INT. J. BIOL. BIOTECH., 2 (3): 583-587, 2005.

STUDY OF GENETIC DIVERSITY AMONG DIFFERENT VARIETIES OF CANOLA (*BRASSICA NAPUS* L.) BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER

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ABSTRACT

Canola oil is a popular oilseed crop due to its low erucic acid and glucosinolate. Being a newly introduce crop, less information is available about its agronomical requirement and genetic diversity. RAPD study was conducted to assess the genetic diversity among eight varieties of canola using ten RAPD primers. Forty five scorable bands were observed, out of which, 33 (73.33%) were polymorphic and only 12 (26.66%) were monomorphic. It was observed that CON-I, Dunckled, Shiralee and Abasin-95 were genetically closely related to each other. Maximum similarity was observed between CON-I and Dunckled (86%), however Hyola-42 and Oscar were genetically distinct from all other varieties.

Key words: Canola, RAPD, genetic diversity, plant biotechnology

INTRODUCTION

Pakistan is spending a huge amount of foreign exchange on the import of edible oil. The total requirement of edible oil during 2003-2004 was about 1.76 million tones, out of which 0.494 million tones (28.08%) was met from local production and remaining 1.2 million tones (71%) was imported at the cost of Rs. 37.9 billion (Anonymous, 2003-2004a; Anonymous, 2003-2004b). Rapeseed and mustard are the second source of edible oil after cotton seed but their oil is of low quality due to the presence of high concentration of erucic acid and glucosinolates (Malik *et al.*, 2002). Newly introduced canola (*Brassica napus* L) cultivars with low erucic acid and glucosinolates also known as 'double zero' varieties made it more popular and more acceptable to the consumer (Malik *et al.*, 2002; Javed *et al.*, 2003). Besides, canola oil has the lowest level of saturated and the highest level of mono and polyunsaturated fatty acids, which reduce cholesterol level. In Pakistan total area under edible oil seed cultivation is 0.698 million hectares of which 0.047 million hectare are under canola, producing 0.059 million tons of edible oil (Anonymous, 2003-2004 a, Malik *et al.*, 2002). Although numerous factors are responsible for low productivity but the most important one is the non availability of high yielding varieties. Hybridization and induced mutation plays a vital role in creating a genetic variability in the breeding material. Being a newly introduced crop in the country little information is available about the genetic diversity of germplasm collected, used for cultivar development (Phan *et al.*, 2003). Due to the lack of information breeders have been using genetically similar parents extensively in crop breeding programme leading to a narrow genetic base (Fouilloux and Bannerot, 1988; Xia *et.al.*, 2004; Rehman *et al.*, 2002).

Present study was conducted to get the genetic information in the available germplasm of the canola varieties which could help out the breeder in selection of the breeding material. RAPD technique was used for this study, which is based on Polymerase Chain Reaction (PCR) developed by Williams *et al.* (1990); Welsh and McClelland (1990). RAPD markers are less expensive and technically less complex to analyse than other techniques. It does not require large amounts of DNA or prior knowledge of genetic structure (sequence) of the genome (Ford-Lloyd *et al.*, 1996). High annealing temperature ranges 50-55°C is used with RAPD primer of Gene Link to avoid the spurious formation of amplification products and to increase reproducibility. (Atienzer *et al.*, 2000).

MATERIALS AND METHODS

The RAPD analysis was conducted to estimate the genetic diversity among eight different canola varieties (CON-I, Shiralee, Rainbow, CON-II, Dunckled, Abasin-95, Oscar, Hyola-42). The information gathered will be useful in marker assisted breeding as well as genome mapping.

DNA extraction:

DNA was extracted from fresh leaves of canola varieties using DNA isolating Kit (Gentra system, Minnesota, USA.). Two hundred mg fresh leaves were ground in liquid Nitrogen; 3 ml of the cell lysis solution (Tris

[hydroxymethyl] aminomethane, ethylenediaminetetra acetic acid and sodiumdodecyl sulfate was added with leaf sample to the 15 ml centrifuge tube and incubated at 65°C for 60 minutes. Fifteen µl of RNase A solution (Gentra Kit, Minnesota, USA.) was then added to the cell lysate and incubated at 37°C for 30 minutes. Protein precipitation solution (GENTRA Kit, Minnesota, USA.) was added and vortex for 20 seconds and the tubes were placed on ice for 30 minutes. The mixture was centrifuged at 2000 x g for 10 minutes. Supernatant containing DNA was poured in the separate 15ml centrifuge tube and DNA was precipitated by centrifuging at 2000 x g with 3 ml of isopropanol absolute. Ethanol (70%) was used to wash the pellet and the DNA samples were then hydrated with TE buffer. DNA was quantified on spectrophotometer (BIOMATE 3).

DNA amplification:

Fifteen primers from Gene Link (NewYork, U.S.A), each ten bases in length, were used to amplify the DNA (Table 1). PCR reaction was carried out in 25µl reaction mixture containing 13ng of template (genomic DNA), 2.5mM $MgCl_2$ (Eppendorf, Hamburg, Germany), 0.33mM of each dNTPs (Eppendorf, Hamburg, Germany), 2.5U of Taq polymerase (Eppendorf, Hamburg, Germany) and 1µM of primer in a 1xPCR reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 min at 94°C, then 32 cycles:1 min denaturation at 94°C; 1 min annealing at 52°C; 2min extension at 72°C. Final extension was carried out at 72°C for 10 min. Amplified products were analyzed through electrophoresis on 1.5% agarose gel containing 0.5X TBE (Tris Borate EDTA) at 72 Volts for 2 hours, the gel contained 0.5µg/ml ethidium bromide to stain the DNA and photograph was taken under UV light using gel documentation system(Vilber Lourmat, France).

Data analysis:

Data was scored as presence of bands as (1) and absence of band as (0) from RAPD of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei and Li (1979):

$$S_{ij} = \frac{2Nij}{N_i+N_j}$$

Where:

N_{ij} = number of bands common in between cultivar of i and j and

N_i and N_j = total number of bands for cultivars i and j

Table 1. Sequence of the primers.

Primer	Sequence	Primer	Sequence
A-15	TTCCGAACCC	B-06	TGCTCTGCCC
A-16	AGCCAGCGAA	B-07	GGTGACGCAG
A-17	GACCGCTTGT	B-10	CTGCTGGGAC
A-20	GTTGCGATCC	B-12	CCTTGACGCA
B-05	TGCGCCCTTC	B-17	AGGGAACGAG

RESULTS AND DISCUSSION

Ten primers were used to amplify the brassica genomes that gave multiple fragments. The total number of scorable bands were 45, out of which 33 (73.33%) were polymorphic and only 12 (26.7%) were monomorphic. The number of fragments produced by various primers ranged from 1- 13, with an average of 4 fragments (Fig.**1**).The length of fragments ranged from 300 bp – 2.43 kbp. Primer B-17 produced 33 fragments and primer A-15 produced only one fragment.

Genetic Similarity:

Similarity coefficient reflected the genetic relationship between clones. The maximum similarity was observed between CON-I and Dunckled varieties (86%) (Table 2). Dendrogram showed that CON-I, Dunckled, Shiralee and Abasin-95 were closely related to each other. Another close genetic relationship was observed between CON-II and Rainbow. Hyola-42 and Oscar were found genetically distinct from other varieties (Fig.**3**).

Chen *et al.* (1997). reported that the molecular marker had been used to tag some agronomical important trait in Brassica. In present experiment some specific RAPD bands have been identified, reflecting the RAPDs application for the identification of canola breeds, which may correlate with any morphological marker. We found that the Oscar variety contains a specific DNA segment of 500bp amplified by primer B-06 (Fig. **2**).

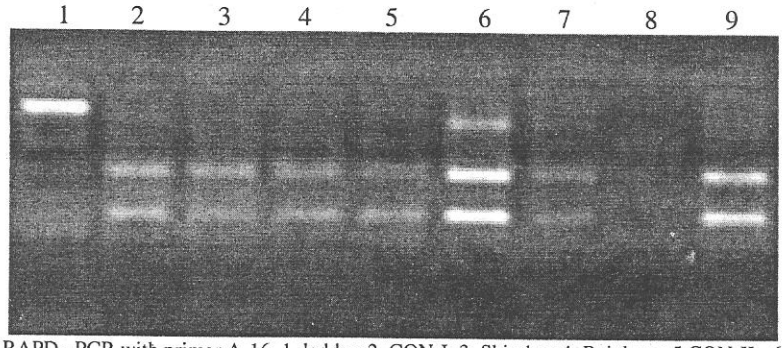

Fig.1. Result of RAPD –PCR with primer A-16. 1, ladder; 2, CON-I; 3, Shiralee; 4, Rainbow; 5,CON-II; 6, Dunckled; 7,Abasin-95; 8,Oscar; 9, Hyola-42

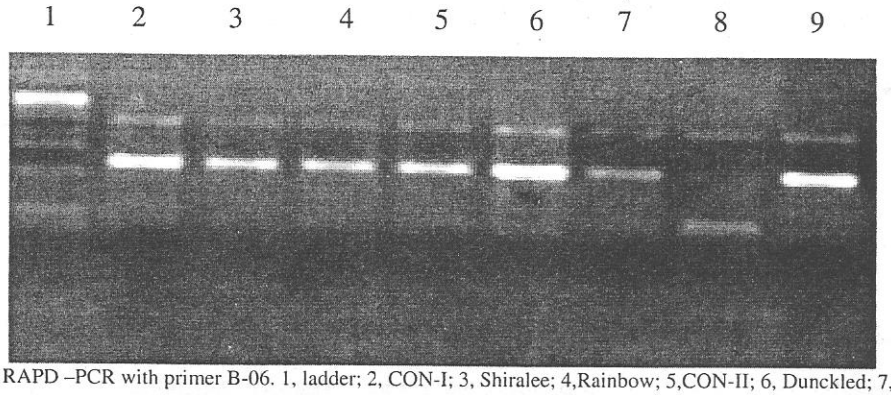

Fig.2. Result of RAPD –PCR with primer B-06. 1, ladder; 2, CON-I; 3, Shiralee; 4,Rainbow; 5,CON-II; 6, Dunckled; 7,Abasin-95; 8,Oscar; 9, Hyola-42

Table 2. Similarity coefficient among the Brassica varieties calculated according to Nei and Li .

Varieties	CON-I	Shiralee	Rainbow	Con- II	Dunckled	Abasin-95	Oscar	Hyola-42
CON- I	1							
Shiralee	0.86	1						
Rainbow	0.73	0.80	1					
Con- II	0.75	0.81	0.82	1				
Dunckled	0.86	0.79	0.71	0.73	1			
Abasin-95	0.81	0.78	0.72	0.76	0.85	1		
Oscar	0.61	0.58	0.44	0.48	0.58	0.60	1	
Hyola-42	0.58	0.67	0.57	0.69	0.66	0.61	0.48	1.00

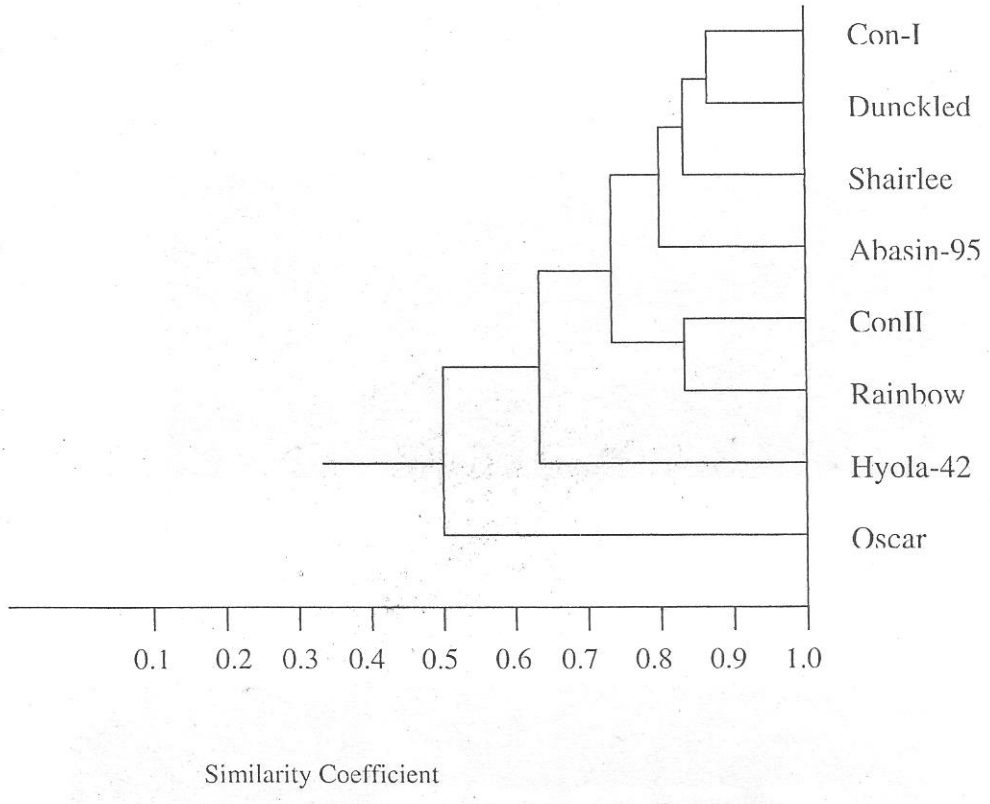

Fig.3. Dendogram of Canola cultivars based on similarity of bands.

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(Accepted for publication June 2005)