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Diversity Analysis of Ethiopian Mustard Breeding Lines Using RAPD Markers

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Abstract

*Ethiopian mustard (***Brassica carinata A. Braun***) is an oilseed crop less known to the other parts of the world. Utilization of the available germplasm of B. carinata for different breeding purposes requires information on genetic diversity. Random amplified polymorphic DNA (RAPD) was employed to characterize the genetic diversity of 22* **B. carinata** *inbred lines derived from accessions collected from eight different geographic areas in Ethiopia and one from Sweden. Forty-three primers were used for amplification. The resulting RAPD pattern was analysed with respect to size and distribution of fragments, reproducibility, genetic diversity and informativeness of the marker for genotype specific amplification. In total, 371 bands were amplified of which 239 (65%) were polymorphic. Band size ranged from 300 to 4000 kb. The number of bands generated by each primer varied from 3 to 15 with an average of 8.6, while number of polymorphic bands varied from 1 to 12 with an average of 5.6. RAPD patterns were reliably reproducible between replicates. Genetic similarity (GS) calculated from the marker data using Jaccard`s similarity coefficient (JCS) ranged from 0.34 to 0.84. Using cluster analysis based on unweighted pair-group method with arithmetic average (UPGMA) and principal coordinate analysis (PCoA), the 21 Ethiopian inbred lines were grouped into three subgroups and the single genotype introduced from Sweden formed a separate group. The clustering pattern failed to show a clear correspondence between geographic and molecular diversities within the Ethiopian gene pool. Generally, RAPD differentiation was higher for the exotic genotype, thus formation of a gene pool distinct from the Ethiopian gene pool could be possible through introduction. Based on the genetic relatedness, selective parental combinations were earmarked as potential parents for the future breeding work. The RAPD assay generated genotype-specific products in 14 of the genotypes studied which could be used as DNA fingerprint for variety identification.*

Introduction

Detecting genetic variation has been important in breeding programmes and is always a major concern for plant breeders. Determining the genetic variation among and within germplasm accessions provide information that is important for choosing accessions to be used in breeding programmes (Williams and St. Clair 1993). Crosses between genetically divergent parents are expected to: (i) result in large genetic variance among progenies in subsequent selfing generations that can be exploited as line cultivars, and (ii) yield higher amounts of heterosis that could be exploited

through breeding of hybrids or synthetic varieties. Populations from separated areas are expected to accumulate higher genetic diversity between them (Chandel and Joshi 1983). Ethiopia is characterized by diverse physiographic and agroclimatic conditions that often occur within a short distance (Hawkes and Worede 1991). According to Linhart and Grant (1996) and Rao and Hodgkin (2002) natural selection acting on heritable phenotypic variation will result in adaptation and differentiation among populations of the same species inhabiting environments differing in their selective regime. Hence, in developing breeding stock of maximum diversity, the inclusion of genotypes collected from different geographic areas is a strategy to capture allelic diversities of a species.

The development of biochemical (allozymes) and DNA based markers has offered new alternatives to study genetic diversity. DNA-based markers reveal differences and relatedness between individuals and discern genomic diversity. These markers have the potential to reveal a large amount of variation with good coverage of the entire genome (Melchinger et al. 1994). Amongst such molecular tools, random amplified polymorphic DNA (RAPD) provides a simple and fast approach in detecting DNA polymorphism (Williams et al. 1990; Welsh and McClelland 1990). The RAPD assay provides multi-locus profiling of DNA sequence differences of genotypes when genetic knowledge is lacking (Rafalski and Tingey 1993). In *Brassica* coenospecies, RAPD markers are considered to be as efficient as restriction fragment length polymorphism (RFLP) markers for estimating intra-specific genetic relationships among genotypes (Phippen 1997; Thormann et al. 1994). RAPD markers have also been used for cultivar identification in *B. napus* (Ren et al. 1995) for gene tagging in *B. napus* Cheung et al. (1997) and genetic mapping in *B. rapa* (Tanhuanpää and Schulman 2002).

B. carinata is among the least tailored oilseed *Brassica* crop by modern plant breeding techniques as its importance in the world has hitherto been localized only to Ethiopia. Hence, it has remained as the most undeveloped with regard to quality attributes and production effeciency. In basic experimental studies, the availability of stable and repeatable experimental materials with the required variability is essential. Inbred lines are most preferred as experimental materials for two major reasons: (i) because of their uniformity, and (ii) because of the repeatability of their identity from one generation to the other. Therefore, 22 inbred lines were developed as experimental materials. Complementing the geographic and phenotypic diversity data by genetic diversity measurements deemed necessary to determine the prospect of the inbred lines as an experimental material and parents in future work. This study was, therefore, conducted with the objectives of evaluating and quantifying the genetic diversity of these 22 *B. carinata* breeding lines developed from accessions collected from eight geographic areas in Ethiopia and abroad; examining repeatability of the RAPD method to analyse genetic diversity in *B. carinata*; and; explore the coincidence of RAPD differentiation along geographic origin within the Ethiopian gene pool.

Materials and methods

Plant Material

The plant material included 21 inbred lines (S_5) developed from 36 accessions of Ethiopian origin and one from Sweden. The accessions from Ethiopia were colleted from eight different geographic areas with large variations of altitude (Table 1) that are distributed over most parts of Ethiopia. The Swedish genotype was obtained from Gatersleben Plant Genetic Resource Centre, Germany. Inbred lines 11 and 12 were developed from the same S_2. DNA extracted from inbred lines 10 and 20 was divided for analyses as replicates (inbred lines 23 and 24, respectively), to determine the repeatability of the RAPD technique.

Table 1. Inbred lines of Ethiopian mustard, their parental accession code along with area of collection and altitude included in the genetic diversity study using RAPD techniques

Inbred line code†	Parental acc. No.‡	Area of collection	Altitude (m)	Inbred line code	Parental acc. No	Area of collection	Altitude (m)
G 1	21278	Wolo	2290	G 12	21071	Bale	2640
G 2	21369	Kefa	1772	G 13	21182	Welega	2120
G 3	21245	Gonder	1860	G 14	21182	Welega	2120
G 4	21080	Arssi	2390	G 15	21253	Gojam	1740
G 5	21068	Bale	2500	G 16	21265	Wolo	1950
G 6	21069	Bale	2450	G 17	21316	Shewa	2430
G 7	21005	Arssi	2450	G 18	21276	Wolo	2290
G 8	21209	Welega	2460	G 19	21007	Arssi	2900
G 9	21224	Kefa	1750	G 20	21192	Welega	2090
G 10	208404	Gojam	1960	G 21	21289	Wolo	2570
G 11	21071	Bale	2640	G 22	43/79	Sweden	-

†G stands for genotype; ‡Accession No. refers the accession identification number of the Institute of Biodiversity Conservation, Ethiopia

Genomic-DNA Extraction

Total genomic DNA was extracted from 0.1 gram of young leaf tissue, frozen in liquid nitrogen and stored at -20 °C. The extraction was carried out using a mini DNA isolation method following Nucleon extraction kit. The DNA quality was assessed by electrophoresis on a 1% agarose gel and the concentration was determined by fluorescent spectroscopy using Hoechst 33258 calf thymus DNA as a standard (following BIORAD's catalogue number 170-2480). The DNA was diluted to 5 ng/µl of working sample with TAE buffer before use.

Polymerase Chain Reactions (PCRs)

Forty-three pre-screened (Adefris and Becker 2005, 2006) 10-mer oligonucleotides that gave clear and consistent amplification were used for PCR amplification (Table 2). The reactions were carried out in a 25 µl volume containing 2 x 1 reaction buffer [200

mM Tris-HCL, pH 8.55, 160 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 3.0 mM $MgCl_2$, 0.4 mM of dNTP, 0.16 µM primer, 1.0 unit of Taq DNA polymerase and 25 ng of genomic DNA template. The remaining volume was filled with distilled, filtered and sterilized water. To protect from evaporation during amplification, two drops of mineral oil were added in each sample tube. DNA amplifications were performed in a Perkin Elmer 480 thermocycler programmed for a preliminary step of 1 min at 94°C, 45 cycles of 30 s at 92 °C, 60 s at 35 °C and 2 min at 72 °C and a final step of 6 min at 72 °C. This was followed by a hold time at 4 °C until samples were removed from the machine. The RAPD fragments were separated by electrophoresis using 1.8% agarose gel and visualized with ethidium bromide and photographed under UV light using a computer printer. Images of DNA profiles on the gels were captured into the PC files using a Camera Module online with the computer using the program Herolab E.A.S.Y. store (Herolab GmbH Laborgeräte, Wiesloch).

Data Scoring and Analysis

The 22 inbred lines and the two duplicates were scored for presence or absence of RAPD fragments. When the band intensity was too weak to determine its presence or absence a score of 9 was given and was consider as a missing value. Molecular size (bp) of the PCR products were estimated by comparing the position of bands with MBI Fermentas # Smo311, gene ruler™ 1 Kb ladder.

Jaccard`s coefficient of similarity (JCS) among the 24 inbred lines (22 inbred line and two duplicates) was calculated in all of the pair-wise comparisons of the inbred lines using the computer software Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc version 2.10q, Rohlf 2001). Reproducibility of RAPD amplification and scoring was determined on the basis of percentage agreement of banding pattern between the replicates in the two genotypes. Similarity matrix from 22 inbred lines (excluding the duplicates) was used to construct the dendrogram based on the unweighted pair-group method with arithmetic means (UPGMA) and principal coordinate analysis (PCoA).

Table 2. Sequence, total number of band (TNB), number of polymorphic band (NPB) and range of band size of the 43 primers used to generate RAPD markers in 22 *Brassica carinata* genotypes

Primer	Sequence (5´- 3´)	No of bands		Molecular size range (in bp)	Primer	Sequence (5´- 3´)	No of bands		Molecular size range (in bp)
		TNB	NPB				TNB	NPB	
OPA-01	CAGGCCCTTC	6	5	1200-2600	OPAH-03	GGTTACTGCC	4	1	650-2500
OPA-04	AATCGGGCTG	9	7	600-3300	OPAH-05	TTGCAGGCAG	7	4	300-1500
OPA-12	TCGGCGATAG	12	10	600-2500	OPAH-06	GTAAGCCCCT	10	7	750-3100
OPB-11	GTAGACCCGT	7	3	600-2500	OPAH-09	AGAACCGAGG	13	9	400-1750
OPB-15	GGAGGGTGTT	6	2	500-2390	OPAH-10	GGGATGACCA	7	5	500-2000
OPC-02	GTGAGGCGTC	11	6	600-2390	OPAH-14	TGTGGCCGAA	3	3	1200-1500
OPC-10	TGTCTGGGTG	8	6	500-2500	OPAH-15	CTACAGCGAG	11	5	500-2600
OPC-11	AAAGCTGCGG	12	8	500-2500	OPAH-16	CAAGGTGGGT	4	2	700-2100
OPC-19	GTTGCCAGCC	9	4	600-3500	OPAH-17	CAGTGGGGAG	15	10	650-4000
OPD-20	ACCCGGTCAC	10	6	600-3400	OPAH-19	GGCAGTTCTC	8	6	750-2100
OPQ-03	GGTCACCTCA	10	6	700-4000	OPAH-20	GGAAGGTGAG	9	6	500-1900
OPQ-15	GGGTAACGTG	5	5	900-2750	OPAJ-1	ACGGGTCAGA	8	7	600-1500
OPR-09	TGAGCACGAG	12	6	750-3100	OPAJ-04	GAATGCGACC	8	6	750-3000
OPR-20	ACGGCAAGGA	6	4	750-2300	OPAJ-06	GTCGGAGTGG	8	7	600-2600
OPU-10	ACCTCGGCAC	12	4	500-3000	OPAJ-08	GTGCTCCCTC	9	7	550-3000
OPU-14	TGGGTCCCTC	6	2	750-2200	OPAJ-9	ACGGCACGCA	7	6	400-2500
OPU-17	ACCTGGGGAG	8	6	800-3100	OPAJ-11	GAACGCTGCC	8	5	650-3000
OPAG-02	CTGAGGTCCT	7	5	550-3000	OPAJ-12	CAGTTCCCGT	12	8	600-3500
OPAG-09	CCGAGGGGTT	11	7	400-2700	OPAJ-13	CAGCCGTTCC	7	3	1200-3000
OPAG-11	TTACGGTGGG	9	7	500-2500	OPAJ-15	GAATCCGGCA	10	7	600-3200
OPAG-13	GGCTTGGCGA	5	4	1000-2800	OPAJ-18	GGCTAGGTGG	7	1	600-3000
OPAG-18	GTGGGCATAC	14	12	400-3000					

Results and Discussion

RAPD Analysis

The 43 pre-screened primers varied in their ability to generate variability among the genotypes (Table 2). Across the 22 genotypes 371 RAPD loci were scored. Of these, 239 (65%) were polymorphic. The number of bands generated by the primers varied from 3 to 15 with an average of 8.6. The number of polymorphic bands (NPBs) ranged from 1 to 12 with an average of 5.6. The most effective primer was OPAH-17 amplifying 15 bands. Nevertheless, OPAG-18 generated the highest NPBs, 12 bands. The number of amplification products per primer as well as the size range conform to those records with other *Brassica* species by Lazaro and Aguinagalde (1996) and Rabbani et al. (1998). The proportion of polymorphic bands (PBs) to the total amplification products observed in this experiment (65%) was lower than the 80.0% reported in *B. campesteris* (Das et al. 1999) and 75.6% in *B. juncea* (Jain et al. 1994) but higher than the 54.0% reported on *B. oleracea* (Cansian and Echeverrigaray 2000). Variation in PBs could be attributed by species differences and/or selecting only very few primers, which gave the highest NPBs, or many primers that show any NPBs.

RAPD Reproducibility

There is a concern in the literature regarding the reproducibility of RAPD pattern (Prevost and Wilkinson 1999; Rafalski and Tingey 1993). Here too, there was not 100% similarity among RAPD bands generated from the two sample-DNA extracts of the same genotype, G10 and G20, whose DNA finger printing was determined in replicates. Out of the 239 polymorphic bands scored, the number of differently scored bands for the two samples of G10 and G20 were eight and five, respectively. On average 6.5 bands were scored differently in the two replicates of the two genotypes. The process of replication has proved that on the average the GS of two genotypes could be wrongly estimated 2.75 times out of 100 (6.5 times out of 239). Of course, the consequences of errors due to limited reproducibility of RAPD markers is less serious in completely homozygous genotypes (Hallden et al. 1996). Thus, in the same laboratory under a stringent reaction condition and careful data scoring, RAPD pattern are reliably repeatable. This is in concordance with previous reports of Link et al. (1995) in faba bean and Prevost and Wilkinson (1999) in potato. The various sources of error that affect the GS values along the various steps of the RAPD technique have been discussed by Hallden et al. (1996). Ignoring doubtful bands from scoring minimized errors that arise during band scoring.

Genetic Similarity among Genotypes

Genetic variability of indigenous (Ethiopian) *B. carinata* accessions has been previously studied by Abebe et al. (1992) and Alemayehu and Becker (2002) using morphological, yield and seed quality traits. However, such parameters of characterizing diversity are labour intensive and time consuming. Furthermore, morphological, yield and seed quality traits are normally subjected to genotype by

environment interaction (Melchinger et al. 1994; Rafalski and Tingey 1993; Welsh and McClelland 1990; Williams et al. 1990).

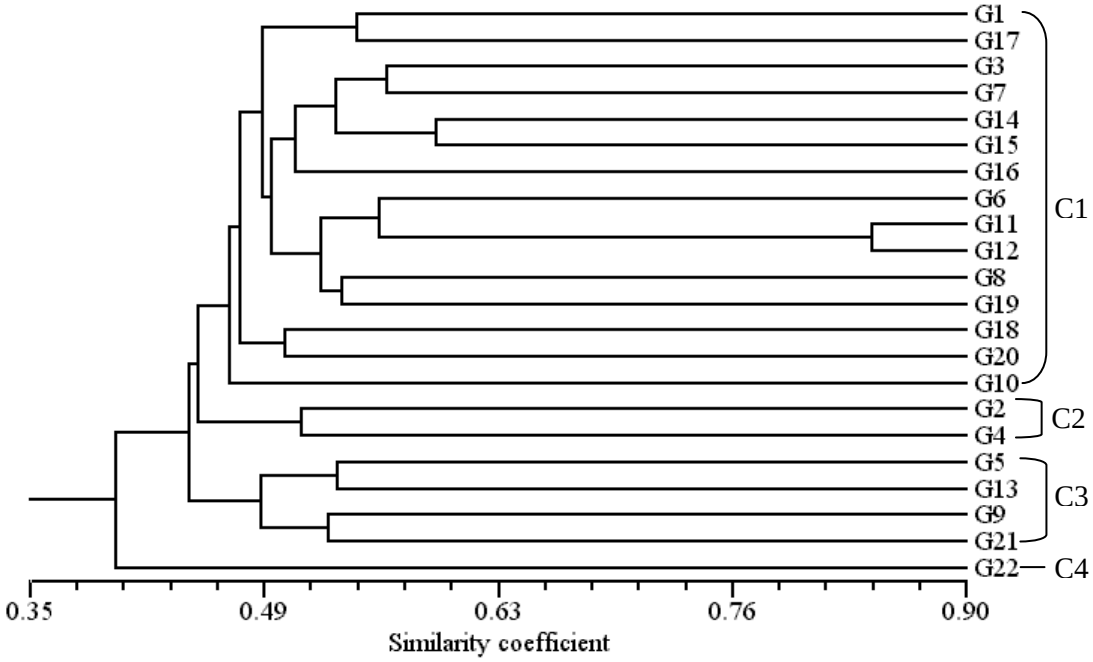
Perusal to Table 3, genetic similarity among pairs of the 22 genotypes ranged from 0.34 (G2 vs. G5 and G5 vs. G22) to 0.84 (G11 vs. G12) with an average value of 0.47. About 34% of the pair wise genetic similarity comparisons were between 0.45-0.49. Of the 231 GS values, 117 were less than the average GS value, 0.47, indicating that most of the genotypes were quite distant each other. But, disregarding the GS value obtained from G 11 and G 12, the remaining GS values varied only between 0.30 and 0.60. This could be an indication for the prevalence of a less differentiated gene pool within the Ethiopian germplasm material from which the inbred lines were developed.

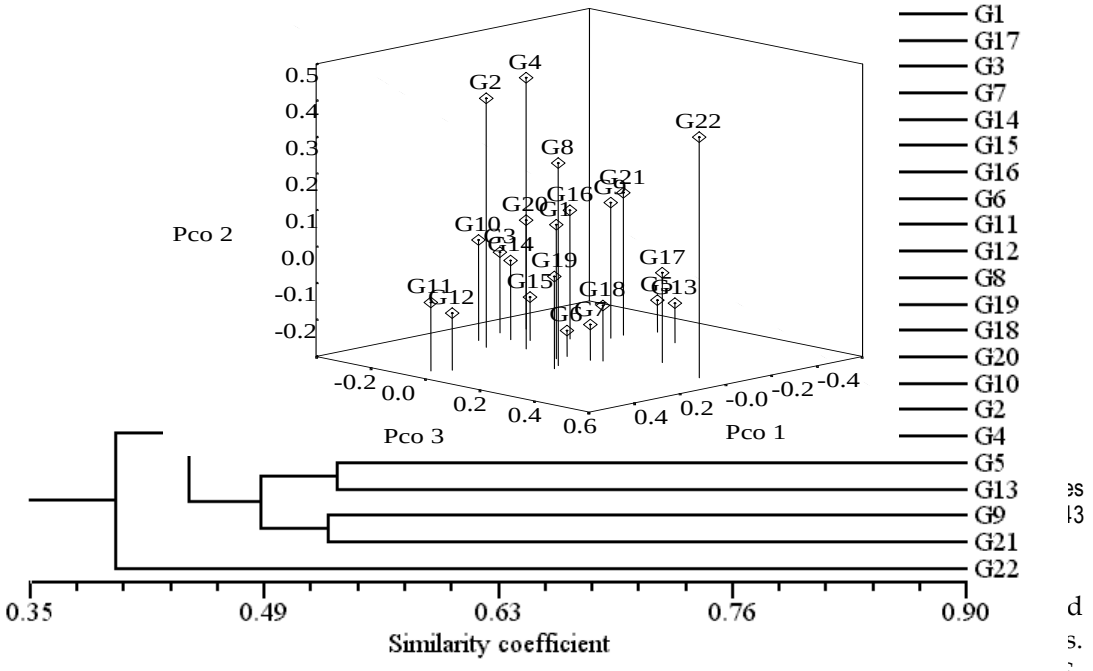
The relationship among the 22 genotypes based on the genetic similarity values were clustered and presented as a dendrogram (Fig. 1). Although the range of Jaccard`s similarity coefficient was substantially high (between 0.34 and 0.84), the dendrogram failed to show a major grouping. G22, which was an introduction from Sweden, shared not more than 41% similarity with the Ethiopian genotypes and formed a solitary cluster. At about 46% similarity index, three groups were formed by the remaining 21 genotypes. G5, G13, G9 and G21 formed a separate group. G2 and G4, sharing about 50% similarity from the rest, formed a separate group. The remaining 15 genotypes clustered together. Under this big group, the two inbred lines G11 and G12 that originated from the same S_2 parent clustered very close to each other. Cluster 1 and 3 could be divided into more similar subgroups at a higher GS values. Topology of the dendrogram failed to separate the genotypes collected from different geographic areas within Ethiopia into different groups or subgroups. Generally, the 21 inbred lines developed from accessions collected from different geographic regions of Ethiopia were randomly distributed to the different groups or subgroups in the dendrogram.

A PCoA was performed based on Jaccard`s similarity estimator to portray the genetic similarity of the 22 genotypes. The PCoA indicated (Fig. 2) that, the first two principal coordinates (PCos) accounted for only 16.6% of the total variation. Adding the third PCo improved the amount of variance explained to 23.3%. In spite of the low contribution of the first three PCos to the total variance, the PCoA depicted relationship that agrees with pedigree, passport data and the clustering pattern observed in the dendrogram. The two genotypes, G11 and G12 that shared common parentage at the S_2 generation were clearly separated from the others on the 1st and 2nd PCos. Similarly G22 was separated from all the others on the 2nd and 3rd PCos and formed a solitary group as in the dendrogram. G2 and G4 were also separated from the others on the 2nd principal coordinate. The other genotypes formed two groups with respect to the 2nd principal coordinate. With respect to the correspondence between geographic and genetic diversity, in the PCoA, like in the dendrogram, overlapping among genotypes collected from different geographic areas was evident.

Table 3. Genetic similarity (as Jaccard`s estimator) for the 22 Ethiopian mustard genotypes obtained from 239 polymorphic RAPD bands

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G
G1	1.00																					
G2	0.51	1.00																				
G3	0.50	0.54	1.00																			
G4	0.45	0.51	0.44	1.00																		
G5	0.42	0.34	0.48	0.43	1.00																	
G6	0.45	0.48	0.49	0.39	0.47	1.00																
G7	0.54	0.44	0.56	0.39	0.41	0.54	1.00															
G8	0.51	0.51	0.47	0.46	0.36	0.49	0.51	1.00														
G9	0.51	0.45	0.47	0.45	0.47	0.48	0.50	0.46	1.00													
G10	0.43	0.41	0.52	0.45	0.39	0.44	0.46	0.45	0.47	1.00												
G11	0.54	0.50	0.50	0.42	0.36	0.53	0.53	0.52	0.41	0.53	1.00											
G12	0.50	0.50	0.50	0.45	0.40	0.58	0.53	0.52	0.44	0.49	0.84	1.00										
G13	0.43	0.37	0.49	0.45	0.53	0.50	0.52	0.42	0.49	0.44	0.39	0.42	1.00									
G14	0.51	0.45	0.56	0.46	0.46	0.49	0.50	0.47	0.45	0.48	0.50	0.53	0.43	1.00								
G15	0.49	0.43	0.53	0.45	0.47	0.54	0.53	0.47	0.49	0.50	0.53	0.52	0.45	0.59	1.00							
G16	0.45	0.46	0.49	0.45	0.46	0.47	0.48	0.51	0.47	0.42	0.42	0.46	0.45	0.50	0.56	1.00						
G17	0.54	0.44	0.45	0.43	0.47	0.47	0.54	0.44	0.47	0.40	0.45	0.50	0.47	0.50	0.47	0.44	1.00					
G18	0.46	0.43	0.45	0.40	0.42	0.50	0.50	0.47	0.41	0.42	0.46	0.49	0.46	0.44	0.56	0.48	0.48	1.00				
G19	0.52	0.44	0.48	0.38	0.40	0.50	0.50	0.53	0.44	0.51	0.53	0.56	0.45	0.49	0.42	0.44	0.44	0.46	1.00			
G20	0.46	0.46	0.40	0.45	0.39	0.52	0.46	0.46	0.48	0.47	0.50	0.48	0.41	0.49	0.51	0.47	0.42	0.50	0.45	1.00		
G21	0.42	0.43	0.51	0.47	0.48	0.48	0.47	0.42	0.53	0.45	0.41	0.44	0.50	0.48	0.50	0.48	0.46	0.38	0.42	0.43	1.00	
G22	0.39	0.39	0.36	0.37	0.34	0.39	0.41	0.47	0.40	0.37	0.42	0.41	0.38	0.40	0.40	0.39	0.44	0.40	0.42	0.39	0.46	1.





seed. G22 was identified by the RAPD pattern as most distant to the remaining 21 genotypes. As it was the only non-indigenous accession, the geographic isolation of

this particular genotype from the rest could have led for a sizeable RAPD differentiation. In both the dendrogram and PCoA analysis, the 21 inbreds developed from accessions collected from different geographic regions of Ethiopia did not form well-defined groups in accordance to their geographic origin. An exception to this broad generalization is the clustering of G6 with G11 and G12 to form a small conglomerate with the same geographic origin. In the dendrogram, two of the three subgroups contained genotypes collected from more than one geographic area and conversely genotypes collected from one geographic area were grouped into different subgroups. This shows weak RAPD differentiation along with geographic regions and can be interpreted as an absence of definite association between genetic diversity and geographic origin in these genotypes. Lack of definite correspondence between the RAPD banding pattern and geographic origin was also reported in *B. juncea* (Rabbani et al. 1998) and *B. napus* (Mailer et al. 1994). In *B. carinata* Alemayehu and Becker (2002) and Adefris and Becker (2005) also found no definite correspondence between genetic and geographic diversity and noted the diversity of population within geographic origin and similarity of population beyond geographic limits. Other diversity studies on sorghum (*Sorghum bicolor*) by Ayana et al. (2000) and on enset (*Ensete ventricosum*) by Birmeta et al. (2002) in Ethiopia similarly indicated the absence of regional RAPD marker differentiation.

The breeder may chose cultivars that are distantly related to obtain transgressive segregants or for superior hybrid combinations. For possible hybrid combination selections of parents with lower GS values are suggested. Successful combination could be created by crossing the introduced genotype, G22 with any of the other inbred lines; G2 with most of the other inbred lines, especially with G5 (GS = 0.34), G13 (GS = 0.37) and G22 (GS = 0.39); G4 with G6, G7 and G22 (GS = 0.39), G19 ((GS = 0.38); G5 with G8 and G11 (GS = 0.36) and G10 (GS = 0.39); G13 with G11 (GS = 0.39); G21 with G18 (GS = 0.38); and G20 with G22 (GS = 0.39).

Detection of genotypic specific bands

The RAPD analysis not only facilitated discrimination of one genotype from the other but also provided the opportunity to look for genotype specific markers (Table 4). Fourteen of the 22 genotypes generated a unique band when amplified by at least one primer. Nine of these 14 genotypes gave one unique band each generated by amplifying their DNA by a single primer. Four other genotypes (G6, G8, G9 and G22) gave each two unique bands at different loci when each genotype was amplified by two different primers. Two other genotypes, G6 (when amplified by primer OPAJ-15, OPC11 and OPAJ6) and G19 (when amplified by Primers OPC-2, OPAH-6 and OPAJ11), each produced three unique bands at different loci. This unique banding pattern resulted from genotype × primer interaction can be used as a DNA fingerprint for variety identification. This would have a paramount importance for the establishment of property rights and determination of variety purity. According to Kresovich et al. (1994) such unique bands would be especially valuable if they are linked to agronomically important traits. Line specific markers also have the potential

of enhancing efficiency of individual breeding programs through accelerated backcrossing, and other genetic studies such as the determination of out crossing.

Table 4. List of genotypes that showed unique bands along with their approximate size (in range) and the primer that generated the bands

Genotype	Generating primer	Approximate molecular marker size (kb) (range)
G1	OPD-19	1000-1500
G2	OPAJ-4	750-1000
G4	OPAH-6	1000-1500
G6	OPAJ-15	3000-3500
G6	OPC-11	2000-2500
G6	OPAJ-6	1500-2000
G8	OPAG-9	2000-2500
G8	OPAH-3	2000-2500
G9	OPC-10	1000-1500
G9	OPAJ-3	1000-1500
G11	OPR-9	2500-3000
G14	OPAJ-6	1000-1500
G15	OPAH-17	1000-1500
G16	OPA-12	750-1000
G18	OPAJ-6	2000-2500
G19	OPC-2	500-750
G19	OPAH-6	2500-3000
G19	OPAJ-11	2500-3000
G20	OPAJ-15	500-700
G22	OPB-15	1000-1500
G22	OPA-4	2000-2500

In conclusion, the RAPD technique provides a means of fingerprinting individual genotypes in a reproducible manner. The study also demonstrated that the RAPD technique could be applied for cultivar identification and estimation of genetic variation among *B. carinata* genotypes. Within the Ethiopian genotypes, factors other than geographic origin were potent sources of RAPD differentiation. Conversely, RAPD differentiation was higher in the exotic genotype, thus formation of a gene pool distinct from the Ethiopian gene pool could be possible through introduction of germplasm.

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