B*) while two primers (OPB19 and OPI 5) failed to produce any amplified products. The polymorphic DNA bands ranged from two (GL-769 and 161-3-C) to a maximum of twelve (E100Ym). A total of 87 polymorphic DNA bands were revealed among all these accessions. No single primer was found that can differentiate all the chickpea accessions analysed. However, unique DNA bands were detected and they varied from one (GL-769, 88-9-B and 175-2) to a maximum of seven (E100Ym). A total of 40 unique amplified DNA bands were found among all the accessions across all the primers analysed (Table 3). The total number of amplified DNA bands with all the primers in each individual accession varied from 220 to 228. There was no difference in the number of amplified bands or the polymorphic bands revealed between the *desi* and the *kabuli* chickpea accessions, however, the mutants of the *desi* accessions analysed produced comparatively more polymorphic DNA bands than their parents (Fig. 3). In case of accession E100Ym which is a spontaneous mutant selected from E100Y, the number of amplified DNA bands were less at 220 compared to 228 amplified bands in E100Y across all the primers. However, E100Ym revealed 12 polymorphic bands with seven unique DNA bands among the 13 polymorphic primers compared to only 6 polymorphic bands in E100Y. In case of accession C235 and its mutants, an

Table 3. RAPD analysis of DNA band polymorphism in different chickpea accessions.

	ICC 4918	GL 769	P 9847	ILC 26	ILC 2506	ILC 3748	E 100Y	E 100Ym	C 235	88-9-B	161-3-C	38-8-B	175-2
Number of bands with all primers	223	226	220	226	227	228	228	220	225	226	225	227	225
Number of polymorphic bands	8	2	6	5	7	8	6	12	8	2	10	10	3
Number of unique bands	2	1	3	2	2	5	5	7	3	1	4	4	1
Average number of bands	5.86	5.94	5.78	5.94	5.97	6.00	6.00	5.78	5.90	5.94	5.90	5.92	5.90

Table 4. Similarity matching coefficients for assessing genetic similarity among the 13 chickpea accessions analysed.

ICC 4918	GL 769	P 9847	E 100Y	ILC 26	ILC2506	ILC 3748	E100Ym	C 235	161-3-C	88-9-B	38-8-B	175-2
1												
0.857	1											
0.693	0.755	1										
0.897	0.877	0.714	1									
0.857	0.877	0.673	0.877	1								
0.755	0.816	0.734	0.775	0.775	1							
0.693	0.795	0.673	0.755	0.755	0.897	1						
0.530	0.510	0.551	0.510	0.551	0.530	0.510	1					
0.673	0.816	0.612	0.693	0.734	0.714	0.693	0.571	1				
0.714	0.775	0.612	0.734	0.816	0.714	0.693	0.571	0.755	1			
0.734	0.877	0.673	0.795	0.836	0.816	0.836	0.469	0.775	0.755	1		
0.693	0.795	0.591	0.755	0.755	0.775	0.714	0.510	0.775	0.693	0.755	1	
0.714	0.857	0.653	0.775	0.816	0.795	0.775	0.448	0.755	0.755	0.938	0.734	1

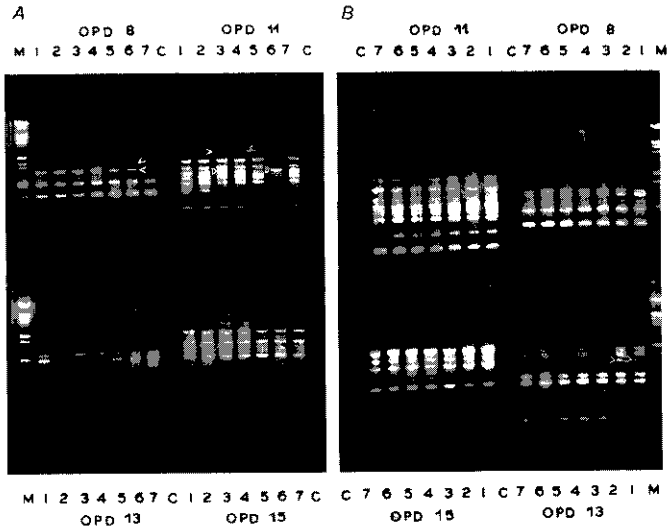

Fig. 2*A*. RAPD profile obtained by amplification of chickpea accessions DNA using primers OPD 8, OPD 11, OPD 13 and OPD 15. *Lanes 1 - 7*: ICC 4918, GL 769, P 9847, E 100Y, ILC 26, ILC 2506, ILC 3748. *Lane C*: Amplification without DNA. *Lane M*: λ *Eco* RI and *Hind* III digest. *Arrows* indicate polymorphic DNA bands.
Fig. 2*B*. RAPD profile obtained by amplification of chickpea accessions DNA using primers OPD 8, OPD 11, OPD 13 and OPD 15. *Lanes 1 - 7*: E 100Y, E 100Ym, C 235, 88-9-B, 161-3-C, 38-8-B, 175-2. *Lane C*: Amplification without DNA. *Lane M*: λ *Eco* RI and *Hind* III digest. *Arrows* indicate polymorphic DNA bands.

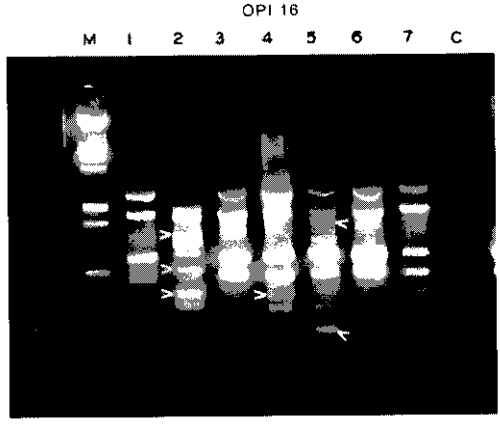

Fig. 3. RAPD profile obtained by amplification of chickpea accessions DNA using primer OPI 16. *Lanes 1 - 7*: E 100Y, E 100Ym, C 235, 88-9-B, 161-3-C, 38-8-B, 175-2. *Lane C*: Amplification without DNA. *Lane M*: λ *Eco* RI and *Hind* III digest. *Arrows* indicate polymorphic DNA bands.

average of 225 bands were amplified with very little difference between the parents and the mutants when number of amplified bands were compared. Two of the mutants, 161-3-C and 38-8-B produced maximum of 10 polymorphic DNA bands compared to 8 in C235 while the other two mutants, 88-9-B and 175-2 produced 2 or 3 polymorphic bands.

Phylogenetic analysis of the RAPD data: Similarity matching coefficient for assaying the genetic similarity according to Nei and Li (1979) revealed estimates of similarity between the 13 chickpea accessions analysed (Table 4). The most conspicuous was the accession E100Y and its mutant E100Ym which were found only 51 % similar and the similarity varied from 53 - 55 % when compared with other accessions. C235 and its mutants, however, were found almost similar to their parent with 75 - 77 % similarity index. Comparison of the *kabuli* (P 9847, ILC 26, ILC 2506 and ILC 3748) and *desi* (ICC 4918, GL-769, E100Y, E100Ym, C235, 161-3-C, 88-9-B, 38-8-B and 175-2) accessions revealed that ILC 26 is closer to the *desi* accessions with 73 - 81.6 % similarity while other *kabuli* accessions were more similar to each other than to any of the *desi* accessions of chickpea. Among the mutants of C235, the accession 161-3-C and 175-2 showed 75.5 % similarity with C235 compared to 77.5 % similarity shown by 88-9-B and 175-2. Accession 38-8-B showed little similarity to 161-3-C compared to other mutants and to *kabuli* accession P9847. This is not unexpected since both 161-3-C and P 9847 are tall plants, have big pods and large leaf size compared to bushy habit, small leaf size and copious anthocyanin pigment in accession 38-8-B. Phylogenetic trees were constructed based on similarities using UPGMA (*NTSYS-PC 1.60*) analysis and neighbour-joining method (Saitou and Nei 1987). The trees obtained on the basis of simple matching coefficients showed E100Ym being grouped separately from other accessions. There were two major groups with P9847 occupying one of them while other accessions being accommodated in a separate group with many sub-groups. The trees

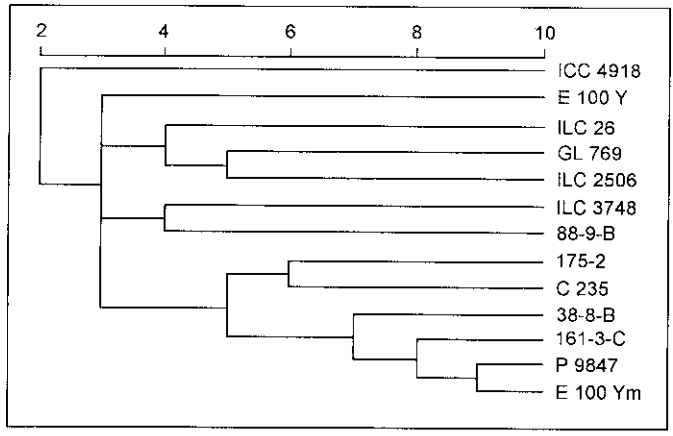

Fig. 4. Phylogenetic tree obtained for 13 chickpea accessions analysed based on UPGMA method using *NTSYS-PC*. The dendrogram was obtained as described in text.

constructed based on UPGMA analysis using *NTSYS-PC* software with ICC 4918 as the outgroup produced three major groups with six sub-groups (Fig. 4). This grouping of the accessions was further confirmed by Principal Co-ordinate (PCO) analysis of the RAPD data. Using this analysis E100Ym was again found separately grouped from E100Y as well as other chickpea accessions. C235 was found a small distance away from its mutants 38-8-B and 161-3-C compared to 88-9-B and 175-2. The other accessions GL-769, ILC 26, ILC 2506 and ILC 3748 grouped together (data not shown). In PCO analysis the *kabuli* and the *desi* accessions of chickpea were not separated into distinct groups and an intermixing of both the types was observed.

The above analysis revealed that RAPD is an efficient technique to fingerprint the chickpea accessions and to reveal similarity or differences among the accessions. The amplification profile of accession C235 and its mutants and their phylogenetic grouping shows the efficiency of RAPD technique. In the absence of a highly polymorphic RFLP probe which can detect sufficient molecular diversity in chickpea, the PCR based analysis using random primers or microsatellites, can help to fingerprint the accessions that has important applications in chickpea breeding. A disadvantage of the RAPD approach lies in the fact that the allelic status of the fingerprint band is usually unknown, and its occurrence has to be treated as a dominant character. Inspite of these difficulties, RAPD markers show great promise for characterising chickpea accessions and examining taxonomical relationships within them.

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