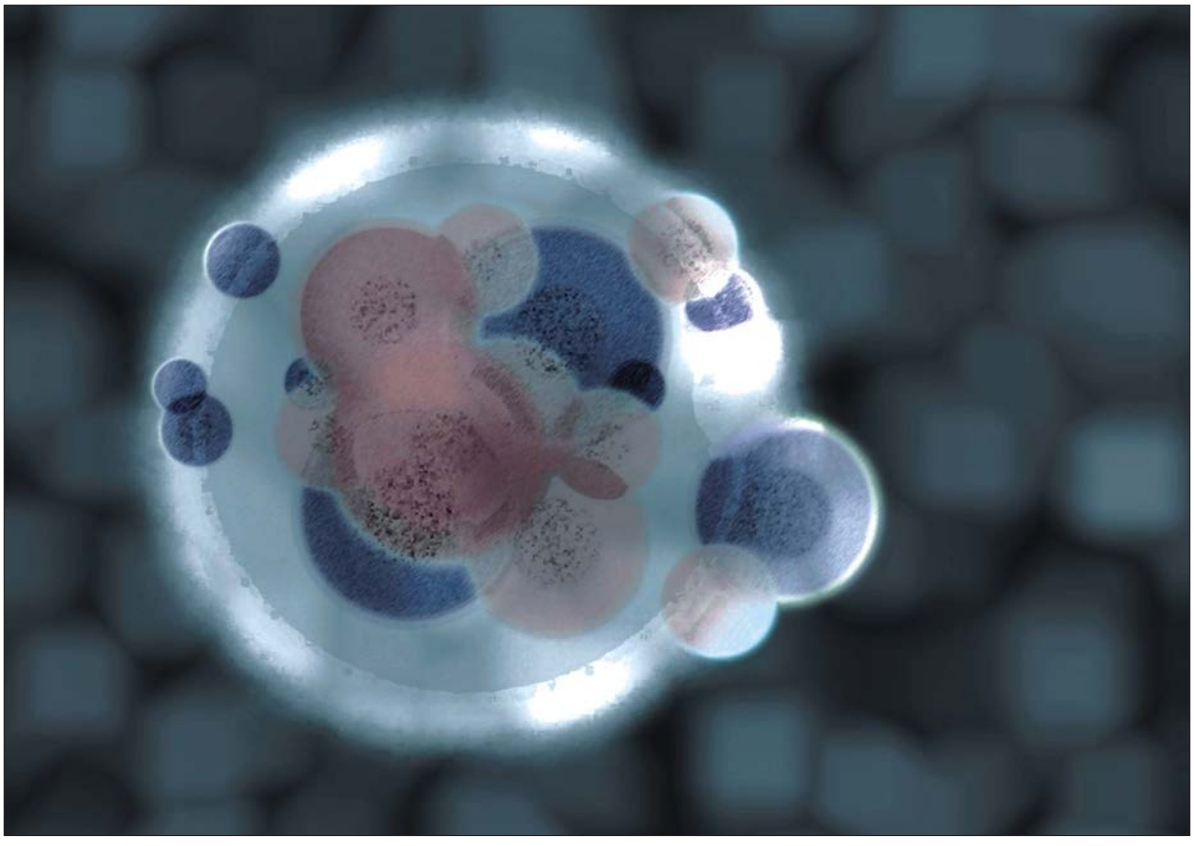

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 4 (2): 239-242, 2004
ISSN 1727-3048

Genetic Variation Within *Brassica rapa* Cultivars Using SDS-PAGE for Seed Protein and Isozyme Analysis

[1,2]Rahman Md. Mukhlesur, [2]Yutaka Hirata and [1]Shah-E-Alam
[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo 183-8509, Japan

Abstract: Genetic relationship were evaluated among thirty two cultivars of *Brassica rapa*, collected from Bangladesh, Japan and China, by employing SDS-PAGE for seed protein and esterase, acid phosphate and peroxidase analysis. SDS-PAGE generated a total of 32 bands of which nearly 31% were polymorphic, followed by esterase of 9 bands of nearly 19% polymorphic and no identifyable polymorphic band was found from both acid phosphate and esterase analysis. However, these polymorphic markers clearly distinguished between yellow sarson and brown seeded cultivars. Cluster analysis using data generated by SDS-PAGE for seed protein, clearly separated the yellow sarson, self-compatible cultivars from the brown seeded, self-incompatible cultivars, as well as different brown seeded cultivars from Bangladesh, Japan and China origin.

Key words: *Brassica rapa*, SDS-PAGE, esterase, acid phosphate, peroxidase

INTRODUCTION

Estimates of genetic relatedness are important in designing crop improvement programmes. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation strategies. Molecular markers are the best tools for determining genetic relationships. Different types of marker systems have been used for biodiversity analysis. The electrophoresis of seed storage proteins is a method to investigate genetic variation and to classify plant varieties[1]. Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. It has been widely suggested that such banding patterns could be an important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivars or when cultivars are to be patented[2]. Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice[3]. However, the information on the SDS-PAGE on different species of Brassica for genetic diversity is still limited.

Isozyme analysis offers a rapid and more reliable means for producing genetic profiles (fingerprints) and elucidation of genetic relationships within and different taxa. This techniques are efficient tools for genetic, systematic and breeding studies, particularly in *Brassica* species because of their high level of polymorphism[4]. Isozyme loci have also been used as markers in a number of genetic studies, such as genetic diversity in *Brassica* juncea[5]; genetic diversity in *B.* rapa[6], testing genome construction of different species of Brassica[7]; isozyme loci and their linkage in *B. campestris*[8] and isozyme markers as seed coat color[9].

Analysis of SDS-PAGE and isozymes are fairly simple and inexpensive, which are added advantages for use in practical plant breeding. The objectives of the study were: (I) To assess the seed protein and isozyme polymorphisms within and different cultivars of *Brassica rapa*, (ii)To elucidate the genetic relationships among cultivars and (iii) to investigate the geographical distribution of their electrophoretic band types.

MATERIALS AND METHODS

Plant materials: Thirty two different genotypes of *B. rapa* were collected from Bangladesh, Japan and China and were subjected to SDS-PAGE for seed protein and isozyme analysis (Table 1).

Electrophoresis: Seed storage proteins were extracted from cotyledon of single seed and mixed with 50-80 μl of extraction buffer of 0.0625 M Tris-HCl (pH 6.8), 8 M Urea, 2% Sodium dodecylsulfate (SDS) and 5% 2-

Corresponding Author: Md. Mukhlesur Rahman, Ph.D. Student, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2 Email: mmr255@yahoo.ca

Mercaptoethanol and then kept for over night. The crude homogenates were then centrifuged at room temperature with 14000 rpm for 20 min. Thereafter, 8.5 µl of the crude extract was directly analyzed by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) using 12% (w/v) mini-slab gel. 0.1% Bromophenol blue (BPB) was used as marker dye to the cathode buffer. Electrophoresis was carried out at 10 mA for stacking gel and 20 mA for separation gel until the dye front head migrated to within 2 mm of the end of the gel. Gels were stained with 0.5% Coomassie Brilliant Blue (CBB) G-250 in acetic acid-ethanol-water (2:5:5 volume ratio) for one hour and distained in acetic acid-methanol-water (7:20:73 volume ratio) for over night. Banding patterns were scored from at least two electrophoregrams for each stain. When ambiguous band patterns were obtained, electrophoresis was further carried out by changing the gel concentration and/or electrophoresis time to determine the protein type.

Isozyme analysis: Isozyme electrophoresis was performed on leaf samples collected from seedlings at two - to three-leaf stage. For peroxidase analysis 500 mg fresh leaf was collected in icebox and homogenized by liquid N_2 and was added 100 µl of 0.2 M Phosphate buffer (pH 7.0 was adjusted by Potassium Phosphate, monobasic) and 10 µl of 2-Mercaptoethanol before centrifugation at 14000 rpm for 15 min at 4°C temperature to clear the cellular debris. The supernatant was recovered and used directly for electrophoresis or stored at a temperature of -20°C. All the extractions were performed at a temperature of approximately 4°C and icebox. For Acid phosphatase and Esterase analysis, enzyme extraction was followed to that of Nozaki *et al.*[8]. Standard polyacrylamide gel electrophoresis (7.5% polyacrylamide separation gel, 2.85% polyacrylamide stacking gel and 0.05M Tris, 0.384M Glycine running buffer) was applied for separation of the enzymes. The electrophoresis was carried out at 10 mA for stacking gel and 20 mA for separation gel. Staining procedure for esterase and acid phosphate were according to Nozaki *et al.*[8]. Peroxidase loci were detected by 0.044 M Phosphate-0.028 M Citric acid buffer (pH 4.4-4.6) and 0.026% (w/v) O-dianisidine and 1% H_2O_2 in an incubator at 37°C temperature until the bands developed sufficiently to permit scoring. The bands were fixed by 7% acetic acid after staining.

Data analysis: Bands were scored as 'a' for presence or boldly exhibited and 'b' for their absence or weakly exhibited across the cultivars to generate a dandrogram. A genetic similarity (GS) was computed based on Nei and Li[10]. GS= 2 X Number of shared fragments between individuals A and B/(Number of fragments in A + Number of fragments in B). GS was converted to the genetic distance by 1-GS.

RESULTS

SDS-PAGE for seed protein: In total, 31-32 bands per cultivar were detected in SDS-PAGE electrophoregrams, of these, 31.3% polymorphism were obtained. The polymorphic bands were appeared in ten positions designated as 'A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I' and 'J' (Table 2 and Fig. 1). Bands in the position 'G', showed presence-or-absence type polymorphism. Bands in position 'I' was divided into two patterns ('I_1' and 'I_2') according to their levels of mobility and banding pattern. Remaining polymorphic bands were differed in the protein intensity among cultivars.

The six Bangladeshi genotypes of yellow sarson of *B. rapa* showed uniquely identifiable banding character at position 'A', 'B', 'C', 'G', 'I' and 'J', respectively (Table 2 and Fig. 1). At the position 'G', all the cultivars of

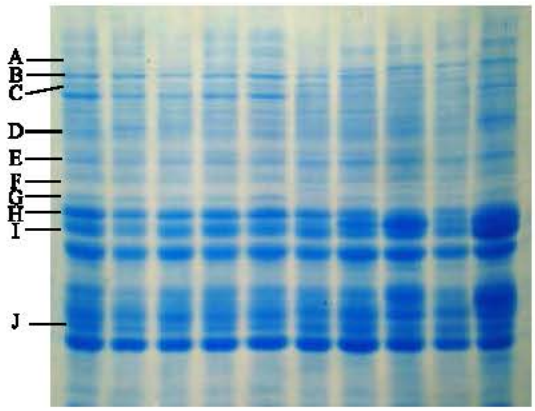

Fig. 1: Electrophoregram types identified by SDS-PAGE of seed protein of different cultivars of *Brassica rapa*

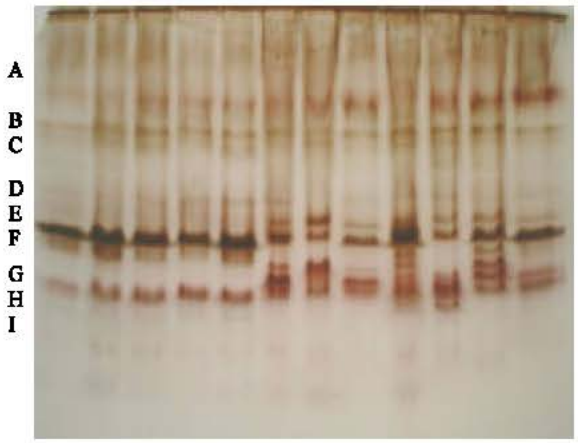

Fig. 2: Electrophoregram types identified by esterage of different cultivars of *Brassica rapa*

Table 1: List of cultivars of *B. rapa* used in this study

Name or code of cultivars	Country of origin	Habit
Sampad, Sonali, Dhali, Agrani, Shafal, YS-1293	Bangladesh	YS, self-compitable
Kallyania, TS-72, BR-0293, BR-0394, BR-0593, BR-3597, BR-5897	Bangladesh	BS, self-incompitable
Tori-7, Tori-94, Tori-2002	Bangladesh	BS, self-incompitable, toria
JR-229, JR-254, JR-255, JR-275, JR-276, JR-285, JR-286, JR-291	Japan	BS, self-incompitable
CR-85, CR-86, CR-89, CR-90, CR-92, CR-94, CR-103, CR-104	China	BS, self-incompitable

Table 2: Number of cultivars showed presence or more stained to the respective banding types by SDS-PAGE for seed protein

											I		

Species	Country of origin	Cultivars	A	B	C	D	E	F	G	H	I_1	I_2	J
YS *B. rapa*	Bangladesh	6	0	6	6	2	4	6	0	6	0	6	0
BS B. rapa	Bangladesh	10	7	7	0	3	10	7	10	10	10	0	10
	Japan	8	8	0	5	2	8	0	8	3	8	0	8
	China	8	8	0	1	0	8	0	8	8	8	0	8

Table 3: Number of cultivars showed presence or more stained to the respective banding types by esterage analysis

Species	Country of origin	Cultivars	A	B	C	D	E	F	G	H	I
YS *B. rapa*	Bangladesh	6	6	6	6	6	0	6	0	0	6
BS *B. rapa*	Bangladesh	10	10	10	10	10	10	10	10	10	10
	Japan	8	8	8	8	8	8	8	4	8	8
	China	8	8	8	8	8	5	8	8	8	8

N.B.: YS= Yellow sarson, BS= Brown seeded

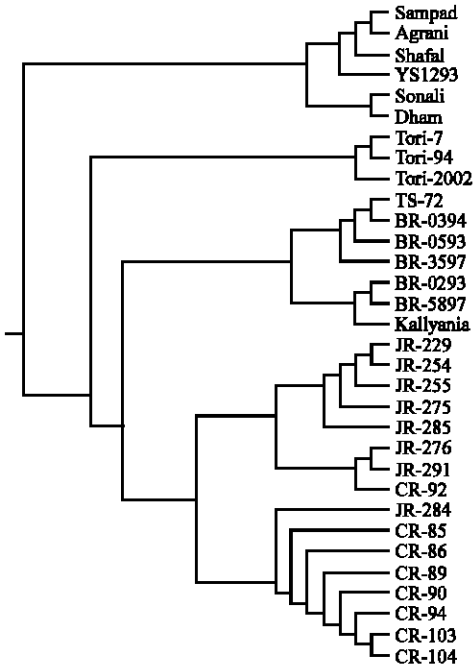

Fig. 3: Phenotypic dendrograms generated by Clustal W based on SDS-PAGE

yellow sarson in *B. rapa* were G-null type. Whereas, Japanese and Chinese cultivars exhibited a band over position G and other Bangladeshi cultivars of *B. rapa* showed distorted pattern i.e. presence-or-absent type in different seeds of the same cultivar with almost equal frequency. The yellow sarson also exhibited very strong bands at position 'B' and 'C', whereas other cultivars of brown seeded showed strong band either at position 'B' or at position 'C' or not exhibited with any strong band at the both positions. However, the cultivars of yellow sarson were unquickly characterized as G-null, J-null, A-less, B-rich, C-rich and I_2- types, where brown seeded types were E-rich, G-rich and I_1 types. All the Japanese and Chinese cultivars scored as B-null and F-null to that of Bangladeshi cultivars.

Esterase: A total nine esterase bands of which 18.8% polymorphism were obtained. The bands were identified as 'A', 'B', 'C', 'D', 'E', 'F', 'G', 'H' and 'I', where polymorphic bands were obtained at position 'E', 'G' and 'H' (Table 3 and Fig. 2). Yellow sarson types showed identifyable H-null type of banding pattern over other brown seeded types. Yellow sarson types also exhibited E-null and G-null types, where around 37% of Chinese cultivars showed E-null and 50% of Japanese cultivars showed G-null type of banding pattern.

Acid phosphate and Peroxidase analysis: The bands of acid phosphate and peroxidase were clearly revealed, but polymorphism was not detected.

Genetic similarity matrix and cluster analysis: The bands obtained with SDS-PAGE were used to compute the similarity coefficient using the Clustal W-pc programme[11]. The dandrogram based on cultivars were grouped into five clusters named A, B, C, D and E (Fig. 3). Cluster A consisted of all six yellow sarson, self-compitable cultivars. Cluster B comprised of three cultivars of toria, where as cluster C of seven brown seeded Bangladeshi cultivars, cluster D of seven Japanese and one Chinese cultivars and cluster E is of seven Chinese and one Japanese cultivars. A similarity matrix, based on the frequencies of band in a group, was used to determine the relationships between the five groups studied (Table 4). The estimation of genetic

Table 4: Similarity matrix (below diagonal) and genetic distance (above diagonal) of five different cluster based on SDS-PAGE analysis

Cluster	A	B	C	D	E
A	-	0.133	0.100	0.138	0.138
B	0.867	-	0.032	0.067	0.033
C	0.900	0.968	-	0.067	0.033
D	0.862	0.933	0.933	-	0.034
E	0.862	0.967	0.967	0.966	-

similarity ranged from 0.862 to 0.968. Cultivars in cluster A of yellow sarson showed highest genetic distance among the cultivars of those of other clusters of brown seeded cultivars. The lowest genetic similarities value (0.86-0.90) were obtained between cluster A and the other cultivars of cluster B, C, D and E (Table 4). The highest almost similar genetic similarities were obtained between cluster B & C, B & E, C & E and D & E, followed by cluster B & D and C & D.

DISCUSSION

The genotypes of *B. rapa* from Bangladesh, Japan and China differ in protein types, this information may help us to establish the diversity of Asian *Brassica.* Thanh and Hirata[3] observed seed storage protein diversity in wild and cultivated rice. Genetic diversity was found in the cultivars of Bangladeshi yellow sarson and other brown seeded *B. rapa,* where yellow sarson showed six unique protein types. The yellow sarsons are special ecotypes from India, belongs to ssp. trilocularis and are self-compatible, whereas other brown seeded *B. rapa* belongs to ssp. oleifera and are self-incompatible. In case of Bangladeshi brown seeded *B. rapa*, different seeds of same cultivar exhibited different protein types at respective positions. The possible explanations for the distribution of protein types in yellow sarson and brown seeded *B. rapa* are as : due to self-compatibility of yellow sarson, it did not show any segregation pattern in same cultivar of different seeds, whereas the brown seeded types are self-incompatible showed different protein types at the positions. The result gives similar agreements with that of Das *et al.*[12] where the yellow sarson and brown seeded were clearly differentiated by RAPD and AFLP analysis. Esterase isozyme polymorphism was clearly obtained and could easily differentiate the yellow sarson and brown seeded types. This is a useful tool to study the segregation population of yellow and brown seeded cultivars at seedling stage.

ACKNOWLEDGMENT

We acknowledges with thanks to the Ministry of Education, Science and Culture, Japan for the financial assistance in form of scholarship to conduct the current research.

REFERENCES

1. Isemura, T., N. Shiyo, M. Shigeyuki, Y. Michihiro, N. Hiroo, I. Masayoshi and 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.
2. Tanksley, S.D. and R.A. Jones, 1986. Application of alcohol bedydrogenase allozymes in testing the genetic purity of F1 hybrids of tomato. Hort. Sci., 16: 179-181.
3. Thanh Vo Cong and Yutaka Hirata, 2002. Seed storage protein diversity of three rice species in the Mecong Delta. Biosphere conservation, 4: 59-67.
4. Chevre, A.M., P. This, F. Eber, M. Deschamps, M. Renard, M. Delseny and C.F. Quiros, 1991. Characterization of disomic addition lines *Brassica napus-B. nigra* by isozyme, fatty acid and RFLP markers. Theor. Appl. Genet., 81: 43-49.
5. Kumar R. and V.P. Gupta, 1985. Isozyme studies in Indian mustard (*Brassica juncea* L.). Theor. Appl. Genet., 69: 1-4.
6. Persson, K., A.S. Falt and R. Von Bothmer, 2001. Genetic diversity of allozymes in turnip (Brassica rapa L. var. rapa) from the Nordic area. Hereditus, 134: 43-52.
7. Chen, B.Y., W.K. Heneen and V. Simonsen, 1989: Comparative and genetic studies of isozyme in resynthesized and cultivated *Brassica napus* L., *B. campestris* L. and *Brassica alboglabra* Bailey. Theor. Appl. Genet., 77: 673-679.
8. Nozaki, T., A. Mineko, T. Takashi and I. Hiroshi, 1995. Analysis of isozyme loci and their linkage in *Brassica campestris* L. Breeding Sci., 45: 57-64.
9. Rahman, M.H., 2001. Introgression of alleles of the isozymic locus glucosephosphate isomerase-2 (GPI-2) from the CC genome of *Brassica carinata* to the CC genome of *Brassica alboglabra* and their independent segregation from seed color. Plant Breeding, 120: 363-364.
10. Nei, M. and W.H. Li, 1979. Mathematical model for studying genetically variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA., 74: 5267-5273.
11. Jeanmougin, F., J.D. Thompson, M. Gouy, D.G. Higgins and T.J. Gibson, 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci., 23: 403-405.
12. Das, S., R. Jyothi, B. Sabhyta, P.S. Srivastava and L. Malathi, 2000. Assessment of genetic variation within *Brassica campestris* cultivars using amplified fragment length polymorphism and random amplification of polymorphic DAN markers. Plant Molecular Biology Division, Tata Energy Research Institute, Lodhi Road, New Delhi, India.