

Genetic Diversity among Selected Varieties of *Brassica napus* (Cruciferae) Based on the Biochemical Composition of Seeds

N. Nasr, M. Khayami*, R. Heidari, R. Jamei

Department of Biology, Faculty of Science, Uroumieh University, Uroumieh, Iran

* Corresponding author, e-mail: khayami26@yahoo.com

(received: 12/9/2004 ; accepted: 2/1/2006)

Abstract

This research deals with the seed contents of some varieties of *Brassica napus* (cvs. Ebonit, Bristol, VDH80001, Option, PF, Colvert, Orient, Cobra, IRA, Rigent×Cobra) using the following approaches: 1) measurement of the total seed protein which yielded protein content ranged from 18.46 to 30.8/100 gr of dry weight in VDH8001 and Bristol varieties, respectively; 2) study of seed storage protein using gel electrophoresis; 3) extraction of the total seed oil yielding contents ranging from 37% to 44% gr/100 of dry weight in Rigent×Cobra and Cobra varieties, respectively; 4) determination of fatty acids contents and their kinds. Among five major isolated fatty acids, oleic and stearic acids have the highest and the lowest values, respectively. The genetic linkage among the seeds belonging to different varieties was investigated based on fatty acids as well as proteins.

Keywords: *Brassica napus*, SDS-PAGE electrophoresis, seed protein, fatty acids.

Introduction

A great deal of research has been focused on oil crops of various plants, especially members of the mustard family (Brassicaceae or Cruciferae) such as species of *Brassica*. Extensive reviews focused on seeds of various plants with respect to their oil extracts. It has been shown that high amount of protein and high percentage of seed oil increase the food value (Stello *et al.* 1990). Moreover, the plants with high food value are important sources of certain drugs. Oilseed rape (*Brassica* and related species, Brassicaceae) is now the second largest oilseed crop in world providing 13% of the world supply. The world commerce is largely supplied by two species, *Brassica napus* L. and *B. rapa* L. Seeds of these species commonly contain 40% or more oil and produce meal with 35-40% protein. Rapeseed oil has a high concentration of oleic acid (60%), and contains moderate levels of linoleic acid (20%) and linolenic acid (10%). This fatty acid composition of a vegetable oil is considered by many nutritionists ideal for human nutrition and superior to that of many other plant oil (Rakow & Raney 2003). Rapeseed oil also has the lowest saturated fatty acid content of any vegetable oil, amounting to about 7% of total fatty acids,

whereby palmitic acid with about 4% and stearic acid with about 2% of the total fatty acids, are the major saturated fatty acids in rape seed oil (Adamska *et al.* 2004).

The food value of canola, one of the many cultivars or so-called "varieties" of *Brassica napus* L., is known for its low amount of saturated fatty acids (less than 4% palmitic acid) and its considerable high amounts of oleic acid (about 60%). Canola oil affects the reduction of total cholesterol, the low density lipoproteins of blood and the composition of blood phospholipids. Its function is to prevent blood coagulation in the blood vessels.

Several biochemical methods, mostly electrophoresis, have been used to estimate the genetic diversity among different plant species (Hames & Richwood, 1990; Mc Donalds & Brewbaker 1974), and estimating the genetic diversity has been used recently in several approaches, such as phylogenetic reconstruction (Fatokun *et al.* 1992, Kaga *et al.* 1996, Manghan *et al.* 1996), plant breeding and relationships between agricultural varieties (Sakar & Bose 1984, Menancio *et al.* 1993, Kaja *et al.* 1999), linkage maps, and identification of markers connected with resistance genes against pests and diseases.

Seed protein electrophoresis has been widely used in chemotaxonomy mainly because of the stability of these compounds (Gorinstein & Moshe 1991, Krishnan & Sleper 1997). Moreover, the determination and identification of varieties of various agricultural crops has been achieved by means of electrophoretic techniques (Moller & Spoor 1993).

This study focuses on the determination of seed oils and proteins in ten varieties (cultivars) of *Brassica napus*, an important crop grown worldwide for seed oil, protein, and as a vegetable. Moreover, the electrophoresis of seed proteins was conducted in order to calculate the genetic diversity among the selected varieties.

Material and Methods

Ten varieties of *Brassica napus* (Brassicaceae) were studied for total seed protein, seed storage proteins, total oil, and type of fatty acids. The seeds were provided by the Seed Oil Cultivation Company, Esfahan-Iran. The experiments were repeated three times to ensure about the reproducibility of the obtained data. The seeds were washed and powdered separately, and the powders were put in an oven for 24 h at 100° C to dehydration. During the experiments the powders were separately kept in special cans. The selected varieties were Ebonit, Bristol, VDH8001, Option, PF, Colvert, Orient, Cobra, IRA and Rigent×Cobra. For determination of total protein content Kjeldahl's standard method was used (Bilborrow *et al.* 1993, Francois 1994).

Extraction and electrophoresis of seed storage proteins: Storage proteins were identified using sodium dodecylsulphate-polyacrylamid gel electrophoresis (SDS-PAGE). The gel was used in 12.5% (Wendell & Weeden 1989). After calculating number of bands of each sample, the relative mobility of each band was determined. The molecular weight of each band is identified by the standard curve obtained from the standard proteins. Standard proteins used and their molecular weight were: cytochrom-C (12384D), myoglobin (16949D), carboanhydraz (30000 D), ovalbumin (42700 D), albumin (66250 D) and ovotransferrin (78000 D).

Determination of the total oil and fatty acids:

Among all chromatography methods, the identification of fatty acids compositions by gas chromatography (GC) and the use of their methyl esters give the most accurate results (Gordon 1990). A similar method as cited by Gordon (1993) has been carried out in the present study using a "GC1000" instrument (Danny Company, Italy). For measurement and determination of different kinds of fatty acids, their methyl esters were injected to GC. Fatty acids were identified by their retention times as compared with those of appropriate standards. Values for individual fatty acids were expressed as a percentage of the total peak area.

Data analysis: The protein bands were scored as 0 for absence or 1 for presence. The obtained data matrix was used for calculating Nei's genetic distance resulting in a dendrogram based on UPGMA method. Version 10 of SPSS program package was used for statistics in this study. The same procedure was applied to fatty acid data.

Results and discussion

The results of total seed proteins are shown in Tab. 1. Concerning with the quantity of proteins the seeds of all examined *Brassica napus* varieties have a high amount of protein and can be regarded as a good source of proteins.

The results obtained from SDS-PAG electrophoresis (Fig. 1) showed that the method provides a powerful tool for reliable variety identification based on genetic differences in seed storage protein composition among different varieties of *Brassica napus*. Variety identification was possible in all samples by using SDS-PAGE electrophoresis of seed storage proteins. The varieties 1 (Ebonit), 4 (Option) and 5 (PF) show the most (19) and varieties 6 (Colvert) and 7 (Orient) show the least (12) protein bands.

The obtained dendrogram classified the varieties in 5 classes (Fig. 2): class I includes the varieties 2, 10, 8 and 3 (Bristol, Rigent×Cobra, Cobra, VDH800 I, respectively); class 2 includes varieties 4, 5 and 1 (Option, PF, Ebonite, respectively), class 3 includes solely the variety 6 (Colvert), class 4 includes variety (Orient); and class 5 includes the variety 9 (IRA). Class 2 varieties show the least genetic

distance and consequently have the most genetic linkage. Moreover, class 2 has the most linkage with class 1 and the least linkage with three other classes. Therefore, according to this test and the result of others the use of storage seed protein is recommended as far as linkage and genetic distance are concerned.

Tab. 1- Total seed protein (gr per 100 gr dry weight) of 10 selected varieties of *Brassica napus*.

Variety	Mean \pm Standard deviation
Ebonit	23.81 \pm 0.05
Bristol	18.46 \pm 0.066
VDH8001	30.47 \pm 0.20
Option	20.53 \pm 0.28
PF	24.74 \pm 0.19
Colvert	21.24 \pm 0.076
Orient	23.00 \pm 0.058
Cobra	22.21 \pm 0.024
IRA	18.99 \pm 0.098
Cobra \times Regent	20.21 \pm 0.17

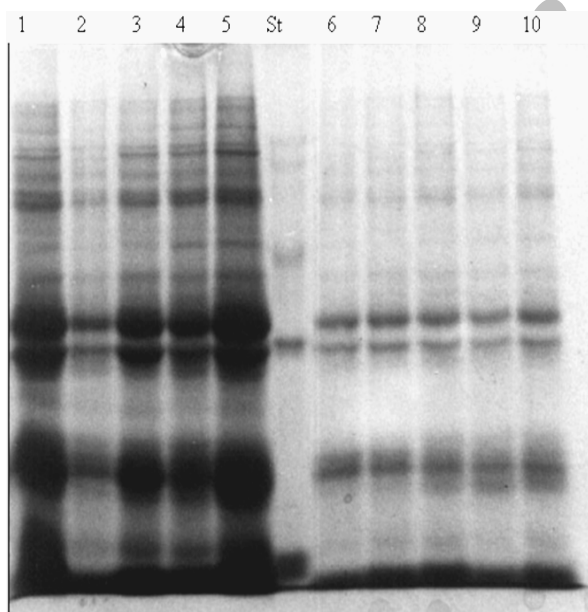


Fig. 1- Seed protein banding patterns of 10 *Brassica napus* varieties. Numbers at the top of the columns indicate the varieties: 1- Ebonit, 2- Bristol, 3- VDH8001, 4- Option, 5-PF, 6- Colvert, 7- Orient, 8- Cobra, 9- Ira, and 10- Rigent \times Cobra. St: standard proteins (see the text).

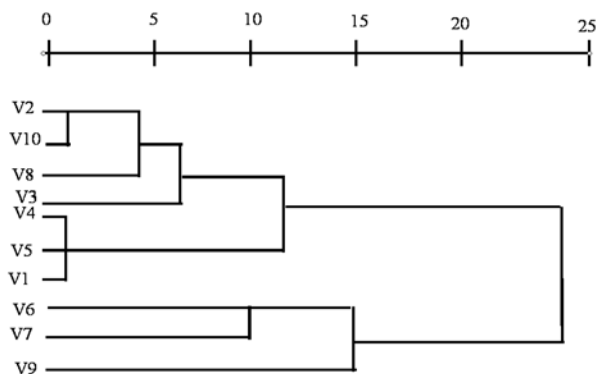


Fig. 2- Dendrogram obtained from Nei genetic distance and UPGMA based on band scoring of SDS-PAGE gel electrophoresis shown in fig. 1. The varieties are V1-Ebonit, V2- Bristol, V3- VDH8001, V4- Option, V5- P.F, V6- Colvert, V7- Orient, V8- Cobra, V9- Ira, and V10- Rigent \times Cobra.

The results of oil and fatty acids obtained from GC (Tab. 2 and Tab. 3) show that all ten varieties have high amounts of oleic and linoleic acid. Linoleic acid is the most important unsaturated fatty acid from nutritional point of view, because it cannot be synthesized in human body and, therefore, should be supplied by food. Oleic acid is one of the most important unsaturated fatty acids with high resistance against oxidation and is considered as highly efficient oil for different applications. Downey (1999) found that fatty acid composition of *Brassica napus* are: 6% palmitic, 1.5% stearic, 58% oleic, 23% linoleic and 7% linolenic acid, that is nearly similar to our results. Moreover, Velasco *et al.* (1999) obtained the mean of oil content about 44% and the percentage of Oleic acid 70.3%. The oil content reported by them is similar to our results but the amount of oleic acid is higher than our results.

The dendrogram obtained from the fatty acids analysis (not shown) do not match with the results obtained from seed storage proteins. The data based on protein analysis are much reliable than those of fatty acids, because the proteins belong to semantic molecules which have more phylogenetic value than the fatty acids.

Tab. 3- The linkage between the total oil percentages of studied varieties. For a list of varieties see Fig. 1. They are considered in 4 classes.

Varieties	Class A	Class B	Class C	Class D
8	37.2000			
3	37.9667	37.9667		
5		40.2667	40.2667	
1		40.3333	40.3333	
9		40.3667	40.3667	
7			41.0333	
2			42.1667	42.1667
6			42.3000	42.3000
4			43.0000	43.0000
10				44.3667

Comparing with other investigations, the obtained results are meaningful and close to other studies. AL-Barzinjy *et al.* (2003) reported the oil and protein contents in a range of 42.3-46.5 and 18.9-19.5 gr per 100 gr dry weight, respectively. Similar results were obtained by Siddiqui (2004) as well as by Declercq & Daun (1998). Asghar Malik *et al.* (2004) concluded that percentage of seed oil content in 7 varieties is 42.3-45.1 gr per 100 gr dry weight and the content of protein in these varieties is 19.2-23 gr per 100 gr dry weight.

References

- Adamska E., Cegielska T., Kakzmark Z., Szala L. 2004: Multivariate approach to evaluating the fatty acid composition of seed oil in a double haploid population of winter oilseed rape (*Brassica napus* L.). *J. Appl. Genet.* **45(4)**: 419–425.
- AL-Barzinjy M., Stolen O., Christiansen J.L. 2003: Comparison of growth, pod distribution and canopy structure of old and new cultivars of oilseed rape (*Brassica napus* L.). *Soil Plant Sci.* **53**: 138–146.
- Asghar Malik M., Imran Aziz Z.K., Ashfaq Wahid M. 2004: Growth, seed yield and oil content response of canola (*Brassica napus* L.) to varying levels of sulphur. *Int. J. Agric. Biol.* **6**: 1153–1155.
- Bilborrow P.E., Evans E.J., Zhao F.J. 1993: The influence of spring nitrogen on yield components and glucosinolate content of autumn sown oilseed rape (*Brassica napus* L.). *J. Agric. Sci. Camb.* **120**: 219–224.
- Declercq D.R., Daun J.K. 1998: Quality of western Canadian canola. *Canadian Grain Commission, Crop Bulletin No. 236*.
- Downey R.K. 1990: Canola: a quality *Brassica* oilseed. Timber Press, Portland. Pp: 217.
- Fatokun C.A., Menancio H.D., Danesh D., Young, N.D. 1992: Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. *Genetics* **132**: 842-846.
- Francois L.E. 1994: Growth, seed yield, and oil content of canola grown under saline condition. *Agron. J.* **86**: 233–237.
- Gordon M.H. 1990: Principles and application of gas chromatography in food analysis. In: Ellis Horwood (ed) Series in Food Science & Technology. New York.
- Hames B.D., Richwood M. 1990: Gel electrophoresis of proteins, a practical approach. Oxford University Press, UK.
- Kaga A., Tomooka N., Egava Y., Hosaka K., Kamijima O. 1996: Species relationship in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. *Euphytica* **88**: 17–24.
- Krishnan H.B., Slepner D.A. 1997: Identification of tall fescue cultivar by sodium dodecyl sulphat polyacrylamid gel electrophoresis of seed proteins. *Crop Sci.* **37**: 215–219.
- Mc Donalds T., Brewbaker J.L. 1974: Isoenzyme polymorphism in flowering plants. *J. Heredit.* **65**: 37–42.
- Menancio H.D., Fatokun C.A. Kumar L. Danesh D., Young N.D. 1993: Comparative genome analysis of mungbean (*Vigna radiata* L.) and cowpea (*Vigna unculata* Walpers) using RFLP mapping data. *Theor. Appl. Genet.* **86**: 797–810.
- Moller M., Spoor W. 1993: Discrimination and identification of *Lolium* species and cultivars by rapid SDS-PAGE electrophoresis of seed storage proteins. *Seed Sci. Technol.* **21**: 213–223.
- Rakow G., Raney JP. 2003: Present status and future perspectives of breeding for seed quality in *Brassica* oilseed crop. Proc. 11th Int. Rape Seed Congress, Copenhagen, Denmark, Pp. 181-185.
- Siddiqui M.H., Oad F.C., Burior U.A. 2004: Yield and oil content of various canola (*Brassica napus* L.). *Asian J. Plant Sci.* **3(2)**: 258–259.
- Velasco L., Moller C., Becker H.C. 1999: Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rape seed (*Brassica napus* L.). *Euphytica* **106**: 79–85.
- Wendel J.F., Weeden N.F. 1989: Visualization and interpretation of plant isozymes. In: Soltis, D.E., Soltis, P.S. (eds.) *Isozymes in plant biology*. Dioscorides Press, Portland, Oregon, Pp. 5–45.