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Evaluation of Genetic Diversity among Different Genotypes of *Brassica napus* Using Random Amplified Polymorphic DNA Markers

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Abstract: In current research, genetic relationships among rapeseed genotypes from several geographical origins including France, Canada, Germany, Iran, Hungary, Denmark, Australia and America were evaluated using RAPD markers. Among generated 86 bands, 80 different polymorphic bands were obtained using 9 random primers. Diversity Index (DI) or Polymorphism Information Content (PIC) was varied from 0.29 to 0.48, showed a relatively high potential of primers among studied genotypes. Dice similarity coefficient between genotypes was calculated using Nei and Li formula. Maximum (0.91) and minimum (0.42) similarity coefficients were observed between Bristol and Amber genotypes, consul and express, respectively. Cluster analysis based on dice similarity coefficient was also carried out. Base on the cluster analysis, genotypes were grouped into five main clusters. Results showed that genotypes with same geographical origin were genetically different. Therefore, geographical origins of genotypes cannot be used as a base to cross parent to obtain high heterosis and it must be carried out by exact genetic studies. Results confirmed that RAPD is a simple, cheap and fast method for evaluation of genetic diversity of *Brassica napus*.

Key words: *Brassica napus*, cluster analysis, genetic diversity, RAPD marker

INTRODUCTION

Canola (*Brassica napus* L.) is one of the most important members of Cruciferae family. Total production of canola seed had reported over 46.4 m t ha^{-1} in 2005 (FAO, 2005). *Brassica napus* is an important source of vegetable oil regarding world and it is the second largest oilseed crop after soybean now (FAO, 2007). Evaluation of genetic diversity among wild and crop plants population is necessary for protection, conservation and useful application of germplasms, identification of suitable parents in order to high quality crosses and identification of genetic content of important breeding traits related to breeding purposes (Kresovich *et al*., 1992; Diers and Osborn, 1994; Halldén *et al*., 1994; Cruz *et al*., 2007). On the other hand, low levels of genetic diversity in studied cultivars increase the potential vulnerability to diseases and pests (Jordan *et al*., 1998). Evaluation of genetic diversity in plants had been carried out using various techniques consist of morphological, protein, isozyme and DNA-based markers (Shengwu *et al*., 2003). DNA-based markers are a powerful tool for studies of genetic diversity, therefore it is used for genetic studies and evaluation of genetic diversity. One of suitable DNA-based markers for genetic diversity studies is RAPD (Random Amplified Polymorphic DNA). This marker has a high potential in order to polymorphic evaluation in all races of plants and animals and for identification and study of races is very valuable (Welsh *et al*., 1991). Davis *et al*. (1995) expressed that RAPD marker was more exact than other markers such as protein and isozyme markers for evaluation of genetic diversity. Although RAPD has not high repeatability, is used for evaluation of genetic diversity in studied populations because of many advantages such as single, need to little genomic DNA, to save in electrophoresis time and cheap costs. Specially, a large number of accessions are commonly investigated in studies of genetic diversity. Using RAPD in *B. napus* indicated that genomic distribution was proportional. On the other hand, rate and single of RAPD was the most important factor for quick development of number of markers related to canola genetic map (Quiros *et al*., 1991). Moreover, RAPD and RFLP (together) were used to determine genetic distances among different Cruciferae species (Thormann *et al*., 1994). RAPD was used for classification of *Brassica*, *Sinapis* and *Raphanus* species and had been indicated that RAPD was suitable for classification of populations to species and genus levels (Demek *et al*., 1992). Mailer *et al*. (1997) used RAPD for evaluation of genetic diversity among 25 Australian canola varieties. Eighty nine bands were generated using 19 random primers in which 6 primers

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(with the most generated bands) were used for varieties distinction. Shengwu *et al*. (2003) collected a high number of canola genotypes from several countries (England, France, Germany, China and Czech Republic) and then used RAPD for evaluation of genetic diversity and studies of genetic relationships among germplasms of these genotypes. Because of important role of canola for vegetable oil production in the world, in the daily diet and totally, for both nutritional and industrial purposes, achievement of studies on in order to canola breeding is essential. This study was carried out to evaluate the genetic diversity and determine of pedigree relationships among different genotypes of *B. napus* using RAPD markers.

MATERIALS AND METHODS

Plant material: The plant material for this study comprised 20 genotypes of rapeseed cultivars (Table 1). Genotypes imported from several geographical origins including France, Canada, Germany, Iran, Hungary, Denmark, Australia and America. Seeds were cultivated in vases and were grown to 10 days.

DNA extraction: DNA extraction was carried out using leaves of seedlings. Total genomic DNA was extracted according to the modified protocol of Dellaporta *et al*. (1983). DNA concentration and quality were estimated by means of spectrophotometer and electrophoresis on 1% agarose gel. All DNA samples were diluted to the working concentration 20 ng μL^{-1} with sterile water.

DNA amplification: The total reaction volume for DNA amplification was 25 μL. Reaction mixtures contained 1x PCR buffer, 2 mM $MgCl_2$, 200 μM each of dNTPs (Sinagene Inc.), 2.5 unit DNA Taq polymerase (Sinagene Inc.), 0.2 μM primer, 60 ng of genomic DNA. DNA amplification was carried out using thermocycler (Model Biometra). Thermocycler was programmed 5 min at 94.0°C primary, 35 cycles consist of 50 sec of 94°C, 50 sec of 37°C (39°C for primer of number 9) and 60 sec of 72°C following by the final extension 10 min at 72.0°C. After amplification, PCR products were separated by electrophoresis in 1.2% agarose gel with 1X TBE buffer and stained with ethidium bromide and photographed under the UV light. For measurement of bands weight, 100 bp DNA ladder (Plus Fermentase) was used.

Table 1: List of *B. napus* genotypes

Name of genotype	Type of genotype	Geographical origin
Consul	Winter	France
Hylite	Spring	Canada
RG-9908	Winter	Germany
(Yanter×tower) F4	Spring×winter	Iran
GK. Helena	Winter	Hungary
Akamer	Winter	Germany
Calibra	Winter	Germany
Turner-1	Winter	Denmark
Pauc 906	Winter	France
Talent	Winter	Germany
Express	Winter	Germany
Turner-2	Winter	Denmark
Hyola 401	Spring	Australia
Bristol	Winter	American
Amber	Spring	Canada
Hysin111×PF 7045	Spring	Iran
H. 42	Spring	Australia
Okapi	Winter	France
Goliath	Spring	Germany
Zarfam	Spring	Iran

Data analysis: For all genotypes, a binary matrix was generated in which band presence and absence was 0 and 1, respectively. Based on binary matrix, pair-wise distances between the genotypes were calculated using Dice distance metrics (Nei and Li, 1979). These calculations were carried out with the use of NT SYS 2.02. Based on Dice distance metrics, clustering analysis was carried out using UPGMA. Diversity Index (DI) or Polymorphic Information Content (PIC) of primers was calculated as (Botstein *et al*., 1980):

$$PIC = \left[1 - \sum pi^2\right]$$

For study of DI and number of polymorphic bands relationship, linear regression was generated using excel software. For each primer, efficiency was also calculated using numbers of polymorphic bands/total generated bands formula.

RESULTS AND DISCUSSION

All used primers produced polymorphic bands (Table 1). Before achievement of calculations, unclear bands were deleted and were not interred in calculations. Total numbers of bands were 1059 that among generated 86 bands, 80 different polymorphic bands were obtained using 9 primers. Average band numbers were 118 and 53 for each primer and genotype, respectively. Weight ranges of bands were from 500 to 1500 bp Fig. 1. Average CG percent of used primers was 66% (Table 2). Six to twelve polymorphic bands were generated for all

Table 2: Primers used for generating RAPDs in *B. napus* genotypes

Primer's name	Sequence (5'–3')
A	5'- GGG CTC GTG G
B	5' TCA GCC AGC G
C	5'- CTA TAG GCC G
D	5'- CGT CAC AGA G
E	5'- CGG TGA CAT C
F	5'- GAG CCA GAA G
G	5'- CCT GGG CTT G
H	5'- TGA CGC GCT C
I	5'- GCG TGT AGG CT

Rajcan *et al*. (1999) and Tanhuanpaa *et al*. (1995)

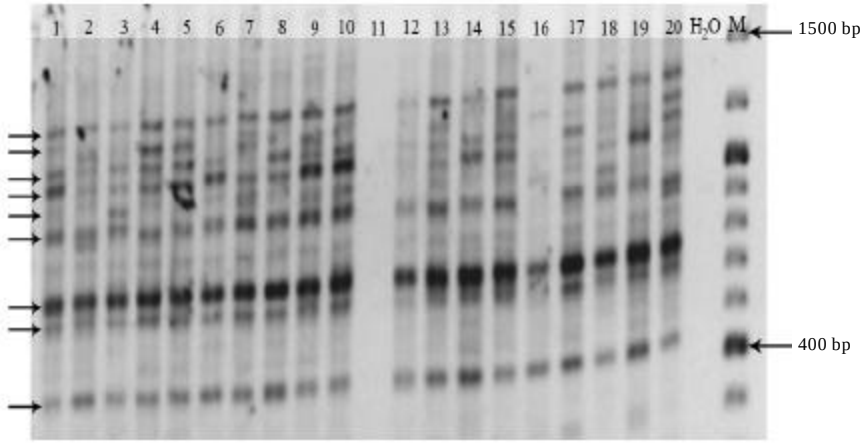

Fig. 1: RAPD pattern obtained by PCR amplification using the primer 1 (5'- GGG CTC GTG G) in the 20 studied genotypes. Lane 1: Genotypes of Consul, Lane 2: Hylite, Line 3: RG-9908, Lane 4: (Yanter×Tower) F4, Lane 5: GK. Helena, Lane 6: Akamer, Lane 7: Calibra, Lane 8: Turner-1, Lane 9: Pauc 906, Lane 10: Talent, Lane 11: Express, Lane 12: Turner-2, Lane 13: Hyola 401, Lane 14: Bristolm, Lane 15: Amber, Lane 16: Hysin111×PF 7045, Lane 17: H. 42, Lane 18: Okapi, Lane 19: Goliath, Lane 20: Zarfam, Lane H_2O: Negative control (H_2O) Lane M: 100 bp DNA ladder (Plus fermentase)

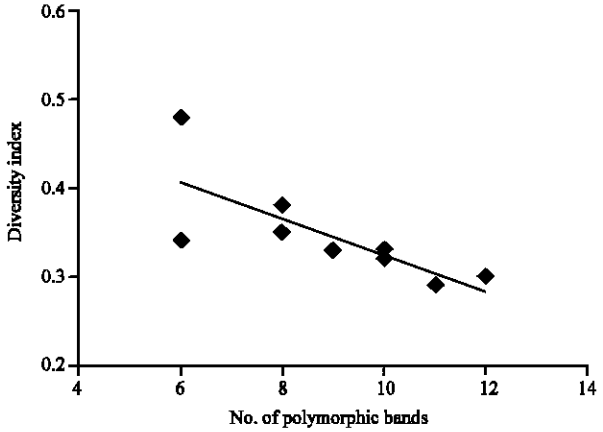

Fig. 2: Regression between Diversity Index (DI) and No. of polymorphic bands

primers in which maximum polymorphism (12 bands) and minimum polymorphism (6 bands) was observed for primer 8 and primers 2 and 3, respectively. Maximum primer efficiency and minimum primer efficiency was calculated for primer 8 and primer 2, respectively in which all bands of primer 8 were polymorphic and 6/8 bands of primer 2 were polymorphic (Table 3). For each primer, reaction was carried out in two repetitive reactions with same complete condition. Same result from both repetitive reactions indicated that PCR reactions were carried out in very suitable condition. For all PCR reactions, if a same fixed and optimized libratory conditions are used and PCR

Table 3: Number of polymorphic bands were generated using each primer and their diversity indexes that was calculated using Botstein *et al.* (1980)

Primer's name	Generated bands No.	No. of polymorphic bands	DI	Max. DI
A	9	9	0.33	0.50
B	8	6	0.34	0.46
C	6	6	0.48	0.50
D	10	10	0.33	0.50
E	9	8	0.35	0.50
F	10	8	0.38	0.50
G	12	11	0.29	0.46
H	12	12	0.30	0.50
I	10	10	0.32	0.48

reactions are also carried out at short term, repeatability of RAPD will be increased (Heun and Helentjaris, 1993).

Diversity index was varied from 0.29 (for primer 7) to 0.48 (primer 3) which showed a relatively high potential of primers among studied genotypes. Results showed that a large number of polymorphic bands would not be due to high diversity index certainly (Fig. 2). For example, primers 7 and 8 had high similarity in the over polymorphic bands them self, in order hands, these primers had 8 polymorphic bands in which for 18 genotypes, monomorphic bands were observed. Totally, RAPD is suitable and high efficient tool for identification and characterized of species such as *B. napus* genetically (Hu *et al*., 1999).

Based on Nei and Li (1979) formula, genetic similarity coefficient of Dice was calculated among 20 studied genotypes. Maximum (0.91) and minimum (0.42) genetic similarity coefficients were observed in genotypes 14 and 15 and genotypes 1 and 11, respectively. Based on

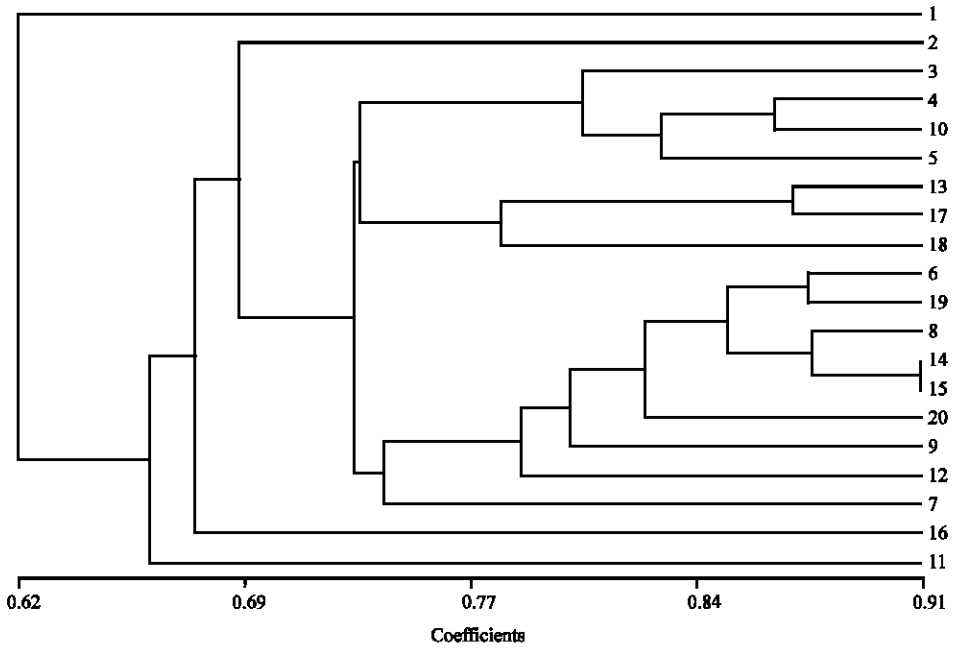

Fig. 3: Dendrogram of UPGMA cluster showing the genetic relationships among genotypes of *Brassica napus* L.

genetic similarity coefficient, clustering analysis was also carried out for all genotypes in which genotypes were grouped into five main groups. In this clustering analysis, genotypes 1, 2, 11 and 16 were grouped into four independent groups and other genotypes were categorized as one group (Table 3). In this group, genotypes 14 and 15 (with minimum similarity coefficient) are placed. Afterward, genotypes 6 and 14 (similarity coefficient = 0.89), genotypes 6 and 19 (similarity coefficient = 0.87), genotypes 13 and 17 (similarity coefficient = 0.87) and genotypes 8 and 15 (similarity coefficient = 0.86) had the most similarity coefficient, respectively. Cross of less genetic distance genotypes will not be due to suitable segregation for breeding traits of interest (such as yield). Genotypes 1 and 11 had minimum genetic different, afterward, genotypes 1 and 12 (similarity coefficient = 0.55) and genotypes 1 and 9 (similarity coefficient = 0.56) had the least similarity genetically. Plants with high heterosis are produced by cross between two parents with the least genetic similarity. Therefore, famous parents of different groups must be selected for interest of crosses. Clustering analysis showed that RAPD is suitable marker to among genotypes even same geographical genotypes (specially, among German genotypes). In current research, Canadian genotypes (with similarity coefficient = 0.69) was grouped into two different groups. Also, genotypes with French origin indicate determine different. In 6 German genotypes, high similarity coefficient (0.87) was observed between genotypes 6 and 19 and other German genotypes (3, 7, 10 and 11) had less similarity. Among Iranian genotypes (4, 16 and 20), genotypes 4 and 16 had a lot of difference. Genotypes 4 and 20 were categorized in one group. High genetic similarity (0.87) was observed between Australian genotypes (Fig. 3). Genetic diversity of genotypes, especially same geographical genotypes showed that studied genotypes had different genetic background and/or different breeding programs. Results indicated that geographical origins of genotypes cannot be used as a base to cross parent to obtain high hetrosis and it must be carried out by exact genetic studies. Results confirmed that RAPD was a simple, cheap and fast method for evaluation of genetic diversity of *B. napus*.

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