

Full Length Research Paper

# Study of seed proteins pattern of *brassica napus* varieties via sodium dodecyl sulfate polyacrylamid gel electrophoresis

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Accepted 27 January, 2011

Canola (*Brassica napus* L.) is an oilseed crop adapted to various environments. The current research was conducted to determine the seed storage protein profiles of 12 *Brassica napus* cultivars (Geronimo, Celecious, Milena, Sahara, Sunday, Zarfam, Dante, SLM-046, Talaye, Talent, ARC-2, Opera) that were analyzed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on biochemical markers. For this purpose total soluble proteins were resolved on 15% SDS polyacrylamide gels. Then seed protein was extracted by extraction buffer. The results of SDS-PAGE were shown 17 bands on gel that they had high polymorphism. Finally genotypes were clustered into three groups by applying un-weighted pair group mean analyses.

**Key Word:** *Brassica napus* L., Seed, SDS-PAGE, Cluster analysis.

## INTRODUCTION

The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties [Isemura et al., 2001]. The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins [Ullah et al., 2010]. However, the information on the SDS-PAGE on different species of *Brassica* for genetic diversity is still limited [Rahman and Hirata, 2004]. Seed storage protein profiles have also been used to study evolutionary relation of several crop plants [Ravi et al., 2003]. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varieties improvement [Sadia et al., 2009]. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. [Mukhlesur and Hirata, 2004] Acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has become one of the most broadly used techniques to separate and characterize proteins [Laemmli 1970].

Since, seed storage protein analysis can be a practical

tool for identification of species, varieties and cultivars.

The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE) Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins [Ullah et al., 2010]. Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding [Sadia et al., 2009].

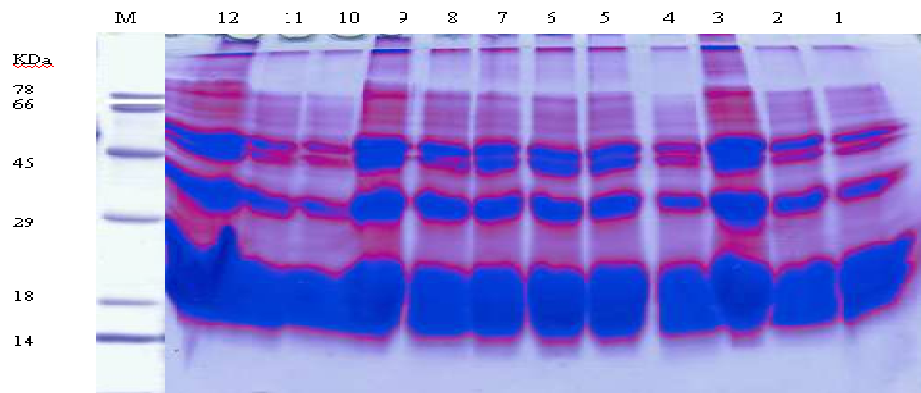
The aim of the current study was to evaluate seed protein variability in different *Brassica napus* genotypes and grouping them.

## MATERIAL AND METHODS

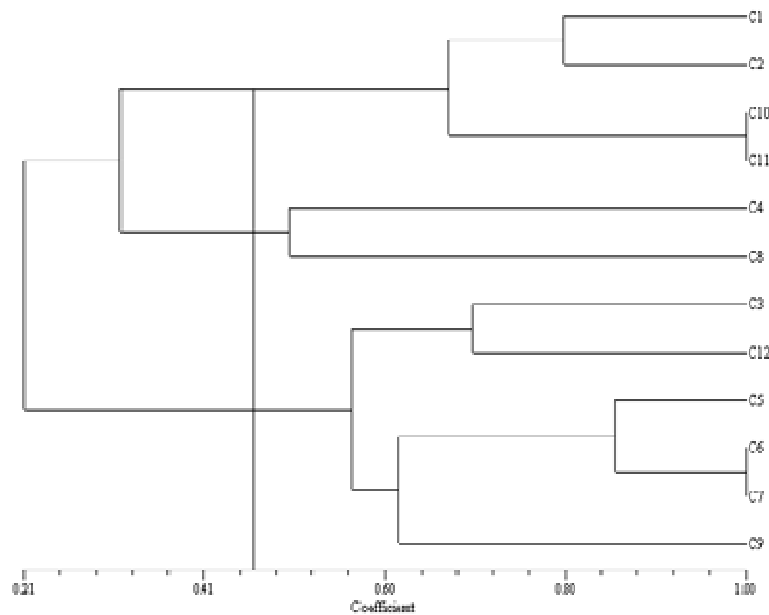
### Protein Extraction, Gel Electrophoresis and Staining

This study was carried out with 12 *Brassica napus* cultivars (Geronimo, Celecious, Milena, Sahara, Sunday, Zarfam, Dante, SLM-046, Talaye, Talent, ARC-2, Opera). These varieties are cultivated more than others in Iran. The seeds were powdered separately. Cultivars seed protein were extracted with extraction buffer (Tris-HCl, pH=8.5; NP-40, /2%; PMSF, 1mM and EDTA, 1mM), following the method described by Xi and collaborators (2006) with some modifications (Kakaei et al., 2010). SDS-PAGE method in resolving gel with 15% acryl amid and stacking gel 5% acrylamid was applied for resolving of these genotypes. At the end

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**Figure 1.** The SDS-PAGE result of seed proteins pattern of *Brassica napus* varieties. (1)Geronimo, (2)Celecious, (3)Milena, (4)Sahara ,(5)Sunday, (6)Zarfam, (7)Dante, (8)SLM-046, (9)Talaye, (10)Talent, (11)ARC-2, (12)Opera



**Figure 2.** Dendrogram of the relationships among 12 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins.

of electrophoresis, protein bands were revealed by Coomassie Brilliant Blue R-250 staining and destaining was done using methanol and acetic acid for 3 hours. The SDS-PAGE was adapted after Laemmli (1970) with some modifications. Protein assay was made according to Bradford (1976). The molecular weight of each band is identified by the standard proteins that their molecular weights were: Ovotransferrin (78 kDa), bovine serum Albumin (66 kDa), Ovalbumin (45kDa), Actinidin (29kDa),  $\beta$  Lactoglobulin (18 kDa) and Lysosyme (14 kDa).

For grouping of genotypes cluster analysis was done and dendrogram drawn based on Jacard's similarity coefficients and UPGMA (unweighed pair group mean analyses) method.

## RESULTS AND DISCUSSION

Electrophoresis of proteins is a powerful tool for detection of the genetic diversity and the SDS-PAGE of seed

protein is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations [Iqbal et al., 2005 and Javid et al., 2004].

Figure 1 showed gel SDS-PAGE, *Brassica napus*. It was detected 17 bands per cultivars in electrophoregrams. The showed polymorphism is on the basis of difference in protein intensity among genotypes. The mostly of diversities such as (added or removed bands) were absorbed in areas with 66-100 kDa and 35-25 molecular weight. The bands with about 35 kDa molecular weight in genotypes numbers 1, 2, 4 and 10 were weaker than other genotypes. The polymorphism in the 15-27 kDa range in most major genotypes is not detectable. Use to SDS-PAGE is reported research [Kakaei et al., 2009: Kakaei and Farshadfar., 2010: Hameed et al., 2009:

**Table 2.** The *Brassica napus* genotypes that have been used in study of seed proteins pattern.

Number	genotypes name	Cluster No.
1	Geronimo	3
2	Celecius	3
3	Milena	1
4	Sahara	2
5	Sunday	1
6	Zarfam	1
7	Dante	1
8	SLM-046	2
9	Talaye	1
10	Talent	3
11	ARC-2	3
12	Opera	1

Farshadfar and Farshadfar 2008; , Kakaei 2009 and Sadia et al., 2009). The relationships among 12 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins has been showed in figure 2 as a dendrogram. The Dendrogram divided genotypes in three clusters. Cluster 1, 2 and 3 comprises of four, two and six genotypes respectively (Table 2). This result showed that there is enough variation for seed protein content among the rapeseed cultivars and we can cross the cultivars that are the most distance (for example cross between 1 and 9) for maximum heterosis achievement.

## ACKNOWLEDGEMENTS

Kermanshah University of Medical Science Medical Biology Research Center, for using laboratory protein electrophoresis.

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