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Original scientific paper

COMPARISON OF ELECTROPHORETIC PROTEIN PROFILES OF *Brassica rapa* SUB-SPECIES BROWN SARSON THROUGH SDS-PAGE METHOD

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Jan Ahmad S., Z. Khan Shinwari, M. Ashiq Rabbani, H. Khurshid, M. Ishaq Ibrahim, M. Adil, M. Ilyas (2017): *Comparison of electrophoretic protein profiles of Brassica rapa sub-species brown sarson through SDS-PAGE method*.- Genetika, Vol 49, No. 1, 95- 104.
Estimation of protein based variability among different *Brassica* sub-species is important for crop improvement. In present study total seed protein based variation among brown sub-specie of *B. rapa* was studied. Twenty different brown types' genotypes were analysed through Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method. The small, medium and large sizes proteins were noted. A total of 12 bands were obtained in which 10 (83.33%) are highly polymorphic while the rest two (15.38%) are monomorphic. The protein size base polymorphism was divided into different groups on the basis of molecular weight that ranges from ~10 kDa to ~180 kDa. The data of variable proteins were analysed through Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method, which clustered all genotypes into four main cluster groups. The cluster I and II, III and IV consisted 3, 3, 6 and 10 genotypes respectively. The similarity coefficient values (40 to 96%) were calculated among different genotypes. The maximum similarity coefficient (96%) was recorded among genotypes Br-607, Br-560, and between Br-589 and Br-607 respectively. Our study showed maximum protein based diversity among brown sub-species of *B. rapa* which may serve as model to search protein based variation in other important plants sub-species.

Key words: Brassica rapa, brown sarson, phylogenetic relationship, SDS-PAGE

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INTRODUCTION

Biochemical markers such as proteins are commonly used to study genetic variability among plant species/sub-species. These protein are actually the end product of gene and highly polymorphic among organisms (ZAHHOR *et al.,* 2015; MUKHERJEE and DATTA, 2008; MUKHLESUR *et al.,* 2004). The identification of protein based variation in *Brassica* species is important to identified improved genotypes (SEMAGN *et al.,* 2006). Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method give quick and accurate proteins based polymorphism among different plant species, as it is free from any environmental effect (DHAWALE *et al.,* 2015; DAS and MUKHERJEE, 1995).

This method has been utilized for many important brassica species. SHINWARI *et al.* (2013) identified new protein sub-units in *Eruca sativa* L. cultivars that sizes ranges from 15-220 KDa. Maximum genetic similarity (60-100%) was calculated for all experimental genotypes. The similar method was also used by ZADA *et al.* (2013) that evaluated 94 different *Brassica carinata* L. (Ethiopian mustard) genotypes. A total of 14 highly polymorphic and 17 monomorphic protein sub-units were reproduced. The overall genetic similarity value 50% to 100% was obtained. KHURSHID and RABBANI (2012) divided the proteins sub units of many brassica species into three sub groups. The first group shows maximum polymorphism followed by group II and III respectively. The first region contain highly polymorphic proteins, the second one showed minor polymorphisms and the third region showed no or very low level of polymorphic pattern. Various morpho-biochemical and molecular methods are used to screen elite genotypes (JAN *et al.,* 2016; PERIC *et al.,* 2014). Protein based polymorphism varies among species and sub-species (DHAWALE *et al.,* 2015; DUDWADKAR *et al.,* 2015).

The brown sub-species of *B. rapa* are grown as oil seed crop in all over the world. The morpho-physiological and biochemical Reponses vary with type of sub-species. However there are few reports available about the protein based diversity among these sub-species. The present study was conducted to study protein based variation among diverse *B. rapa* sub-species brown sarson.

MATERIALS AND METHODS

Experimental Materials

The present research work was performed at Plant genetic resources institute (PGRI), National agriculture research centre (NARC), Islamabad Pakistan. The fresh seeds of brown ecotype of *B. rapa* were acquired from the gene bank of PGRI, NARC, Islamabad, Pakistan. The list of genotypes is given in Table 1.

Procedure of Protein Extraction

The fully mature 15 seeds were ground with the help of mortar and pistal for 2-3 minutes continuously with great care, not to produce too many oils. The crushed materials were transferred to each eppendorf tube with addition of 400 µl protein extraction buffers (0.5M Tris-HCl (pH 8.0), 0.2% Sodium dodecyle sulphate (SDS), 5M urea, 1% 2-mercaptoethanol, and bromophenol blue dye). The samples were then vertexes for 1-2 minutes for proffer mixing and store in refrigerator at -20 ºC for 20 hours. After providing proffer incubation time, the samples were centrifuged at high speed 12000 rpm for 10 minutes at 25 ºC.

Table 1. List of Accessions of Brown B. rapa

Sr No.	Accession No.	Source
1	Br-560	NARC, Islamabad, Pakistan
2	Br-561	NARC, Islamabad, Pakistan
3	Br-564	NARC, Islamabad, Pakistan
4	Br-565	NARC, Islamabad, Pakistan
5	Br-577	NARC, Islamabad, Pakistan
6	Br-578	NARC, Islamabad, Pakistan
7	Br-585	NARC, Islamabad, Pakistan
8	Br-586	NARC, Islamabad, Pakistan
9	Br-588	NARC, Islamabad, Pakistan
10	Br-589	NARC, Islamabad, Pakistan
11	Br-607	NARC, Islamabad, Pakistan
12	Br-608	NARC, Islamabad, Pakistan
13	Br-609	NARC, Islamabad, Pakistan
14	Br-610	NARC, Islamabad, Pakistan
15	Br-611	NARC, Islamabad, Pakistan
16	Br-612	NARC, Islamabad, Pakistan
17	Br-613	NARC, Islamabad, Pakistan
18	Br-614	NARC, Islamabad, Pakistan
19	Br-615	NARC, Islamabad, Pakistan
20	Br-616	NARC, Islamabad, Pakistan

Electrophoresis

The separation and staking gel was prepared by using protocol of KHURSID and RABBANI (2012). 10 µL upper protein layers were loaded to each well along with protein marker (page-ruler SM#0671) at 100 V. The gels were then transferred into staining solution and kept for 6-8 hour on shaker. After proffer staining the sating solution was removed and destaining solution was poured into same plates and kept on shaker for about 1-2 days.

Data Analysis

The following equation of was used to study similarity coefficient study among ecotypes by using pair-wise comparison. The genetic similarity estimates (F) were designed by using following equation of NEI and LI (1979)

Similarity (F) = 2Nab/ (Na+Nb)
Where Na = the number of scored fragments of individual ‘a’
Nb = the number of scored fragments detected in individual ‘b’
While;
Nab = the number of shared fragments between ‘a’ and ‘b’

Mark the clear bands as 1 and absence of band is 0 and developed a sheet of whole banding pattern. The dedogram were made by using UPGMA (Unweighted pair-group method with

arithmetic averages) (SNEATH and SOKAL, 1973) with the help of bioinformatics software NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

RESULTS

Genetic Variation of Brassica rapa ssp Brown Sarson Genotypes Based on SDS-PAGE of Total Seed Proteins

In this study, protein based variability was studied in 20 different brown sub-species of *B. rapa* was noted. Proteins profiling showed highly protein variability among all brown sarson *B. rapa* genotypes. A total of 12 protein bands were recorded in which two are monomorphic (16.66%) while the rest of 10 bands (83.34%) are highly polymorphic. The protein regions were divided into three main groups. The region I consist large size proteins, region II have medium size proteins and region III have low molecular weight proteins. The regions I, II and III consist proteins, that size ranges from ~100 to ~170 KDa, ~50 to ~99 KDa and ~10 to ~49 KDa respectively (Figs. 1a-b).

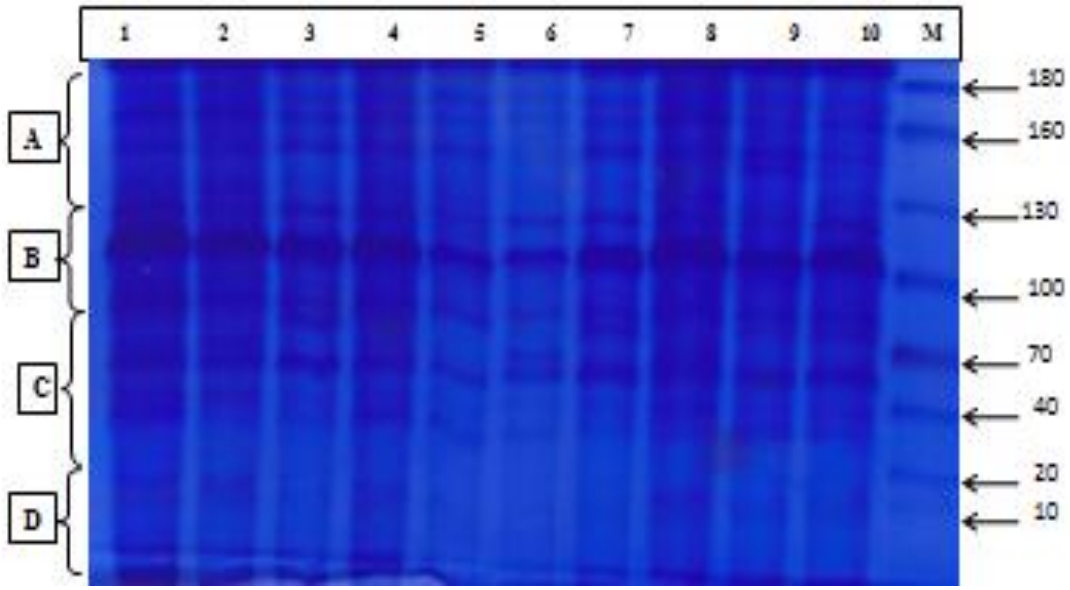

Figure 1(a): Electrophoretic banding pattern of *B. rapa* genotypes generated through SDS-PAGE of total seed storage proteins. M represents molecular size marker, while numbers from 1-10 represent accessions Br-588, Br-589, Br-586, Br-560, Br-616, Br-564, Br-561, Br-585, Br-577 and Br-610, respectively

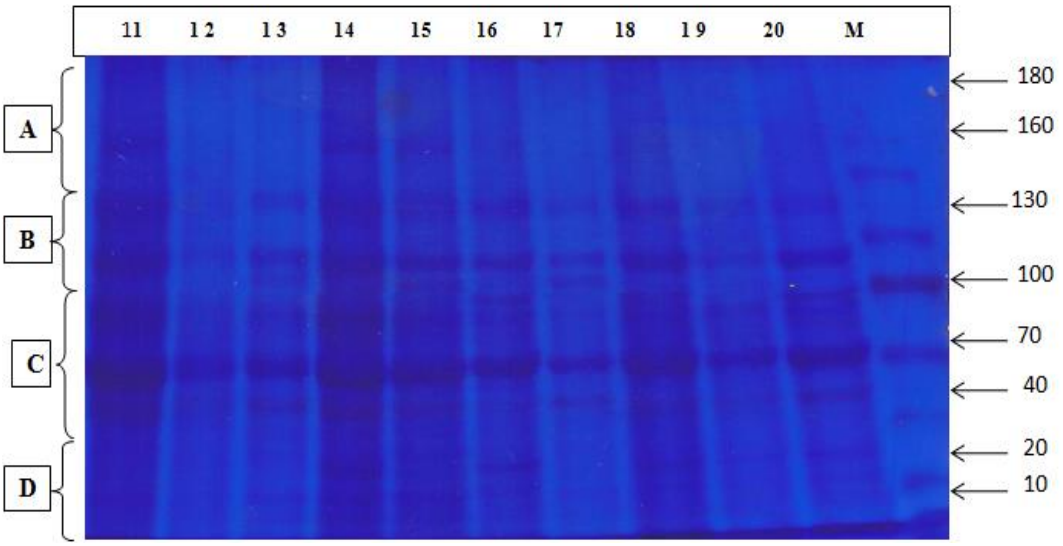

Figure 1(B): Electrophoretic banding pattern of *B. rapa* genotypes generated through SDS-PAGE of total seed storage proteins. M represents molecular size marker, while numbers from 1-10 represent accessions Br-611, Br-615, Br-613, Br-612, Br-565, Br-614, Br-578, Br-607, Br-609 and Br-608, respectively

Genetic Similarity Matrix and Cluster Analysis Study of B. rapa ssp Brown Sarson

Similarity coefficient of different proteins was calculated by following method of NEI and LI (1979). The similarity coefficient values ranges up to 40 to 96% (Table 3). The highest similarity coefficient 96% was noted among genotypes Br-607, Br-560, and Br-608 and between Br-589 and Br-607. The lowest similarity coefficient value 40% was recorded between Br-588 and Br-614. Our results showed that there are considerable differences occurred between these genotypes.

Table 2. Grouping of 20 genotypes of Brassica rapa through cluster analysis based on SDS-PAGE method

Clusters	No. of genotypes	Genotypes
I	8	Br-560, Br-561, Br-564, Br-577, Br-585, Br-586, Br-588, Br-589
II	1	Br-616
III	6	Br-565, Br-607, Br-609, Br-613, Br-614, Br-615
IV	5	Br-578, Br-608, Br-610, Br-611, Br-612

The 20 different brown genotype of *B. rapa* was clustered into four major groups. The Phylogenetic tree was constructed by using UPGMA (Unweighted Pair Groups Method with Arithmetic averages) method (Figure 2, Table 2). The Cluster-I, II, III, IV and V consisted 8, 1, 6 and 5 genotypes, respectively. Maximum dissimilarity was found between group I and IV. These two groups show highest level of diversity from each others. These genotypes were arranged into groups on the basis of similarity coefficient levels. The maximum similarity was observed among Br-560/Br-562, Br-585/Br-586 and Br-620/Br-622 as compared to other genotypes.

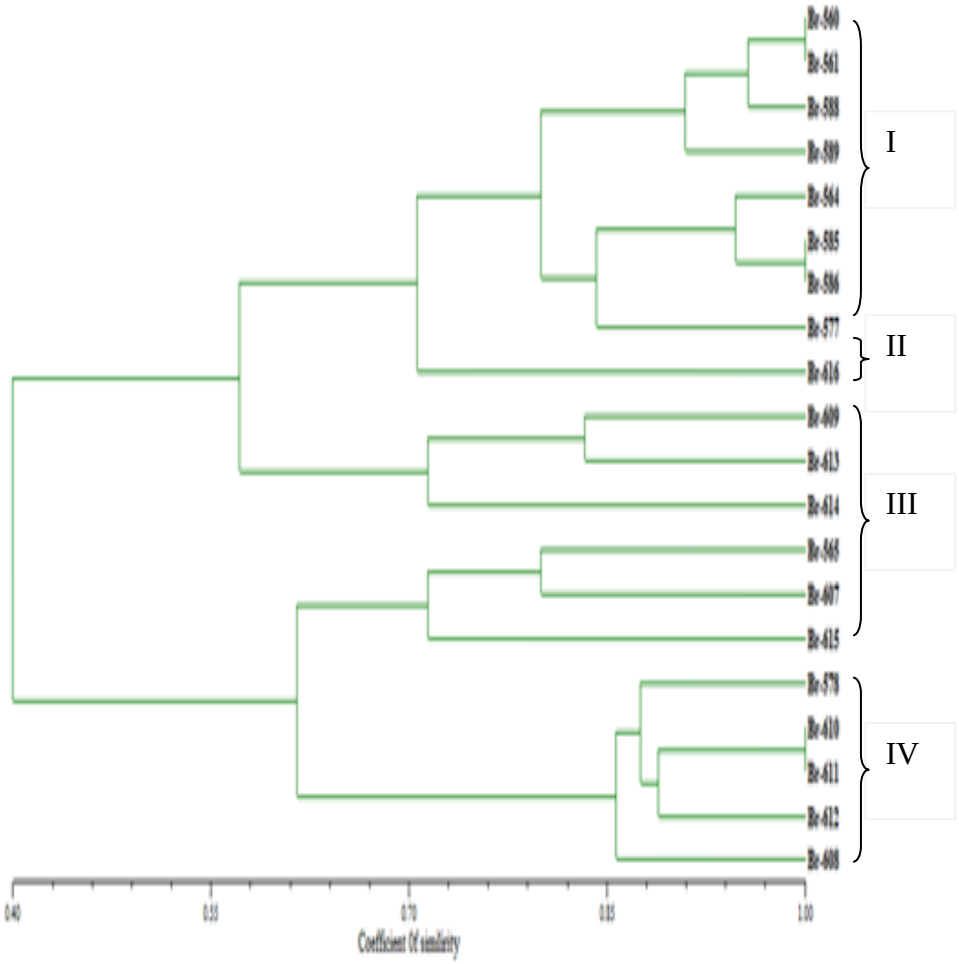

Figure 2. Dendrogram indicating the phylogenetic relationships among *B. rapa* ssp brown sarson

Table 3. Dice coefficient of similarity among B. rapa ssp brown sarson

Genotypes	Br-560	Br-608	Br-564	Br-565	Br-577	Br-610	Br-615	Br-586	Br-607	Br-589	Br-588	Br-561	Br-609	Br-578	Br-611	Br-612	Br-616	Br-614	Br-585	Br-613
Br-560	1																			
Br-608	0.9	1																		
Br-564	0.9	0.9	1																	
Br-565	0.84	0.84	0.82	1																
Br-577	0.9	0.9	0.89	0.82	1															
Br-610	0.78	0.78	0.63	0.67	0.63	1														
Br-615	0.95	0.95	0.95	0.78	0.84	0.71	1													
Br-586	0.95	0.95	0.95	0.78	0.84	0.71	1	1												
Br-607	0.96	0.96	0.86	0.8	0.86	0.74	0.91	0.91	1											
Br-589	0.91	0.91	0.8	0.74	0.8	0.67	0.86	0.86	0.96	1										
Br-588	0.78	0.78	0.63	0.8	0.63	0.71	0.71	0.71	0.74	0.67	1									
Br-561	0.78	0.78	0.63	0.67	0.63	0.86	0.71	0.71	0.74	0.67	0.71	1								
Br-609	0.71	0.71	0.67	0.71	0.67	0.62	0.63	0.63	0.67	0.71	0.46	0.46	1							
Br-578	0.8	0.8	0.67	0.71	0.67	0.88	0.74	0.74	0.86	0.8	0.75	0.88	0.53	1						
Br-611	0.8	0.8	0.67	0.71	0.67	0.88	0.74	0.74	0.86	0.8	0.75	0.88	0.53	1	1					
Br-612	0.9	0.9	0.78	0.82	0.78	0.88	0.84	0.84	0.86	0.8	0.75	0.88	0.67	0.89	0.89	1				
Br-616	0.71	0.71	0.67	0.57	0.67	0.62	0.63	0.63	0.67	0.71	0.46	0.46	0.83	0.53	0.53	0.67	1			
Br-614	0.74	0.74	0.59	0.5	0.71	0.67	0.67	0.67	0.8	0.84	0.4	0.53	0.71	0.71	0.71	0.71	0.71	1		
Br-585	0.71	0.71	0.67	0.71	0.67	0.62	0.63	0.63	0.67	0.71	0.77	0.62	0.5	0.67	0.67	0.67	0.67	0.43	1	
Br-613	0.84	0.84	0.71	0.63	0.71	0.67	0.78	0.78	0.8	0.74	0.67	0.67	0.57	0.59	0.59	0.71	0.71	0.63	0.57	1

DISCUSSION

SDS-PAGE method is one of the key methods, which differentiate *Brassica* specie/sub-species on the basis of protein size and provide a clear protein profile of important diverse genotypes. In present work the brown sub-species of *B. rapa* was analysed through this method and maximum genetic diversity was noted among different genotypes. The SDS-PAGE method provides maximum protein based diversity among genotypes**;** it is cheap and quick method then other molecular methods (ISEMURA *et al.,* 2001; GEPTS and BLISS, 1988; IQBAL *et al.,* 2005; JAVID *et al.,* 2004; RAHMAN and HIRATA, 2004). Various biochemical and molecular methods are used to study genetic diversity among different crop species/sub-species (VERMA *et al.,* 2015; GOODARZI *et al.,* 2015; IZADPANAH *et al.,* 2015; MARJANOVIC-JEROMELA *et al.,* 2014; HENAREH *et al.,* 2016).

The genetic divergence among brown genotypes of *B. rapa* was characterized through SDS-PAGE method. A total of 12 bands were obtained in which 10 are highly polymorphic. All brown type genotypes showed different size of protein bands, that is the evidence that seed total storage protein vary with plant sub-species/ecotype. ZADA *et al.* (2013) reported 31 different protein sub units in *Brassica carinata* genotypes. SHINWARI *et al.* (2013) recorded 17 different polymorphic and 1 monomorphic protein sub-units in important *Eruca sativa* genotypes. TURI *et al.* (2010) reported four different types of protein through similar method in many *Brassica* species. The SDS-PAGE and RAPD markers give maximum polymorphism among soybean genotypes (BARAKAT, 2004). The similar type of protocol was also used by AHMAD *et al.* (2003); to study protein based polymorphism in local Pakistani cultivar *Hyppophae rhamnoides* L. ssp

Turkestanica. The protein bands pattern vary with type of genotypes used (KAKAEI and KAHRIZI, 2011; RABBANI *et al.*, 2001; DHAWALE *et al.*, 2015; DUDWADKAR *et al.*, 2015).

The genetic similarity coefficient was calculated for all twenty different brown type of *B. rapa*. The similarity coefficient value ranges from 40% to 96% was recorded among different genotypes (Table 3). The phylogenetic tree was constructed that classified all twenty genotypes into four major groups (Fig; 3 Table 2). TURI *et al.* (2010) reported 98% similarity value for different *Brassica* cultivars. Our finding shows deviation from the results of SHINWARI *et al.* (2013) those recorded 60% to 100% similarity coefficient for *Eruca sativa* species. That might be due to different plant species used. Different clustering groups from our study were recorded by NASR *et al.* (2006) classified *Brassica napus* genotypes into different clustered group on the basis of their closed relationship with each other in genetic tree. MUKHLESUR *et al.* (2004) also classified different cultivars of *Brassica* into different clustered groups by using similar method.

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REFERENCES

AHMAD, S.D., A.W. JASRA, A. IMTIAZ (2003): Genetic Diversity in Pakistani Genotypes of *Hyppophae rhamnoides* L. ssp. *Turkestanica*. Int. J. Agri. Biol., *5(1)*:10-13.

BARAKAT, H. (2004): Genetic fingerprinting and relationships of six soybeans (*Glycine max* L.) cultivars based on protein and DNA Polymorphism. Int. J. Agri. Biol., *6(5)*:877-883.

DAS, S. and K.K. MUKHERJEE (1995): Comparative study on seed protein of Ipomoea. Seed Sci. Technol., *23*:501-509.

DHAWALE, R.N., M.D. MAHALLE, N.S. PAUL (2015): Biochemical Marker (Protein) Based Characterization of Rice Accessions Bio-Diversity in International Rice Molecular Breeding Programme. Asian J. Biomedical and Pharma Sci., *5(43)*:31-37.

DUDWADKAR, S., M. PARAB, S. SINGH (2015): Diversity analysis among few cucurbitaceae using seed protein profile.Int. J. Plant. Animal and Env. Sci., *5(1)*:146-151.

GEPTS, P. and F.A. BLISS (1988): Dissemination pathway of common bean deduced from phaseolin electrophoretic variability. Europe and Africa. Econ. Bot., *42*: 86-104.

GOODARZI, F., A. HASSANI, R. DARVISHZADEH, H. HATAMI MALEKI (2015): Genetic variability and traits association in castor bean (*Ricinus communis* L.). Genetika, *47(1)*:265-274.

HENAREH M., A. DURSUN, B. A. MANDOULAKANI, K. HALILOGLU (2016): Assessment of genetic diversity in tomato landraces using ISSR markers. Genetika, *48(1)*:25-35.

IQBAL, S.H, A. GHAFOOR, N. AYUB (2005): Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pak. J. Bot., *37*:87-96.

IZADPANAH, F., S. KALANTARI, M. E. HASSANI, M. R. NAGHAVI, M. SHOKRPOUR (2015): Molecular and morphological variation in some Iranian saffron (*Crocus sativus* L.) accessions. Genetika, *47(2)*:711-722.

ISEMURA, T., N. SHIYO, M. SHIGEYUKI, Y. MICHIHIRO, N. HIROO, I. MASAYOSHI, K. OSAMU (2001): Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. Breeding Sci., *51*:225-230.

JAN, S.A., Z. K. SHINWARI, and M. A.RABBANI (2016): Morpho- Biochemical evaluation of *Brassica Rapa* sub-species for salt tolerance. Genetika., *48(1):*323 -338.

JAN, S. A., Z.K. SHINWARI, M.A. RABBANI, S.H. SHAH, M.I. IBRAHIM, and M. ILYAS (2016): Optimization of an efficient SDS-PAGE protocol for rapid protein analysis of *Brassica rapa*. J. Bio. Env. Sci., *9 (2): 17, 24*.

JAN, S. A., Z.K. SHINWARI, and M.A. RABBANI (2016): Determining genetic divergence among *Brassica rapa* ecotypes through electrophoretic mobility of total seed proteins*:* J. Animal. Plant Sci., *26*(6):1758-1764.

JAVID, A., A. GHAFOOR, R. ANWAR (2004): Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pak. J. Bot., *36*:25-29.

KAKAEI, M. and. D. KAHRIZI (2011): Study of Seed Proteins Pattern of *Brassica napus* varieties via Sodium Dodecyl Sulfate Polyacrylamid Gel Electrophoresis. *Int. Res. J. Biotech., 2(1)*: 26-28.

KHURSHID, H. and M.A. RABBANI (2012): Comparison of Electrophoretic Protein Profiles from Seed of Different Oilseed *Brassica* Cultivars. J Public health. Biol Sci., *1(2)*:36-42.

MARJANOVIC-JEROMELA A., R. MARINKOVIC, M. JOCKOVIC, P. MITROVIC, Z.MILOVAC, N. HRISTOV, J. SAVIC, B. STAMENKOVIC (2014): Evaluation of genetic variance components for some quantitative traits in rapeseed (*Brassica napus* L.). Genetika, *46(1)*:179 -185.

MUKHLESUR, R.M., Y. HIRATA, S.E. ALAM (2004): Genetic Variation within *Brassica rapa* cultivars using SDS-PAGE for Seed Protein and Isozyme Analysis. J. Bio. Sci., *4(2)*:239-242.

MUKHERJEE M. and A.K. DATTA (2008): Evaluation of genetic diversity in five species of *Ocimum* by SDS-PAGE. Indian J. Genet. Plant Breed., *68*:212.

NASR, N., M. KHAYAMI, R. HEIDARI, R. JAMEI (2006): Genetic diversity among selected varieties of *Brassica napus* (Cruciferae) based on the biochemical composition of seeds. Just., *32(1)*:37-40.

NEI, M. and W.H. LI (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci, USA, *76(10)*:5269-5273.

PERIĆ, V., A. NIKOLIĆ, V. BABIĆ, A. SUDARIĆ, M. SREBRIĆ, V.DORDEVIĆ, and S.MLADENOVIĆ DRINIĆ (2014): Genetic relatedness of soybean genotypes based on agromorphological traits and RAPD markers. Genetika., *46*:839-854.

RAHMAN, M.M. and Y. HIRATA (2004): Genetic diversity in Brassica species using SDS-PAGE analysis. J. Biol. Sci., *4*: 234-238.

RABBANI, M.A., A.A. QURESHI, M. AFZAL, R. ANWAR, S. KOMATSU (2001): Characterization of Mustard [*Brassica juncea* (L.) Czern. & Coss.] Germplasm by SDS-PAGE of Total Seed Protiens. Pak. J. Bot., *33(2)*:173-179.

SEMAGN, K.A, BJORNSTAD, M.N. NDJIONDJOP (2006): An overview of molecular marker methods for plants. Afr. J. Biotechnol., *5(25)*:2540-2568.

SHINWARI, S., F. AKBER, M.A. RABBANI, A.S. MUMTAZ, Z.K. SHINWARI (2013): Evaluation of genetic diversity in different genotypes of Eruca *sativa* from Pakistan by SDS-PGE analysis. Pak. J. Bot., *45(4)*:1235-1240.

SNEATH, P.H. and R.R. SOKAL (1973): Numerical Taxonomy; The Principles and Practice of numerical Classification. W.F. Freeman & Co.,San Francisco, USA. 573.

TURI, N.A., FARHATULLAH, M.A. RABBANI, N.U. KHAN, M. AKMAL, Z.H. PERVAIZ, M.U. ASLAM (2010): Study of total Seed Storage Protein in Indigenous *Brassica* species based on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Afr. J. Biotech., *9(45)*: 7595-7602.

VERMA, N., S. SHUKLA, K. YADAV, B. K. MISHRA, A. RASTOGI (2015*)*: Biochemical characterization based on SDS-PAGE analysis and correlation among traits in opium poppy *(Papaver somniferum L.)* germplasm. Genetika, *47(3)*:1029 -1050.

ZADA, M., Z.K. SHINWARI, N. ZAKIR, M.A. RABBANI (2013): Study of total seed storage proteins in Ethiopian mustard (*Brassica carinataa*. Braun) Germplasm. Pak. J. Bot., *45(2)*: 443-448.

ZAHOOR, M., M. NISAR, N. ISLAM (2015): Genetic variations of *Robinia pseudoacacia* plant using SDS-PAGE. Pak. J. Bot., *46(6)*:2335-2338.

POREĐENJE PROTEINSKIH PROFILA PODVRSTA *Brassica rapa* METODOM SDS-PAGE

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Izvod

Ocena varijabilnosti na bazi proteina kod podvrsta roda *Brassica* je veoma značajna. U ovom radu pručavana je varijabilnost ukupnih proteina semena kod braon podvrsta roda *B. rapa*. Metodom *Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)* analizirano je 20 različitih genotipova. Dobijeno je ukupno 12 traka, od kojih je 10 (83.33 %) bilo visoko polimorfno, a ostale dve (15.38%) bile su monomoorfne. Izdvojeni proteini bili su mali, srednji i veliki i na osnovu njihove molekulske težine, koja je varirala od ~10 kDa do ~180 kDa, formirano je pet grupa. Na osnovu proteinskih podataka, metodom *Unweighted Pair Group Method with Arithmetic Mean (UPGMA)*, svi genotipovi grupisani su u četiri klastera. Klasteri I, II, III i IV sastojali su se od 3, 3, 6 i 10 genotipova. Koeficijenti sličnosti bili su od 40% do 96%. Maksimalni koeficijent sličnosti (96%) zabeležen je između genotipova Br-607, Br-560, i Br-608 i između Br-589 i Br-607. Ovo istraživanje pokazalo je maksimalan diverzitet između braon podvrsta *B. Rapa*, zasnovan na proteinima, koji se može koristiti kao model za izučavanje drugih biljnih podvrsta.

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