SHORT COMMUNICATION

SABRAO Journal
of Breeding and Genetics
47 (1) 14-20, 2015

GENETIC DIVERSITY OF *Brassica napus* USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

R. CHOUDHARY[1], G. K. RAI[1*], S. K. RAI[2], A. PARVEEN[1], P. K. RAI[3] and R. K. SALGOTRA[1]

[1]School of Biotechnology, SKUAST-Jammu, Chtaha, Jammu 18009, (J&K) India
[2]Division of PBG, SKUAST-Jammu, Chtaha, Jammu 18009 (J&K) India
[3]DLRSS, SKUAST-J, Dhiansar, Jammu, (J&K) India
*Corresponding author's email: gkrai75@gmail.com

SUMMARY

Genetic diversity was studied in 7 genotypes of *Brassica napus* for total seed protein content using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Ten reproducible bands were used for cluster analysis and to estimate the genetic diversity. Out of these bands, five bands were observed polymorphic. A dendrogram was constructed and the genotypes were divided into two main groups comprising 4 clusters. The results obtained from these clusters showed genetic diversity in these accessions on SDS-PAGE level. The results showed that the technique of SDS-PAGE was feasible to distinguish the different species of *Brassica napus* genotypes.

Keywords: *Brassica napus,* sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), genetic diversity, cluster analysis

Short summary statement: Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The highest similarity was recorded in RSPN-25 and RSPN-29, followed by RSPN-28, GSL-1 and DGS-1, GSL-1 (89%). However, the lowest similarity estimates (63%) were recorded in GSC-101 and HNS-0901, followed by 67% in GSL-1 and HNS-0901. The diverse accessions may be used for crossing in breeding programs.

Manuscript received: May 13, 2014; Decision on manuscript: October 28, 2014; Manuscript accepted: January 8, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Brassica is an economically important genus in the *Brassicaceae* family (Syn. Cruciferae). *Brassica* species are widely used in the cuisine of many cultures as recognized as a valuable source of dietary fibre. *Brassica* vegetables contain little fat and are sources of vitamins, minerals and fiber. Oleiferous brassica species viz. *Brassica napus, Brassica campestris, and Brassica juncea* constitute the world's third most important source of edible oils (Gupta and Pratap, 2007). Among the oilseeds in India, *Brassica* ranks second in area and production after groundnut and contributes 26% after total vegetable oil seed output. Among the 3 cultivated species, *Brassica napus* (AACC 2n = 38) is the most important oileferous crop and it is gaining importance because of its high yield potential of 20 qha^{-1} (Anonymous, 1991), wide adoptability and high oil content (44.6%) and good quality (Rai *et al.,* 2007). 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 and 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 rapeseed oil (Adamska *et al*., 2004).

In India, rapeseed mustard is cultivated in an area of 6.51 million ha with a production of 7.67 million tons and with an average yield of 1179 kg/ha. In Jammu and Kashmir (India) 60,000 ha area cultivated under rapeseed-mustard with a production of 48,000 q and 788 kg/ha productivity. (Anonymous, 2010). Looking at the production and productivity of Indian mustard, there is an urgent need for the improvement of *Brassica* crops. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varietal improvement. Quantitative genetic markers are helpful in the estimation of genetic variation. It is helpful in appropriate use of gene pool in specific programs (Sadia *et al.,* 2009). Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.,* 2001; Turi *et al.,* 2010). Seed protein is not sensitive to environmental fluctuations and banding patterns therefore are very stable, and can be used for cultivar identification (Tanksley and Jones, 1981; Nasr *et al.,* 2006; Rai *et al.,* 2011). However, the information on the use of SDS-PAGE on different species of *Brassica* for genetic diversity is still limited (Rahman and Hirata, 2004). Therefore, this study was undertaken to assess the protein polymorphisms and determine the genetic diversity of *Brassica* species using SDS-PAGE.

MATERIALS AND METHODS

Plant Material

Brassica napus (seeds) were collected from Division of Plant Breeding and genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India. Seven genotypes/varieties of *Brassica napus* were electrophoretically characterized using SDS-PAGE at the School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. Genotype/varieties are shown in Table 1).

Table 1. List of *Brassica napus* genotypes used.

Genotype	Source
DGS-1	SKUAST-Jammu
GSL-1,	SKUAST-Jammu
RSPN-25	SKUAST-Jammu
RSPN-28	SKUAST-Jammu
RSPN-27	SKUAST-Jammu
HNS-0901	SKUAST-Jammu
GSL-101	SKUAST-Jammu

Protein extraction and purification

Collected seeds of 7 genotype viz., DGS-1, GSL-1, RSPN-25, RSPN-28, RSPN-27, HNS-0901 and GSL-101 were crushed and grounded with the help of mortar and pestle using CTAB method (Doyle and Doyle, 1987). The seed flour was taken in to a 10 ml test tube. A volume of 5 ml of chloroform, methanol and acetone mixture (2:1:1) was added and mixed well by vortexing. Then the samples were kept at room temperature for overnight. After centrifuging the sample the solvent was removed and taken the defatted seed powder was placed in 1.5 ml eppendorf tubes.

Then the protein extraction buffer (0.6M Tris HCL buffer-pH 6.8 mixed SDS and β-mercaptoethanol) was added. Bromophenol blue was added to extraction buffer as a dye to point out the movement of protein in the gel. All these chemicals were mixed together then the solution was purified and homogenated. The samples were thoroughly by vortexed and centrifuged at 12,000 rpm for 10 minutes at room temperature (RT). After centrifuging the samples, the crude protein recovered as clear supernatant on the top of the tube. Then supernatant were transferred into new 1.5 ml eppendorf tubes and stored at -20^0 C until gel electrophoresis. Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970).

Electrophoresis

Crude protein samples were directly analyzed by SDS-polyacrylamide gel electrophoresis using 12.0% polyacrylamide as resolving gel and 4.5% stacking gel. 20 µg protein samples were loaded with the help of micropipette into the wells of the stacking gel. Electrophoresis was carried out at 20 V for staking gel and 100 V for as resolving gel, until the bromophenol blue (BPB) reached to the bottom of gel plate.

Staining and Distaining

After completion of electrophoresis, the gels were placed in fixing solution (15% TCA) in staining box for overnight. After decanting the fixing solution, pored the 2.0% (w/v) coomassie brilliant blue (CBB) R250 in box. When the staining procedure was completed, then the gel was de-stained by washing with a solution containing acetic acid, methanol and water in the ratio of 5:20:75 (v/v), so that the blue color of the coomassie brilliant blue (CBB) R disappears and the electrophoresis band on gels clearly visible.

Scoring of data and analysis

The protein bands were scored as 0 for absence or 1 for presence for polymorphism. The Jaccard's similarity index was calculated using NTSYS-pc version 2.02e (Applied Bio-Statistics, Inc., Setauket, NY, USA) package to compute pair wise Jaccard's similarity coefficients and this similarity matrix was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) clustering algorithm to obtain a dendrogram.

RESULTS AND DISCUSSION

Bulk seed samples of 7 *Brassica napus* genotypes were used for total protein comparisons. The banding pattern of some of the total seed protein showed close relationship among these studied genotypes, while the difference in banding pattern showed the range of geographic differences (Figure 1 and Table 1). Total 10 bands were detected and which in 5 bands were observed in all genotypes. The ratio of the polymorphic banding was 50%. However, dendrogram was constructed on the basis of these polymorphic bands.

After the study of the banding patterns in these genotypes, 3 zones were observed (A to C) showing variations. As a result, Zone-A was having protein weight from 225 - 90 kDa. Zone-A having total 4 bands which in 2 bands were polymorphic. However, protein bands in Zone-B ranged from 75 to 25 kDa. Protein bands detected in this region were 4, among which 50 kDA protein band were polymorphic. Zone- B comprised both light and dark stained bands, while Zone-C ranged from 15 to 10 kDa. Two Protein bands were produced in this region and single protein band (15 kDA) showed polymorphic (Table 2).

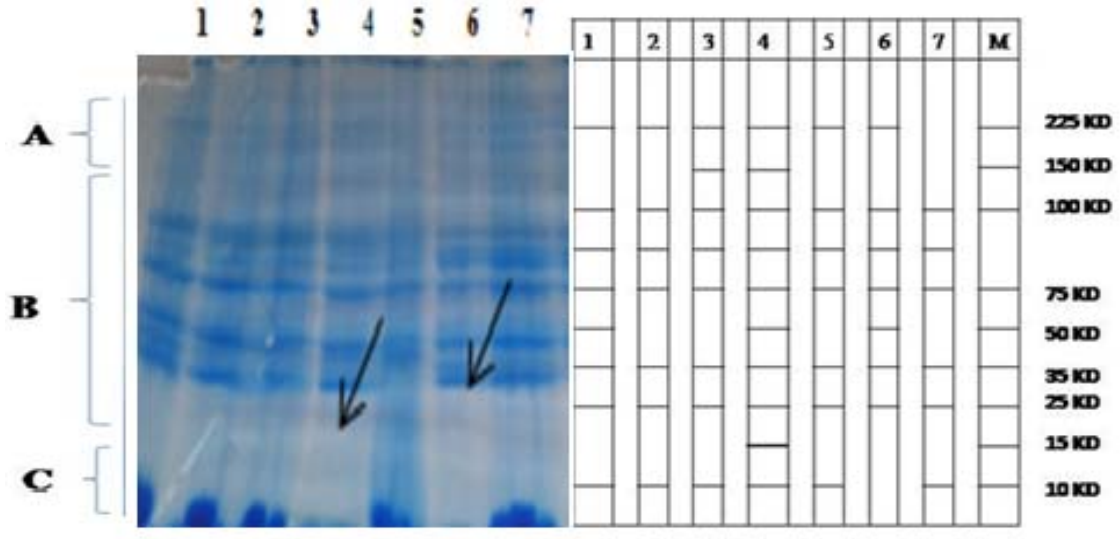

Figure 1. Zymogram of various cultivars of *Brassicas napus* based on SDS-PAGE of total seed proteins. Lane 1 RSPN-28, Lane 2 RSPN-29, Lane 3 DGS-1, Lane 4 GSL-1, Lane 5 RSPN-25, Lane 6 HNS-0901, Lane 7 GSC-101respectively, and M-Molecular weight marker.

Table 2. Presence and absence of protein bands in SDS-PAGE analysis of *Brassica napus.*

Protein region	Protein bands	No. of genotypes/varieties	
		Presence	Absence
GROUP-A	1	6	1
	2	2	5
	3	7	0
	4	7	0
	5	7	0
GROUP-B	6	3	4
	7	7	0
	8	7	0
GROUP-C	9	1	6
	10	6	1

Table 3. Dice Coefficients between different protein types based on SDS-PAGE.

	RSPN-28	RSPN-29	DGS-1	GSL-1	RSPN-25	HNS-0901	GSC-101
RSPN-28	1.00						
RSPN-29	0.88	1.00					
DGS-1	0.78	0.88	1.00				
GSL-1	0.89	0.78	0.89	1.00			
RSPN-25	0.88	1.00	0.88	0.78	1.00		
HNS-0901	0.88	0.75	0.67	0.78	0.75	1.00	
GSC-101	0.75	0.86	0.75	0.67	0.86	0.63	1.00

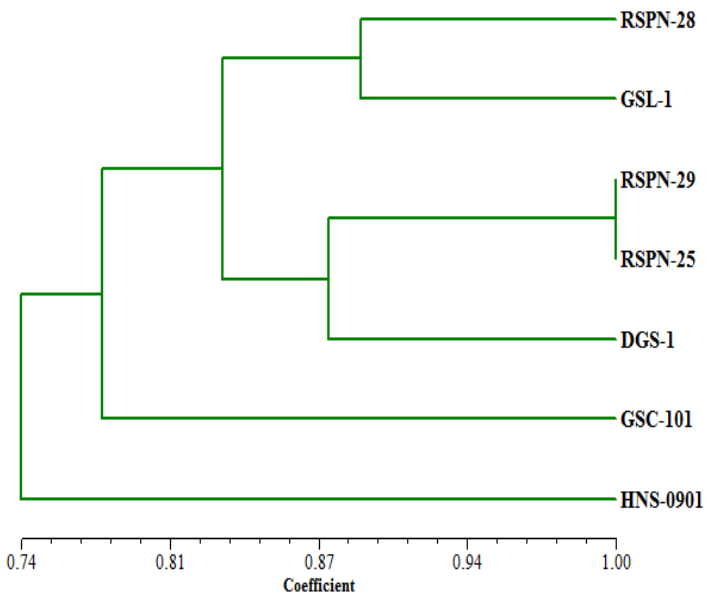

Figure 2. Dendrogram showing the relationships among 7 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins.

Table 4. Cluster pattern of *Brassica napus* genotypes on their genetic divergence.

Protein region	Genotypes/varieties
Class- I	RSN-28 and GSL-1
Class- II	RSPN-29, RSPN-25 and DGS-1
Class- III	GSC-101
Class- IV	HNS0901

Consequently, the region showed both light and dark stained bands. Similar results were found by Turi *et al.,* 2010 and Rabbani *et al.,* 2001 who also reported the same banding pattern in *Brassica species*. Similarly, Kour and Singh (2004) and Nasr *et al.* (2006) obtained the same results.

The results obtained from SDS-PAGE electrophoresis (Figure 1) showed that this method provides a powerful tool for reliable variety identification based on genetic differences in seed storage protein composition among different varieties of *Brassica napus*. Varietal identification was possible in all samples by using SDS-PAGE electrophoresis of seed storage proteins. The genotypes 'GSL-1' showed the highest protein bands (10) followed by 'RSPN-1' and 'DGS-1' (8); 'RSPN-29', 'RSPN-25' and 'HNS-0901' (7) and genotype 'GSC-101' (6). Based on similarity indices, the genotype ranged from 63 to 100%. The highest similarity estimates were recorded in RSPN-25 and RSPN-29, followed by RSPN-28, GSL-1 and DGS-1, GSL-1 (89%). However, the lowest similarity estimates (63%) were recorded in GSC-101 and HNS-0901, followed by 67% in GSL-1 and HNS-0901 (Table 3).

A dendrogram was constructed on the basis of total seed proteins (Figure 2) and classified the varieties into 4 classes. The class -I comprised of 2 genotypes i.e. RSPN-28 and GSL-1; class II includes 3 genotypes (RSPN-29,

RSPN-25 and DGS-1), class III includes the genotype GSC-101 and class IV includes single genotype i.e. HNS-0901. Class II varieties RSPN-29 and RSPN- 25 showed the least genetic distance and consequently have the most genetic linkage. Moreover, class II has the most linkage with class I and the least linkage with three and four classes. Therefore, according to the results in this study, and the result of others, the use of storage seed protein can be recommended for linkage and genetic diversity studies. To study the generic and species level, polyacrylamide gel electrophoresis provides a useful method (Ladizinsky and Hymowitz, 1979) for classical taxonomic classification. However, protein kinds and their differences using SDS-PAGE analysis help the diverse genotypes for breeding programs at seed level and to dig up the record of transparency of genetic resources (Rahman and Hirata, 2004).

From the cluster analysis, it is clear that there is less variation in genotypes. Four clusters and 2 groups were constructed after the analysis of these accessions on a qualitative level (Table 4). The study's finding is further strengthened by the early report of Ghafoor *et al.* (2003), which also concluded the similar results. The results were further strengthened by the earlier findings of Javaid *et al.* (2004) who also reported minimum genetic diversity in groundnut for SDS-PAGE and suggested 2 dimensional (2D) electrophoresis. Turi *et al.* (2010) were also reported similar observations in *Brassica* species. The diverse accessions, having different banding patterns, are suggested to be used in breeding programs. The less divergent may be preserved in the gene bank for use in breeding program (Celis and Bravo, 1984; Beckstrom-Sternberg, 1989).

CONCLUSION

Seed storage protein profiled could be useful marker for genotype identification and diversity analysis (between and within *Brassica* species). Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The electrophoresis of seed storage protein is a method to investigate genetic variation. Out of these bands, 5 bands were observed polymorphic. A dendogram was constructed and the genotypes were divided into 2 main groups. The genotypes 'GSL-1' showed the highest protein bands (10) followed by 'RSPN-1' and 'DGS-1' (8); 'RSPN-29', 'RSPN-25' and 'HNS-0901' (7) and genotype 'GSC-101' (6). Based on similarity indices, the genotype ranged from 63 to 100%. The identified diverse genotypes can be used in future breeding programs for the development of variety.

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.). *Journal of Applied Genet*. 45: 419−425.

Anonymous (2010). Digest of Statistics, Directorate of Economics and Statistics, Govt. of J&K (2010-2011).

Anonymous (1991). Package of practices for Rabi crops. Pp139. Punjab Agricultural University Ludhiana, India

Beckstrom SSM (1989). Two-dimensional gel electrophoresis as a taxonomic tool: evidence from the *Centrospermae*. *Biochem. Syst. Ecol*. 17: 573-5 82.

Celis JE, Bravo R (1984). Two-dimensional gel electrophoresis of proteins; methods and applications. Acad. Press, New York.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19:11-15.

Ghafoor A, Gulbaaz FN, Afzal M, Ashraf M, Arshad M (2003). Interrelationship between SDS-PAGE markers and agronomic traits in chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany.* 35: 613-624.

Gupta SK, Pratap A (2007). History, origin and evolution. *Advances in Botanical Research*, 45:2-20.

Isemura T, Shiyo N, Shigeyuki M (2001). Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. *Breeding Science*, 51: 225-230.

Javaid A, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for

evaluating genetic diversity. *Pak. J. Bot.* 30(1): 25-29.

Kour A, Singh SP (2004). Evaluation of genetic diversity in different genotypes of *Brassica juncea* by SDS-PAGE. 4th Int'l Crop Science Congress, 26th Sep. - Oct. 01, Brisbane, Australia.

Ladizinsky G, Hymowitz T (1979). Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet*. 54: 145-151.

Laemmli UK (1970). Cleavage of structure proteins assembly of the head of bacteriophage T4. *Nature*, 22: 680-685.

Nasar N, Khayami M, Heidari R, Jamei R (2006). Genetic Diversity among Selected Varieties of *Brassica napus* (*Cruciferae*) Based on the Biochemical Composition of Seeds. *JUST.* 32(1): 37-40.

Rabbani MA, Qureshi AA, Afzal M, Anwar R, Komatsu S (2001). Characterization of mustard (*Brassica juncea* L.) germplasm by SDSPAGE of total seed proteins. *Pak. J. Bot.* 33: 173-179.

Rahman MM, Hirata Y (2004). Genetic diversity in *Brassica* species using SDS-PAGE analysis. *Journal* of *Biological Sci.* 4: 234-238.

Rai B, Gupta SK, Pratap A (2007). Breeding methods in oilseed Brassicas. *Advances in Biological Research*, 45: 2-17.

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.

Sadia M, Malik S A, Rabbani M A, Pearce SR (2009). Electrophoretic characterization and the relationship between some brassica Species. *Electronic Journal of Biology,* 5(1): 1-4.

Tanksley SD, Jones RA (1981). Application of alcohol dehydrogenase allozymes in testing the genetic purity of F1 hybrids of tomato. *Horts Science*, 16: 179-181.

Turi NA, Farhatullah, Rabbani MA, Khan NU, Akmal M, Pervaiz ZH, Aslam MU (2010) Study of total seed storage protein in indigenous *Brassica* species based on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). *African Journal of Biotechnology,* 9(45): 7595-7602.