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Evaluating genetic diversity of canola cultivars using morphological traits and molecular markers

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Abstract

This study was conducted to evaluate genetic diversity of canola cultivars using morphological traits and molecular markers. In this study, 18 spring and winter type cultivars were sown in randomized complete block design with 3 replications at research farm of Mohaghegh Ardabili University in 2007. In each replication at flowering stage, 5 plants were selected and leaf samples collected to DNA extraction for RAPD analysis and chlorophyll measurement. At maturity stage, in selected plants, also morphological traits were measured. Significant differences were observed among cultivars in all studied traits. Hyola401 and Ebonite cultivars had high yield, yield components and leaf chlorophyll. Using cluster analysis (UPGMA method), the 18 canola cultivars were categorized in 3 groups. Using RAPD data, Nei's average genetic diversity varied from 0.05 (Hyola308 cv) to 0.16 (PF/1045/91 cv). Mean of genetic variation within (H_S), Total (H_T) and degree of genetic differentiations (G_{ST}) were 0.12, 0.27 and 0.56, respectively. The results showed that high variation exists within and between cultivars.

Keywords: Genetic diversity, Canola, Molecular markers, RAPD.

Introduction

Rapeseed (*Brassica napus* L.), the most important crop species of *Brassica* genus, having 40-45% oil in seeds and at least 46.5% protein, 3.5% fat and 0.35% phosphorus in the meal, is a valuable oil crop in oil industry and animal feed [1]. Also, this plant has a good adaptation to a wide range of climates. On a count of these reasons, in comparison to other oil plants, many studies are being run around the world for improving that. A main prerequisite of each breeding project is availability to genetic variation [2, 3]. Comprehensive information about genetic structure of populations may be obtained, if a study on genetic diversity was done by morphological, chromosomal, biochemical and molecular markers as complementary to each other [4, 5]. In recent years, DNA markers along to other markers have been well applied to identify genotypes and to assess genetic diversity. DNA markers reflect directly individual differences at the level of DNA molecules [6] and cover coding and none-coding regions of genome. They are not affected by environment, developmental stage, certain tissue and organ, and have high genomic frequency, high polymorphism and mostly a random genomic distribution [7, 8, 9]. RAPD markers may be used in genetic diversity studies, relationships and QTL tagging. Also, they quickly provide useful information around different parts of genome for polymorphism [10, 11, 12, 13]. Despite the many benefits, these markers are relatively low repeatable. However, their appropriate repeatability has been reported in oily *Brassica* species by SOMERS & al. [14].

KIMURA & al. [15] applied RAPD technique for estimating genetic diversity among 50 varieties of canola, including 13 varieties of Chinese, 26 Japanese and 11 varieties of Korean, European and Canadians. In this study, most varieties of Chinese and Japanese were

put in a single group and showed the same genetic background. They were separated from European varieties. DIVARET & al. [16] used these markers for assessing the level of genetic diversity in seed bulks of seven canola populations along a F_1 and a doubled haploid variety. The results showed that variation within the population was less than between. In this study, the genetic distances were estimated from the bulks data found a high correlation with distances obtained from single-plant method. Therefore, it was concluded that RAPDs can be used for identification and study of genetic diversity between populations using seed bulks. HALLDEN & al. [17] applied RAPD and AFLP markers to determine genetic relationships improved in breeding lines of *B. napus*. Based on obtained results, the both markers estimated very similar genetic relationships among the lines that were consistent to the pedigree information of the lines. Other markers such as microsatellite (SSR), ISSR, AFLP, Isozymes, protein and composition of these markers have been widely used for genetic diversity, linkage and QTL mapping in Canola [18, 19, 20, 21, 22, 23]. This study was also designed to assess genetic diversity of canola cultivars using RAPD markers and morphological traits.

Materials and methods

In this study, 18 spring and winter type cultivars were sown in randomized complete block design with 3 replications at research farm of Mohaghegh Ardabili University (Iran) in 2007 (table 1). Each plot contained 6 rows with 4 m long, 40 and 10 cm space between rows and plants in each row, respectively. At flowering stage, 5 plants from each replication were selected and leaf samples were collected to DNA extraction and chlorophyll measurement [24]. Also, at maturity stage, some morphological traits (plant height, number of pods per plant, pod length, number of seeds per pod, seed weight, number of main and sub branches) were measured in selected plants.

The DNA of leaf samples was extracted using the CTAB procedure according to Saghai-Maroof & al [25]. The quality and quantity of DNA samples were assessed using spectrophotometer (Techne, England) and 0.8 percent agarose gel electrophoresis. All of the DNA samples were diluted to 25ng/µl and used in PCR reactions. 50 RAPD primers (Metabione, Germany) were used to analyze polymorphism in the plants and polymorphic primers were used to genotyping. PCR reaction for RAPD analysis was performed in a volume of 15µl contained 50ng of DNA template, 2mM MgCl2, 0.05mM each dNTP, 0.132 µM primer, 1U *Taq* DNA polymerase and 1x PCR buffer. For RAPD primers the amplification profile consisted of a 5-min initial denaturation step at 94^{o}C followed by 40 cycles of denaturing at 94^{o}C for 30 seconds, annealing at 37^{o}C for 30 seconds, extension step at 72^{o}C for two min and a final extension step at 72^{o}C for 10 min. RAPD amplified products were analyzed using 1.5 % agarose gels and ethidium bromide staining. Each polymorphic marker bands were scored as 1 (presence) and 0 (absence) and obtained data were analyzed using $SPSS_{16}$ NTSYS pc 2.0 and POPGENE 1.31 software.

Table 1. The name and growth type of studied cultivars

Name	Growth type	Name	Growth type	Name	Growth type
Opera	Autumn	Elite	Autumn	Hyola401	Spring
Zarfam	Autumn	Elvis	Autumn	Option500	Spring
Orient	Autumn	Slmo46	Autumn	Hyola308	Spring
Jewel	Autumn	Slmo43	Autumn	RGS003	Spring
Tallaye	Autumn	Licord	Autumn	Hyola60	Spring
Okapi	Autumn	Ebonite	Autumn	PF/1045/91	Spring

Results and discussion

Morphological traits

Analysis of variance for the measured traits showed significant differences among all the cultivars (Table 2). Duncan's test of mean comparisons appeared the higher yield (it was calculated from the product of yield components values) and yield components in cultivars of Hyola 401 and Ebonite than remained cultivars (Table 3).

The two varieties had also the highest leaf chlorophyll content.. The yield per plant was high in varieties of Talaye and RGS003.

Cultivar of RGS003 had the highest values for number of major and minor branches and pod number per plant. In this variety, the number of seeds per pod, seed weight and chlorophyll content were less than those of varieties Ebonite and Hayola 401. Talaye, regarding to long pods and large seeds, was a superior variety for grain yield. Among the studied cultivars, varieties of Ebonite and Hayola 308 showed the highest (122 cm) and the lowest (60.6 cm) height, respectively. Despite having long pods in varieties of Jewel, Hayola 308, Okapi, Licord, they produced a low yield per plant. It might be caused by lower pod number per plant (Table 3).

The values of phenotypic correlation coefficients between traits appeared to lack associations among leaf chlorophyll content and yield components. Also, pod number per plant was significantly positive correlated to pod length and number of main and sub-branches, while that had a significant negative correlation with seed weight. This indicates that increasing the number of main and sub-branches are along (???) to evaluate of the pod number per plant but leading to form a small grain size. Also, it was significantly observed a negative correlation between grain weight and pod length. Relation of chlorophyll a content with chlorophyll b content was significant (Table 4).

Table 2. Analysis of variance for studied traits

Source of variations	df	Mean of squares										
		height	pods/plant	Pod length	Seed/pod	Seed weight	Yeild	Main shoots	Sub shoots	Chlorophyll a	Chlorophyll b	Chlorophyll ab
Repeat	2	13.7^{ns}	4136.9^{ns}	0.3^{ns}	52.1^{ns}	2.7^{ns}	2477.3^{ns}	1.2^{ns}	19.7^{ns}	0.002^{ns}	0.001^{ns}	0.003^{ns}
Cultivar	17	693.8^{**}	28780.3^{**}	0.76^{*}	375.5^{**}	1.7^{**}	5118.9^{**}	4.5^{**}	136.3^{**}	0.003^{**}	0.001^{**}	0.004^{**}
Error	34	173.9	2478.41	0.34	21.96	0.27	1188.3	0.59	5.1	0.0003	0.0001	0.001
C.V	-	13.62	18.47	8.7	15.61	15.2	29.2	17.3	14.5	57.5	21.9	16.7

^{Ns} no significant and ** significant at $p<0.01$ by ANOVA.

Table 3. Mean of measured traits in studied cultivars and LSD values for mean comparison.

Cultivars	Height (cm)	Pod/plant	Pod length (cm)	Seed/Pod	Seed weight (mg)	Yield/plant (gr)	Main branch	Sub branch	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Chlorophyll ab (µg/g)
Talaye	100.4	280	6.8	10.2	4.03	374.2	3.7	14	0.05	0.049	0.084
Jewel	75.6	116.8	6.2	6.17	4.49	96.91	4.6	13.9	0.098	0.052	0.149
PF	82.4	238.8	6.4	7.3	3.16	160.4	5.4	17.4	0.119	0.067	0.186
Licord	109.5	194.6	6.96	7.7	3.26	147.4	3.5	9.2	0.101	0.055	0.156
SLMO43	111.1	298	6.95	8.5	3.49	257.4	3.2	11	0.114	0.062	0.177
SLMO46	110.3	195.1	6.1	6.3	4.82	178.1	3.9	10.9	0.096	0.054	0.109
Elvis	112.3	339.3	7.2	9.4	2.85	284.9	4.2	17.8	0.102	0.057	0.160
Zarfam	95.1	207.8	6.96	16.9	2.75	298.5	3.9	17.1	0.102	0.060	0.160
RGS003	93.5	463.8	6.95	9.5	3.24	428.2	8.2	34.2	0.092	0.050	0.142
Opera	109.8	433.1	7.3	6.9	3.11	257.8	5.8	18.3	0.036	0.038	0.104
Okapi	99.8	214.4	6.8	8.3	2.96	156.7	3.3	9.3	0.134	0.083	0.217
Ebonit	122	344.5	7.6	15.7	2.64	550.7	3.8	10.6	0.093	0.057	0.150
Elit	98.1	309	7.4	10.8	2.8	284.5	4	16.7	0.111	0.065	0.176
Orient	101.1	272	7.2	8.8	2.36	171.1	3.6	7.4	0.120	0.072	0.192
Option500	82.9	389	6.7	8.3	2.4	278.5	4.1	19.8	0.063	0.032	0.095
Hyola60	89.6	159.3	5.7	11.2	4.45	242.2	4.5	13.4	0.068	0.039	0.107
Hyola308	60.6	140	7.2	8.9	3.60	138.3	4.8	13.7	0.082	0.024	0.105
Hyola401	88.8	257.1	7.3	15	4.69	550.8	5.9	28.2	0.110	0.063	0.174
LSD 5%	21.94	82.84	0.97	7.80	0.86	57.36	1.28	3.76	0.029	0.017	0.053

Clarke and SIMPSON [26] found that increasing the pod number per plant led to grow up seed number per pod and the down of seed weight. Also, they reported a weak negative correlation between the seed number per pod and 1000 seed weight. MENDHAM & al. [27] also reported a direct relation between the amount of radiation received by each pod and the final number of seeds per pod. ALI & al. [5, 28] studied the relationship between yield and yield components of rapeseed and found a significant positive correlation between these traits.

The varieties of rapeseeds were categorized in three groups using UPGMA cluster analysis based on a Euclidean distance (Figure 1). First group was formed from varieties of Licord, SLMO46, Zarfam, Okapi, Hayola 60, Hayola 308 and Jewel. Varieties of RGS003 and Opera placed in a single cluster. Remained varieties were clustered in a same group.

Among the fifty RAPD primers used, nine primers produced a one hundred two polymorph and high resolution bands, in total (table 5). Banding average for each primer was 11.3 bands. Maximum and minimum number of bands obtained in primers of Oligo-1 and Oligo-5, respectively. Okapi had the most bands while the least bands were found in cultivar of Hayola 308. Banding pattern and polymorphism of Oligo-1 primer have been displayed in Fig. 2.

Table 4. Values of phenotypic correlation coefficients between studied traits

Trait	1	2	3	4	5	6	7	8	9	10
Height (1)	1									
Pod/plant (2)	0.291*	1								
Pod length (3)	0.174	0.338*	1							
Seed/Pod (4)	0.187	0.119	0.212	1						
Seed weight (5)	-0.224	-0.282*	-0.325*	-0.154	1					
Main branch (6)	-0.252	0.365*	0.077	0.027	0.130	1				
Sub branch (7)	-0.255	0.513**	0.044	0.098	0.160	0.762**	1			
Chlorophyll a (8)	0.061	-0.220	0.123	0.073	-0.155	-0.210	-0.144	1		
Chlorophyll b (9)	0.185	-0.099	-0.030	-0.057	-0.067	-0.166	-0.161	0.239	1	
Chlorophyll ab (10)	0.139	-0.068	0.160	0.132	-0.269*	-0.177	-0.093	0.901**	0.259*	1

* and ** significant at at p<0.05 and at p<0.01, respectively

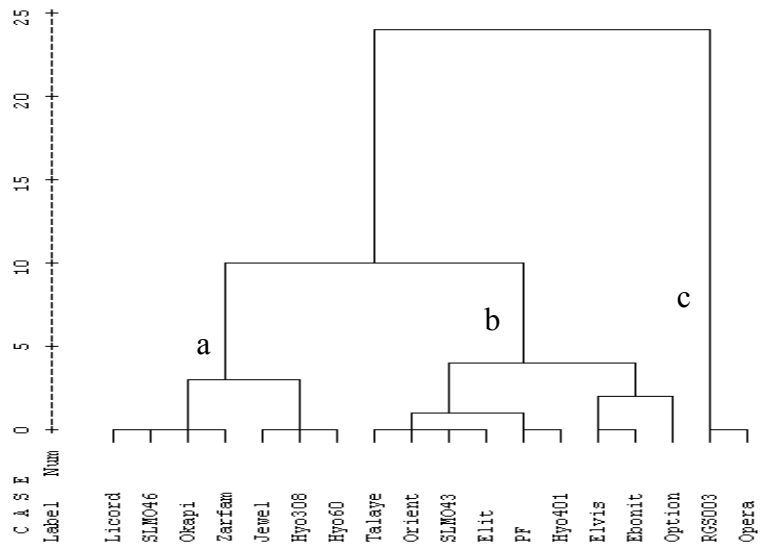

Figure 1. Grouping 18 rapeseed cultivars using cluster analysis based on UPGMA method.

Molecular analysis

Mean of genetic diversity within cultivars was computed based on Nei's gene diversity [29]. The highest (0.16) and the lowest (0.05) diversity were found in cultivars of PF/1045/910 and Hayola 308, respectively (Table 6). Average variation of total (H_T=0.27) and within (H_S=0.12) along to degree of gene differentiation among the cultivars over all loci

(G_{ST}=0.56) showed that there was a good genetic variation within and between the studied rapeseed cultivars. LAZARO & al. [30] reported that genetic variation among and within a population of rapeseed were about 67 and 33 percent, respectively. Also, there was a correspondence between the genetic and the geographical diversities. The results revealed the heterogeneity of the cultivars. Therefore it may be stated that the rapeseed cultivars are a potential source of various genes.

Grouping of the cultivars for molecular data was done using UPGMA cluster analysis based on Nei's genetic distance (Figure 3). The cultivars of SLMO46 and RGS300 were placed in the furthest distance (0.4261) from each other but the least distance (0.0969) was obtained among cultivars of Orient and Option500. The dendrogram revealeda little correspondence between the molecular and morphological grouping.

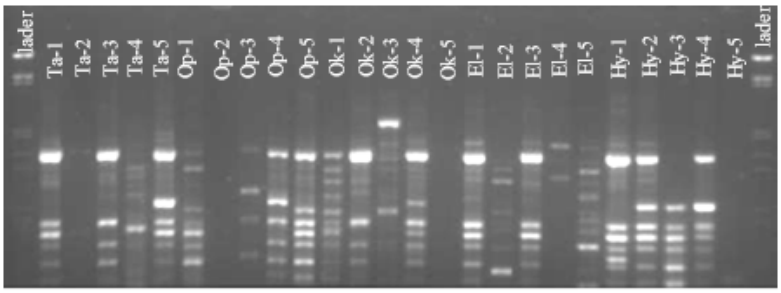

Figure 2. Banding pattern and polymorphism of Oligo-1 primer in some of the studied cultivars

Table 5. The name, sequence and total bands of studied primers

Primer name	Sequence	Total bands
Oligo-1	5' CCT GGG CTT C 3'	276
Oligo-3	5' CCT GGG CTTA 3'	168
Oligo-4	5' CCT GGG CTG G 3'	259
Oligo-5	5' CCT GGG TTC C 3'	106
Oligo-6	5' CCT GGG CCT A 3'	124
Oligo-12	5' CCT GGG TCC A 3'	118
Oligo-13	5' CCT GGG TGG A 3'	160
Oligo-16	5' GGT GGC GGG A 3'	206
Oligo-17	5' CCT GGG TTT A 3'	191

Table 6. Mean of genetic diversity within cultivars based on Nie's gene diversity coefficient [29]

name	Genetic diversity	name	Genetic diversity	name	Genetic diversity
Hyola401	0.09	Elite	0.15	Zarfam	0.15
Hyola308	0.05	Ebonite	0.14	Elvis	0.11
Hyola60	0.11	Okapi	0.06	Slmo46	0.09
Option500	0.15	Opera	0.13	Slmo43	0.13
Orient	0.13	RGS003	0.14	Licord	0.12
PF/1045/91	0.16	Jewel	0.14	Tallaye	0.07

It may be caused by random distribution of the RAPD markers over rapeseed genome that resulted in lack of correlation among the molecular and morphological data. In this grouping, five spring cultivars of Hayola 60, Hayola 308, Hayola 401, Option 500 and PF were placed in a distinct cluster. In other words, the RAPD markers were able to separate the cultivars for their growth types, spring and winter types. RGS003, among the spring cultivars,

classified in a single cluster. MOHEBALIPOUR [31] could detected spring and winter rapeseed cultivars using RAPD markers and also found that primer of 430 (from NAPS company), in contrast to the winter cultivars, had no band in the springs. ASGHARI & al. [32] reported a QTL for cold resistance in the vicinity of the said primer. The RAPD markers could be well applied to locate two QTL for pod length, explained 22 percent of variation, in a DH rapeseed population [33].

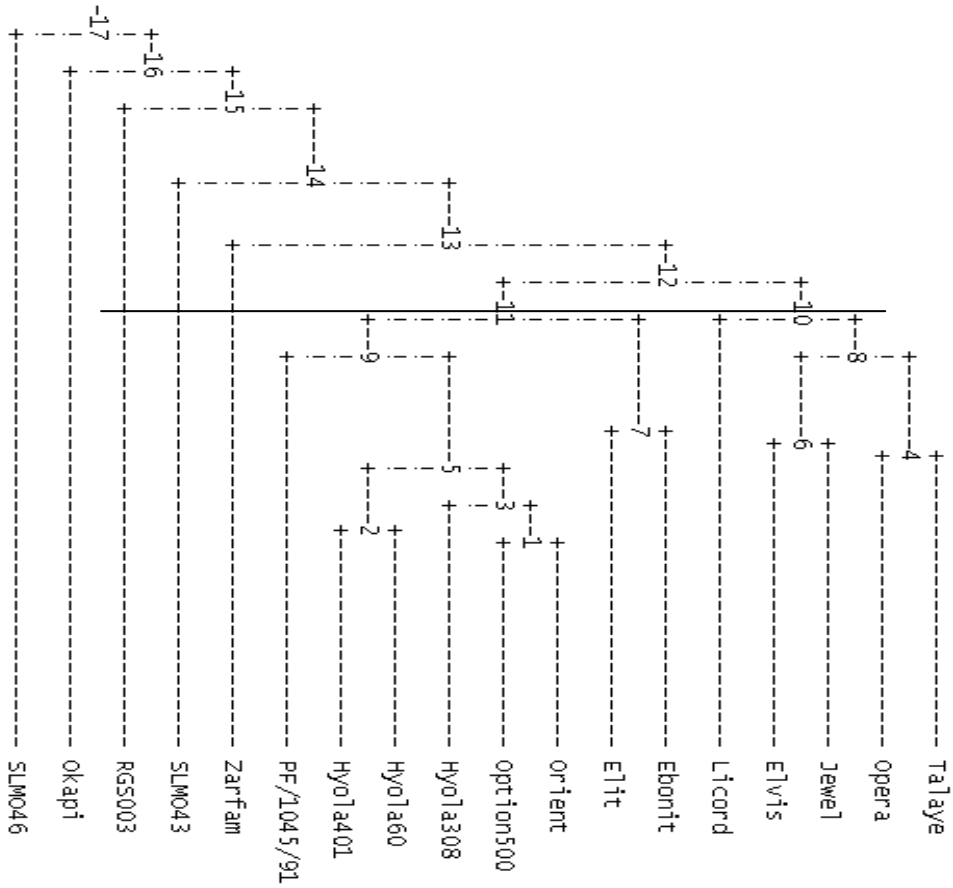

Figure 3. Grouping of the cultivars for molecular data using UPGMA cluster analysis method based on Nei's genetic distance (NEI, [29])

Conclusions

The clustering results and the genetic variation within and between the studied genotypes and the degree of gene differentiation suggest a relatively high genetic diversity that can be used in rapeseed breeding programs. Considering the diversity of the genotypes, the cultivars with a wide genetic distance may be usedas parents to crosses for taking advantage of heterosis and for making mapping populations in the QTL mapping studies. In this study, the RAPD molecular data provided a lot of information. However, application of the other DNA markers as well as biochemical markers such as isozymes and seed storage proteins would efficiently support the genetic studies and led to find valuable information. It is recommended to use multi -environmental experiments to get morphological data for estimation of GE interaction. Totally, rapeseed genetic diversity and identification of heterotic groups should be studied by various markers for being used in breeding plans.

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