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Research Article

## Evaluation of Genetic diversity and heritability of some rapeseed (*Brassica napus*) genotypes using agronomical and molecular traits

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**Abstract:** High level of heritability and variability were estimated for all traits among genotypes. The genetic similarity of nine genotypes *Brassica napus* was estimated by random amplified polymorphic DNA (RAPD), which ranged from 69% to 94%. The phylogenetic tree resulted in two different clusters; the first including two genotypes which also showed high similarity in agronomic traits and the second included the rest of the genotypes. The genetic diversity was discussed in relation to molecular and agronomical traits. High and positive correlation were recorded for all traits. The high coupled of heritability and variability is considered as a powerful source for the selection of donors in the breeding program, where the genetic gain depends on the availability of genetic variation and high heritability.

**Keywords:** *Brassica napus*, Heritability, Genetic diversity, RAPD-PCR.

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### 1. Introduction

Oilseed rape is an important crop grown in cool, moist climates throughout the world. *Brassica* oilseed species in the recent years hold third position among oilseed crops and present important source of vegetable oil. The most common *Brassica* oilseed crops grown for commercial purposes are rape seeds (*B. campestris* L. and *B. napus*) and mustards (*B. juncea* L. and *B. carinata*). The other *Brassica* species are grown on a small scale. Rapeseed (*B. napus* L.; genome AACC,  $2n = 38$ ) is a relatively young species that originated through spontaneous hybridization between turnip rape (*B. rapa* L.; AA,  $2n = 20$ ) and cabbage (*B. oleracea* L.; CC = 18) (1).

Efficient breeding program depends on the availability of knowledge about genetic variation and the high heritability of traits (2). Genetic diversity is an essential and important factor when we need to select the donors to recover transgressive segregates, facilitate the prediction of heterosis and would be helpful in making preliminary screening before field trails (3-4). Different molecular marker techniques have been used to study and evaluate the genetic diversity among the

genus *Brassica*. Marker assisted breeding is now used to enhance flexibility, choice of the donor or breeding material of classic breeding program in genetic improvement of plant (5-10). Random Amplified Polymorphic DNA (RAPD) was used because it is a simple, quick and convenient procedure requiring smaller quantities of template DNA, reproducibility and disclosure of multiple markers from a single two-primer reaction (11-13).

The objectives of the present work were how to choose the donors for genetic improvement, measure morphological traits, estimate the heritability for all characters under study, calculates the genetic diversity for several *B. napus* genotypes using RAPD markers, and to find possible correlations between molecular and agronomic traits in these genotypes.

### 2. Materials and Methods

#### 2.1 Plant materials

Seeds of nine genotypes rapeseed, *B. napus*, (Hanna, MS 13, MS16, MS31, 6/19, 35/9, H8, Drakkar and Duplo), were planted in the field at Faculty of Agriculture Farm, Fayoum University, Fayoum, Egypt.

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Some of physiological characters were measured at the time of maturity such as plant height, height of the first branch, No. of branches, No. of pods, and seed yield per plant.

## 2.2 DNA isolation

Total genomic DNA was isolated from the seeds using Qiagen mini kit according to Ausubel *et al.*, (14). DNA concentration was quantified by visual comparison of DNA standard on ethidium bromide-stained agarose gel.

## 2.3 RAPD profiling

Polymerase chain reactions (PCR) were performed using 20ng template DNA, 0.5µM 10-mer primer, 1.5mM MgCl<sub>2</sub>, 0.1mM of each dNTPs, 0.5 units of *Taq* DNA polymerase and 10x reaction buffer. The PCR reaction was run with 45 cycles of 1 min. at 94°C, 30 sec. at 94°C, 1 min. at 36°C, 2 min. at 72°C followed by 12 min. at 72°C for extension. A set of 10 decamer primers was used in the PCR reaction (Table 1) and only the clear bands in all genotypes were used in the analysis.

Table 1. Primers used in the study and their sequences.

Primer	Sequence
OP A-01	CAGGCCCTTC
OP A-02	TGCCGAGCTG
OP A-03	AGTCAGCCAC
OP A-04	AATCGGGCTG
OP A-05	AGGGGTCTTG
OP A-06	GGTCCCTGAC
OPAH-17	CCTGGGCTTA
OPAI 04	CCTGGGTTTC
UBC-230	TGAGTGGGTG
UBC-536	ACTTGGCCAC
UBC-223	GGGCAGGGGA
UBC-234	GGGCGGAGT

## 2.4 Data analysis

PCR amplification products (bands) were scored as 1 (present) or 0 (absent) in each primer and calculated the percentage of polymorphic bands. Reproducible and clearly distinguished bands were used in the analysis. Genetic similarity among all genotypes based on RAPD markers was calculated using the similarity index:

$$s = 2N_{ab} / (2N_{ab} + N_a + N_b)$$

Where,  $N_{ab}$  is the number of bands shared by genotypes  $a$  and  $b$  in each pairwise comparison, and  $N_a$  and  $N_b$  are the numbers of bands present in the respective genotypes.

## 3. Results and discussion

### 3.1 Agronomic traits

Analysis of variance among the different genotypes revealed significant differences for plant height, yield, No. of the buds and highest of the 1<sup>st</sup> branch but not for No. of branches (Table 2). The analysis of variance showed high variability regarding plant height between genotypes, Hanna, Drakkar and H8 were the highest

while Duplo, 6/18 and 35/9 were the lowest values. Plant height is an important trait and widely used as an index in determining the effect of chemical and physical mutagens on biological organism (15).

No. branches per plant ranged from 6.9 in Drakkar to 4.2 in 26/18 but no significant differences were found. For the No. pods, the analysis of variance was highly significant where Drakkar was the highest and Duplo and 26/18 showed the lowest number. The first branch and yield (g/plant) had the same tendency with the different genotypes; Hanna had the highest values in the two traits followed by MS31 and Drakkar while Duplo, 26/18 and 35/9 were the lowest (Table 2). Hanna, Drakkar and MS31 showed the highest values and high similarity in all traits under study and Duplo, 26/18 and 35/9 with the lowest values (Table 2). The interactions between genotypes and environmental factors have the highest influence on the formation of seed yield per plant in rapeseed (16).

### 3.2 Heritability

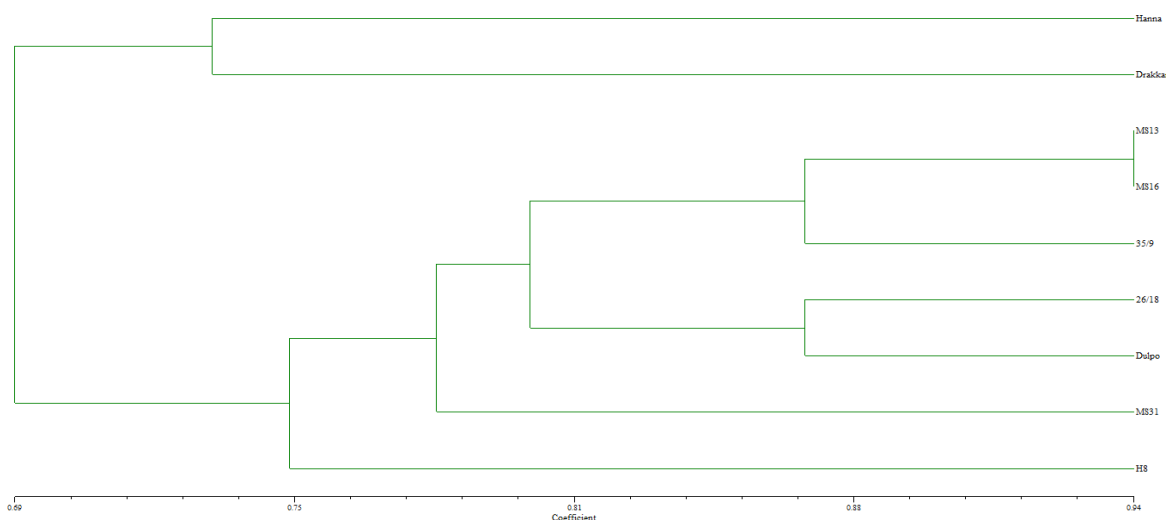
The heritability was calculated for all traits. The highest broad sense heritabilities under the study conditions were observed for No. branches and plant height (99.1 and 95.9 respectively), whilst the seed/plant (g) had the lowest heritability estimates (84.7). The genetic advancement as percent mean was found to be the highest for No. branch and 1<sup>st</sup> branch followed by plant height and No. pods, the lowest genetic advancement was for yield (seed/plant). The higher degree of heritability calculated indicates the effect of additive gene(s). The high coupling of heritability and variability is considered as a powerful source for selecting the donors in the breeding program which the genetic gain depends on the availability of genetic variation (17-18).

### 3.3 RAPD-PCR

Among 10 decamer primer tested, 6 amplified clear bands for RAPD profiling. The RAPD polymorphism in each primer ranged from 4 to 12 bands. The clear bands amplified with different primers were scored ranging from 150 bp to 2027 bp. A total of 67 bands was scored in all primers and genotypes. The analysis of genetic similarity among the nine genotypes and sex RAPD primers was carried out in order to provide some helpful information on the application of molecular markers to rapeseed breeding program. The similarity ranged from 0.7 to 0.94. The results of the dendrogram indicate the presence of two clusters; the first including only Hanna and Drakkar and the second includes the rest of genotypes (Fig. 1). The high similarity was found between MS13 and MS16 (94%) and the high distance was between H8 with Hanna and Drakkar (69%). High relationship between the molecular and agronomical traits which was reported by Seyis *et al.*, (19) indicated that the genetic differences were correlated with the heterotic yield potential in experimental hybrids.

**Table 2.** Mean values of plant height (cm), No. of buds, Yield (g/plant), No. of branch, higher of 1<sup>st</sup> branch (cm) and heritability.

Genotype	plant height (cm)	No. branch	No. buds	higher 1 <sup>st</sup> branch (cm)	yield (g/plant)
Hanna	182 a	6.7 a	166 ab	100 a	17.5 a
MS13	136 bc	5.8 a	145 b	75 b	14.2 c
MS16	137 bc	5.9 a	146 b	79 b	14.3 c
MS31	165 a	6.7 a	164 ab	97 a	16.7 ab
26/18	115 c	4.2 a	85 cd	62 c	12.1 d
35/9	133 c	5.7 a	103 c	65 bc	10.9 d
H8	160 ab	5.6 a	162 ab	73 bc	14.3 c
Duplo	110 c	5.2 a	63 d	52 d	11.7 d
Drakkar	166 a	6.9 a	199.6 a	71 bc	15.1bc
H <sup>2</sup>	95.9	99.1	86.2	88.1	84.7

**Fig. 1.** Dendrogram using Average Linkage (Between Groups) for 9 genotypes based on RAPD data of all (6) primers.

Genetic diversity analysis using molecular markers has the potential to identify novel genetic variation that might assist in the future improvement of heterotic potential in *B. napus*. Molecular markers linked to numerous agronomical important traits have been reported, and a number of them are now successfully integrated into oilseed breeding programs (10, 19-20). *B. napus* has a short history of domestication and high similarity demonstrated between the materials was expected due to its narrow gene pool.

### 3.4 Correlation studies

The correlation coefficients generally highlight the pattern of association among seed/plant components and growth attributes, depicting how yield, as a complex character is expressed. There are high and positive correlations between all traits under study. Seed/plant is positively correlated with the No. branches, No. pods, plant height and 1<sup>st</sup> branch (0.397\*, 0.731\*\*, 0.744\*\* and 0.7565\*\*, respectively) indicating that seed/plant is highly affected by all of these traits. Plant height showed positive correlation with No. branches, 1<sup>st</sup> branch and No. pods (0.676\*\*, 0.767\*\* and 0.790\*\*, respectively) which revealed that higher genotypes would have a high number of branches and pod and subsequently high yielding. Similar findings have also been reported (21-25).

## 4. Conclusion

The results of ANOVA analysis demonstrated high variability of all traits studied except No. branches. High heritability was estimated for all traits among genotypes. The traits with high heritability, high variability and high genetic advance are important and should be considered for genetic improvement. The phylogenetic tree resulted in two different clusters, the first including two genotypes which showed high similarity in agronomic traits, and the second included the rest of genotypes. The higher genetic diversity index should be addressed as a potential cross material in different breeding programs. Hybrids from crossing between the higher genetic distances might have high heterosis for the desired traits. Knowledge of genetic variation is useful and helpful to select hybrid breeding programs but further study is needed to deeply investigate and link breeding traits to DNA markers for *Brassica* breeding and improvement.

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