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Genetic Variation in Iranian Germplasm of rapeseed (*Brasica napus* L.)

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ABSTRACT: Evaluation of genetic diversity among wild and crop plants population is necessary for protection, conservation and useful application of germplasms, identification of genetic content of important breeding traits related to breeding purposes. There are various techniques available, which allow study the genetic variability of crop germplasm; Morphological traits, total seed protein, isozymes and several types of DNA markers examples. The experiment was conducted at the research farm of the Tehran University in Karaj in simple lattice design 9 × 9 were planted. The number of days to the beginning of flowering, the number of days to the completed of flowering and the number of days to the end of flowering were evaluated. Hyola401 with 4680 kg/ha was the most high yielding variety and would most probably have high oil yield per unit area due to its great oil content.

Key words: Days to the beginning of flowering, day to the completed of flowering, day to the end of flowering

INTRODUCTION

Canola is one of the most important oil crops in the world (Bybordi, 2010). Winter rapeseed (*Brassica napus* L.) is an important agricultural crop, grown commonly for oil or biofuel production. After oil extraction, the high protein seed residue can be used as animal feed. Winter rapeseed is mainly cultivated in Europe, Asia, North America and Australia, but has a limited acreage in Turkey. Presently, over 50% of vegetable oil consumed in Turkey is imported from abroad. Rapeseed production has potential as an alternative income source for the Turkey producer. Although its production is still limited, this crop has large expansion possibilities. It is an alternative principally in areas where wheat (*Triticum aestivum* L.) is the only winter crop or in marginal areas for this cereal (Assare *et al.,* 1995). Oilseed canola plant (*Brassica napus* L.) is an important agricultural crop grown primarily for its edible oil and the meal that remains after oil extraction has value as a source of protein for the livestock feed industry (Jensen *et al.,* 1996). Canola container valuable fatty acids and amino acid required by the human body, with 40-49 percent and 35-39 percent protein (after oil extraction) and oil respectively. Canola (*Brassica napus* L.) is considered as an economically important crop of world. But erratic rainfall and scarcity of water for irrigation during the growing season significantly lowers its yield and quality. Water stress affects both vegetative and reproductive stages in canola. The effects of water stress were more severe during reproductive growth than vegetative growth in rapeseed (Ghobadi *et al.,* 2006). Previous studies showed that drought stress significantly decreased the seed oil content of canola (Sinaki *et al.,* 2007). One of the major problems to high yield and production is the lack of synchronized crop establishment in canola due to poor weather and soil conditions (Mwale *et al.,* 2003). The seeds are occasionally sown in seedbeds having unfavorable moisture because of the lack of rainfall at sowing time (Heydecker and Coolbaer, 1977) which results in poor and unsynchronized seedling emergence (Mwale *et al.,* 2003). The success in breeding program of a crop species largely relies to the presence of genetic diversity in the germplasm and knowledge about the characteristics of the genotypes and their genetic relationships (Moghadam *et al.* 2009). Evaluation of genetic diversity among wild and crop plants population is necessary for protection, conservation and useful application of germplasms, identification of genetic content of important breeding traits related to breeding purposes (Kersovich *et al.* 1992; Diers and Osborn. 1994; Hallden *et al.* 1994; Cruz *et al.* 2007). There are various techniques available, which allow study the genetic variability of crop germplasm; Morphological traits, total seed protein, isozymes and several types of DNA markers examples (Shengwu *et al.* 2003). The genetic base of oilseed rape (*Brassica napus*) is quite narrow due to its limited geographic range and intensive breeding (Girke *et al.* 2012).

Research on *Brassica* germplasm and evaluation of its genetic diversity could accelerate the efficient use of genetic variation through establishing a breeding programmer (Stokes *et al.* 2010; Harper *et al.* 2012). Heterosis in hybrids is based on genetic completion between divergent parents, so the information on genetic diversity could help breeders better understand the genetic structure of germplasm and to predict which cross combinations would produce good F1 hybrids (Yu *et al.* 2007). Breeders currently choose components for hybrid combinations based on desirable characteristics without any information about their affinity, although the genetic distance is a prerequisite for heterosis to a certain extent. Recently, numerous markers for description of genetic resources have been developed such as isozymes, storage proteins or DNA based markers (Curn 1995; Zhao & Becker 1998; Schlötterer 2004). At present, molecular methods have become essential parts of most studies on genetic diversity. Molecular methods are very useful for estimating features such as gene flow, genetic drift and degree of out breeding, while other marker systems may be very useful for studying adaptive variation (Rao & Hodgkin 2002). To ensure efficient rapeseed production breeders have aimed to produce highly yielding and high quality cultivars. The information on the genetic diversity in *B. napus* could help breeders and geneticists to understand the structure of *B. napus* germplasm and help them to predict which combinations would produce the best offspring. Rapeseed cultivars used in Europe are generally of very high quality, but some desirable traits are missing in European gene-pool. It was proved that Chinese lines contain some genes, which make production of hybrid seed easier without genetic manipulations. There are various techniques available, which allow study the genetic variability of crop germplasm. Morphological traits, total seed proteins, isozymes and several types of DNA markers are well known examples. DNA based markers provide powerful and reliable tools to reveal variations within crop germplasm and to study evolutionary relationships (Gepts 1993). Among molecular markers, random amplified polymorphic DNA (RAPD) has been employed in genetic research owing to their speed and simplicity (Welsh and McClelland 1990). RAPD analysis has been widely used in recent studies on Brassica crops: (1) for determining the genetic relationships between different related species (Demeke *et al.* 1992, Thormann *et al.* 1994, Ren *et al.* 1995), (2) for the identification of cultivars (Hu and Quiros 1991) and the percentage of hybridity (Marshall *et al.* 1994). Sufficient genetic diversity is very important for plants to survive in changing climate conditions, withstand diseases and pests, etc. Many molecular markers are used for studies of genetic diversity in Brassica: restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), sequence-related amplified polymorphism (SRAP), etc. (Halldén *et al.,* 1994; Riaz *et al.,* 2001; Negi *et al.,* 2004; Hasan *et al.,* 2006). RAPD markers are used for genetic diversity analysis of many plant species (Galovic *et al.,* 2006; Vyšniauskiene *et al.,* 2011; Zybartaite *et al.,* 2011; Vyšniauskiene *et al.,* 2013). One of suitable DNA based markers for genetic diversity studies is RAPD (Random Amplified Polymorphic DNA). This markers have a high potential in order to polymorphic evaluation in all race of plants and for identification and study of races is very valuable (Welsh *et al.* 1991). Although this method has low repeatability, but because of its simplicity and speed, it has been used extensively for variety identification, determination of genetic variability, relationship among the crop genotypes and construction of linkage maps (Young 2000; Jaroslava *et al.* 2002). ISSR or Inter Simple Sequence Repeat marker is another PCR-based marker. This marker has wide application for all organisms, regardless of the availability of information about their genome sequence (Shi *et al.* 2010). ISSR markers are more reproducible than RAPD (Thimmappaiah *et al.* 2009) and have been proven to be a simple and reliable marker system for many organisms, especially plants, with highly reproducible results and abundant polymorphisms (Machkour-M'rabet *et al.* 2009).RAPD and ISSR were used to assessment of genetic diversity in many studies. RAPD was used for evaluation of genetic diversity among different genotypes of *B. napus* and results confirmed that RAPD is a simple, cheep and fast method for evaluation of genetic diversity of *B. napus* (Fazeli *et al.* 2008). Shengwu *et al.* (2003) used RAPD markers for evaluation of genetic diversity of *B. napus* germplasm for China and Europe and reported the occurrence of a considerable genetic variation between Chines and European accessions. Jabbarzadeh *et al.* (2010) used ISSR markers for genetic diversity analysis of rose species and reported that ISSR markers were chosen because the technique is very simple, fast, cost effective, highly discriminative, reliable and require small quality of template DNA. Also, RAPD and ISSR markers were used together and result showed that the genetic variation among ash gourd inbred lines examined, herein, defined a marker array for the development of a standard reference for further genetic analysis, and the selection of potential parent for predicting hybrid performance and heterosis (Veerendra *et al.* 2007).

MATERIAL AND METHODS

In this study, 97 varieties of canola seed were prepared from seed and plant improvement institute oilseeds sector. The experiment was conducted at the research farm of the Tehran University in Karaj in simple lattice design 10 × 10 were planted. Composite soil sampling was made in the experimental area before the imposition of treatments and was analyzed for physical and chemical characteristics.

Table 1: List cultivars in the first year.

ACSN-1	Bristol	Eureka	Licord	Olpro	Rasmus	Talent
ACSN-3	Brown muster	Express	Lirandra	Opera	RGS-003	Taparoo
Adder	C.V. Stas	Fornax	Loras	Option-500	Roby	Tor
Agat	Calibra	Geranimo	Maluka	OR2-8/99	Ryder	Turner
Akamar	Cheyenne	Hayola330	Midas	Orient	Sedo	Ural
Alice	Cobra	Herald	Modena	oriental muster	Sinatra	Valesca
Ascona	Colvert	Hopper	Mozart	Orkan	SLM-046	VDH-8003-98
Astus	Consul	Hylite	NDE-078	Oro	Sonja	VDH-8003-98
Atilla	Dexter	Hyola 308	NSA-1	Parade	Starlite	Westar
Aviso	Digger	Hyola 401	NSA-2	PAU-C61	Sunday	Wotan
Banjo	Ebonite	Hyola 420	Okapi	Pf.6098	SW 5001	WW-559
Belinda	Elite	Hyola 60	Olano	PF7045/91(Sarigol)	SW High Level	Yantar
Bellini	Elvis	Jupiter	Olara	Premier	SW-C3160	Zarram(R/C)
Boomrang	Embleme	Kova	Olga	R.S.S-963	SWC-3-H-97	

Thinning operations to achieve the proper density of wintering and in the rosette stage was performed. To control weeds, plant cultivation was done in the early reproductive stage. Data collected were subjected to statistical analysis by using a computer program MSTATC and SAS. Based on the results of Correlation and stepwise regression, path analysis was performed using SPSS software and the direct and indirect effects traits were obtained. To describe the genetic diversity and group of societies based on the traits, genotypes NTSYS software using the method of classification of full correlation and similarity coefficient using the Euclidean distance as the similarity criterion were classified. The experiment was conducted at the research farm of the Tehran University in Karaj in simple lattice design 9 × 9 were planted.

Table 2: List cultivars in the second year.

Code	**Variety**	**Code**	**Variety**	**Code**	**Variety**	**Code**	**Variety**	**Code**	**Variety**	**Code**	**Variety**
1	ACSN1	14	Cobra	27	Herald	40	Mozart	53	Parade	66	Sunday
2	Adder	15	Colvert	28	Hopper	41	Mustard	54	PAU-C61	67	SW 5001
3	Agat	16	Consul	29	Hylite	42	NDE-078	55	Pf.6098	68	SW High Level
4	Akamar	17	Dexter	30	Hyola 308	43	NSA-2	56	Sarigol	69	Talent
5	Atilla	18	Digger	31	Hyola330	44	Okapi	57	R. S. S-963	70	Taparoo
6	Aviso	19	Ebonite	32	Hyola 401	45	Olara	58	Rasmus	71	Turner
7	Banjo	20	Elite	33	Hyola420	46	Olpro	59	RGS-003	72	Ural
8	Bellini	21	Elvis	34	Hyola 60	47	Opera	60	Roby	73	Valesca
9	Bristol	22	Embleme	35	Kova	48	Option-500	61	Ryder	74	VDH-8003-98
10	B. Mustard	23	Eureka	36	Licord	49	OR2	62	Sedo	75	Wotan
11	CV Stas	24	Express	37	Lirandra	50	Orient	63	Sinatra	76	Y. Mustard
12	Calibra	25	Fornax	38	Midas	51	Orkan	64	Sinatram	77	Zarfam
13	Cheyenne	26	Geranimo	39	Modena	52	Oro	65	SLM-046		

RESULTS AND DISCUSSION

A. The number of days to the beginning of flowering

Among the studied genotypes, genotypes number 23, 30, 59, 1, 32, 78 and 3 (155 day for genotype number 3 and 159.8 day for genotype number 23) lowest value was observed. In contrast, this trait for genotypes 66. 27, 73, 68, 8, 75, 19, 6 and 10 (185 day for genotype number 66 and 180 day for genotype number 10) highest value was observed.

Table 3: Anova analysis of the rape seed.

S.O.V	df	The number of days to the beginning of flowering	The number of days to the completed of flowering	The number of days to the end of flowering
R	1	390.45	367.51	426.97
Block	16	1.5883	3.7388	0.8927
Uncorrected treatment	80	83.0682**	76.0374**	100.04**
Error	64	1.9729	1.8465	2.0117
*, **, ns: significant at p<0.05 and p<0.01 and non-significant, respectively.				

Table 4: Comparison of different traits of canola varieties.

	The number of days to the beginning of flowering		The number of days to the completed of flowering		The number of days to the end of flowering	
Genotype	Rank	Mean	Rank	Mean	Rank	Mean
1	VWXY	157.5	BCD	163.36	PQ	179.25
2	DEFGHIJK	176	ABCDEFGHK	181.35	CDEFGHIJ	199.5
3	Y	155	CD	161..97	PQ	179
4	HIJKLMN	173.5	EFGHIJKLMNOPQRS	178.15	ABCDEFGHI	200.75
5	IJKMNOP	171.75	KLMNOPQRSTUVW	175.32	ABCDEFG	201.75
6	ABCDEFG	180	ABCDEFGH	182.23	ABCD	205
7	JKMNOPQ	169.75	PORSTUVWX	173.69	ABCDEFG	202.5
8	ABCDE	181	ABCDEF	183.07	ABCDEFG	202
9	EFGHIJKLM	175	CDEFGHIJKLMNOP	180	ABCDEF	203
10	ABCDEFG	180	ABCDEFG	182.79	AB	206.25
11	QRSTU	163.75	TUVWXY	171.28	IJKLM	194.75
12	CDEFGI	177	ABCDEF	183.15	ABCDE	204
13	HIJKLM	172	FGHIJKLMNOPQRST	177.09	CDEFGHIJ	199.5
14	IJKLMNOP	175.25	DEFGHIJKLMNOP	179.25	BCDEFGHI	200.5
15	EFGIKM	171	TUVWXY	174.67	ABCDEFGHI	201
16	DEFGHIJKL	175	ABCDEF	179.13	ABCDEF	203
17	FGHIJKLM	175.75	CDEFGHIJKLMNO	180.51	ABCDEFGHI	201.25
18	FGHIJKLM	174	DEFGHIJKLMNOPQRST	179.18	FGHIJK	197.25
19	ABCDEFG	180	ABCDE	183.41	ABCDEFGHI	201.5
20	HIJKMNO	172.75	IJKLMNOPQRSTUV	175.9	EFGHIJ	198
21	EFGHIJKLM	175	EFGHIJKMNOPQRS	178.53	DEFGHIJ	199
22	MNOPQR	168.75	LMNOPQRSTUVW	174.96	ABCDEF	203
23	UVWXY	159.75	BCD	163.62	NO	187.25
24	FGHIJKMN	174	EFGHIJKLMNOPQRS	177.79	ABC	206
25	HIJKLMOB	173	NOPQRSTUVWX	174.5	ABCDE	204
26	MNOPQQR	169.25	LMNOPQRSTUVW	174.88	DEFGHIJ	199
27	AB	184	AB	186.8	ABCDEFG	201.75

Any two means not sharing a common letter differ significantly from each other at 5% probability

B. The number of days to the completed of flowering

Among the studied genotypes, genotypes number 79, 59, 32, 78 and 3 (160.54 day for genotype number 79 and 161.97 day for genotype number 3) lowest value was observed. In contrast, this trait for genotypes 27, 73, 12, 8 and 49 (186.8 day for genotype number 27 and 182.01 day for genotype number 49) highest value was observed.

C. The number of days to the end of flowering

Among the studied genotypes, genotypes number 78, 32, 30, 31 and 3 (177.24 day for genotype number 78 and 179 day for genotype number 3) lowest value was observed. In contrast, this trait for genotypes 74, 41, 10, 60 and 46 (207.25 day for genotype number 74 and 202.25 day for genotype number 46) highest value was observed.

Table 5: Comparison of different traits of canola varieties.

	The number of days to the beginning of flowering		The number of days to the completed of flowering		The number of days to the end of flowering	
Genotype	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**
28	EFGHIJKLM	.175	BCDEFGHIJKMNO	180.73	BCDEFGHIJ	200
29	IJKMNOP	171.25	MNOPQRSTUVW	174.97	GHIJK	196.25
30	UVWXY	159	BCD	163.35	PQ	178
31	TUVWX	161	ABCD	164.28	PQ	178.5
32	WXY	156.5	CD	161.2	Q	177.5
33	STUVW	162	ZABCD	164.86	OP	183.5
34	OPQRST	167	UVWXYZ	170.32	MNO	189
35	JKLMNOP	170	OPQRSTUVWX	174.45	FGHIJK	196.75
36	JKLMNOP	171.75	HIJKMNOPQRSTU	176.38	BCDEFGHIJ	199.75
37	HIJKLMNO	173	EFGHIJKLMNOPQRST	177.48	BCDEFGHIJ	200
38	HIJKLMN	173.5	CDEFGHIJKLMNOP	179.97	ABCDEFGHI	201.25
39	HIJKLMNOP	172	JKMNOPQRSTUV	175.6	ABCDEFGHI	201.25
40	RSTUV	163.25	VWXYZ	170.03	KMN	191
41	DEFGHI	176.75	BCDEFGHIJKM	180.94	AB	206.25
42	DEFGHIJK	176	BCDEFGHIJKLMN	180.86	ABCDEF	203
43	JKLMNOP	171	JKMNOPQRSTUV	175.57	BCDEFGHIJ	200
44	IJKLMNOP	170.75	KMNOPQRSTUVW	175.2	IJKM	194.75
45	EFGHIJKM	175	EFGHIJKLMNOPQRS	178.12	FGHIJK	197.25
46	CDEFGHI	177	ABCDEFGHIJKL	181.21	ABCDEFG	202.25
47	EFGHIJKM	175	EFGHIJKLMNOPQRS	177.7	ABCDEFGHI	200.75
48	PQRST	166.25	XYZAB	168.58	LMN	190
49	DEFGHIJ	176.25	ABCDEFGHI	182.01	BCDEFGHIJ	200
50	IJKLMNOP	171	MNOPQRSTUVW	174.68	BCDEFGHIJ	202.25
51	KLMNOPQ	169.5	HIJKLMNOPQRSTUV	176.09	ABCDEFGHI	201
52	FGHIJKMN	174	HIJKLMNOPQRSTU	176.3	KMN	191
53	FGHIJKMN	174	EFGHIJKLMNOPQR	178.68	ABCDEF	203
54	PQRST	166.25	QRSTUWX	172.86	DEFGHIJ	199

Any two means not sharing a common letter differ significantly from each other at 5% probability

Table 6: Comparison of different traits of canola varieties.

	The number of days to the beginning of flowering		The number of days to the completed of flowering		The number of days to the end of flowering	
Genotype	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**	**Mean**
55	GHIJKLMN	173.75	HIJKLMNOPQRSTUV	176.02	EFGHIJ	198
56	IJKMNOP	170.75	RSTUVWX	172.76	JKLM	194
57	HIJKLMNOP	172	MNOPQRSTUVW	174.64	HIJKLM	195
58	IJKLMNOP	171	KMNOQPRSTUVW	175.32	DEFGHIJ	199.25
59	UVWXY	158.25	D	160.74	NO	187.75
60	IJKLMNOP	170.5	KLMNOQPRSTUVW	175.15	ABCDEFG	202.5
61	GHIJKLMN	173.75	EFGHIJKLMNOPQRS	177.61	DEFGHIJ	199
62	HIJKMNOQP	172	HIJKLMNOPQRSTUV	176.24	HIJKLM	195
63	IJKMNOQP	171.25	HIJKLMNOPQRSTUV	175.92	FGHIJK	197
64	IJKMNOQP	171.5	HIJKLMNOPQRSTUV	176.04	FGHIJK	197
65	NOPQRS	168	STUVWXYZ	172.28	ABCDEFGHI	201
66	A	185	A	187.16	ABCDEFG	202
67	BCDEFGH	178.5	ABCDEFGHIJ	181.84	DEFGHIJ	199
68	ABCD	181.75	ABC	185.76	AB	206.25
69	HIJKLMNO	173	EFGHIJKLMNOPQRS	178.17	DEFGHIJ	199
70	PQRSTU	166.25	WXYZ	169.38	MNO	189
71	JKLMNOP	171.75	JKLMNOPQRSTUV	175.76	CDEFGHIJ	199.5
72	EFGHIJKM	175	BCDEFGHIJKLM	180.94	EFGHIJ	198
73	ABC	183	ABCD	185.26	ABC	206
74	DEFGHIJ	176.25	ABCDEFGHIJ	181.75	A	207.25
75	ABCDEF	180.25	ABCD	185.13	ABCDEFGHI	201
76	DUWX	176	CDEFGHIJKLMNO	180.31	NOPQ	200
77	HIJKLMNO	173	IJKLMNOPQRSTUV	175.72	GHIJKL	196
78	WXY	156.28	CD	161.17	Q	177.24
79	UVWXY	158.18	D	160.54	NO	187.34
80	EFGHIJKM	175.2	DEFGHIJKMNOP	179.2	BCDEFGHIJ	200.45
81	HIJKLMNO	172.98	IJKLMNOPQRSTUV	175.69	GHIJKL	195.98

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