International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 6 (5): 437-443, 2011
ISSN 1816-4897 / DOI: 10.3923/ijar.2011.437.443

SDS PAGE Electrophoresis in Mustard Cultivars

[1]V. Vijaya Geetha and [2]P. Balamurugan
[1]Department of Seed Science and Technology, Krishi Vigyan Kendra, Vriddhachalam 606-001, Tamil Nadu, India
[2]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu India

Corresponding Author: V. Vijaya Geetha, Department of Seed Science and Technology, Krishi Vigyan Kendra, Vriddhachalam 606-001, Tamil Nadu, India

ABSTRACT

The main aim of the study was to discriminate the cultivars of mustard based on the nature of seed protein present in the seeds. Analysis of total soluble proteins (Tris-HCl Soluble) in seeds was done by Polyacrylamide Gel Electrophoresis (PAGE) method. Electrophoresis of seed proteins showed a total of 7 bands in Maya, GM-2, Varuna, PCR 7 and Pusa Bold whereas for rest of the genotypes some bands were absent. The critical bands for identification were the bands at Rf values 0.237, 0.327 and 0.667 which formed the 1st, 3rd and 7th band, respectively. The intensity of the band also varied among all the genotypes. Hence the present study obviously indicated the use of SDS page profile through electrophoresis for discrimination of mustard genotypes.

Key words: Mustard, SDS PAGE, seed protein, genotypes, varietal identification

INTRODUCTION

The success of electrophoretic procedures depends on the wide ranging polymorphism of seed protein and isozymes and the fact that these proteins represent primary gene products. Analysis of protein composition has proved to be a good indicator unless altered by growth condition only to a relative minor extent. SDS-PAGE of proteins is the most commonly used method to discriminate the varieties. The protein banding pattern is unique for the particular genotype and is independent of seed vigor and physiological seed activity (Kamel *et al*., 2003). SDS-PAGE was used for characterization of mustard genotypes, as this produced denatured protein. The advantage of examining denatured proteins is that, it is independent from seed vigor and physiological activity. Denaturing system provides a simple reproducible technique for cultivar identification as reported by Devi (2000) in sunflower and Chehregani and Kavianpour (2007) in beans. The criteria for distinctness among the cultivars were the presence or absence of a particular band or set of bands occurring at a position on the electrophoretic gel and the band intensity. Rapidity and repeatability of results are the foremost among its advantages. Roy *et al*. (2001), in grass pea, used the SDS-PAGE of total seed proteins for the purpose of varietal identification. SDS PAGE was used by Kumar *et al*. (2001) for establishing the genetic identity of sunflower hybrids and determining percentage genetic purity of F_1 hybrid. Similar results were found by Milka Anjana (2002) in Groundnut; Kamel *et al*. (2003) in cruciferae; Mukhlesur *et al*. (2004) in *Brassica rapa*; Kour and Singh (2004) in *Brassica juncea*; Shete (2004) in Castor hybrid TMVCH 1 and it parental lines and Hassan *et al*. (2007) in triticum species. Hence, there is a need to develop protein profiles for the cultivars for varietal characterization. Keeping in view the above facts, the present study was

initiated with the main objective of characterization of mustard genotypes through electrophoresis technique.

MATERIALS AND METHODS

Genetically pure seeds of mustard genotypes were obtained from National Research Centre on Rapeseed and Mustard, Bharatpur, Rajasthan. The experiment was carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore between 2004 and 2008.

Analysis of total soluble proteins (Tris-HCl Soluble) in seeds through Polyacrylamide gel electrophoresis (PAGE): Tris-HCl soluble proteins in the seed were separated using SDS-PAGE, following the method of Varier *et al.* (1992).

Preparation of solution

Extraction buffer (Tris-HCl pH 7.5): Tris base (1.21 g) was dissolved in 50 mL distilled water and pH adjusted with concentrated HCl to 7.5 and then the volume made upto 100 mL.

Protein sample buffer (5x concentration)

Tris-HCl buffer (pH 6.6) 0.6M: Tris- base (7.2g) was dissolved in 50 mL of distilled water and pH adjusted with concentrated HCl to 6.6 and volume made upto 100 mL with distilled water.

Stock-sample buffer

Tris-HCl buffer (pH 6.6) 0.6M	:	10.4 mL
Distilled water	:	7.9 mL
Sodium dodecyl sulphate	:	4 g
Glycerol	:	10.0 mL

The above mentioned components were mixed thoroughly.

Working sample buffer

Stock-sample buffer	:	4.25 mL
β-mercapto ethanol	:	0.75 mL

These components were mixed thoroughly and the volume was made upto 10 mL to this a pinch of Bromophenol blue was added, to act as a tracking dye.

Stock gel solution (30% acrylamide)

Acrylamide	:	30.08 g
Bis-Acrylamide	:	0.80 g

Dissolved in distilled water and volume made up to 100 mL.

Defatting solution

Chloroform	:	2 parts
Methanol	:	1 part
Acetone	:	1 part

Mixed thoroughly and used for defatting.

Separating gel buffer (pH 8.8) 0.5 M: Tris-base (22.69 g) was dissolved in 70 mL distilled water. The pH was adjusted to 8.8 with concentrate HCl or with KOH and volume made up to 100 mL with distilled water.

Stacking gel buffer (pH 6.8) 0.5 M: Tris-base (6.05 g) was dissolved in 70 mL of distilled water, pH adjusted with concentrated HCl to 6.8 and volume made up to 100 mL distilled water.

SDS 10%: SDS (Sodium Dodecyl Sulphate) 1.0 g was dissolved in 6 mL of distilled water and volume made up to 10 mL.

Preparation of separating or running gel mixture

Solutions	:	Gel concentration (10%) for 15 mL
Acrylamide solution (C)	:	5.0 mL
Separating gel buffer	:	3.75 mL
Distilled water	:	6.25 mL
10% SDS (G)	:	100 μL
10% APS (H)	:	100 μL
Tetramethyl Ethylene Diamide (TEMED)	:	20 μL

The separating gel mixture was poured to the glass sandwich assembly to a level of about 4 cm from the notch. One ml of butanol saturated with distilled water was gently applied over the separating gel. After polymerization, (10 min) the outer layer of butanol was washed off by inverting the casting gel and washed 3 to 4 times with distilled water to remove traces of butanol. The water droplets were removed using a filter paper without touching the separating gel.

Preparation of stacking gel mixture (10 mL)

Stock acrylamide solution (C)	:	1.3 mL
Stacking gel buffer (F)	:	2.5 mL
10% SDS (G)	:	100 mL
10% APS (H)	:	50 mL
Distilled water	:	6.1 mL
Tetramethyl Ethylene Diamide (TEMED)	:	10 μL

The above solution were mixed thoroughly and poured on the top of the separating gel. An acrylic well forming comb was inserted, ensuring no air bubble trapped beneath. The top portion of the gel solution was overlayed with 1 mL butanol saturated with distilled water. The gel was allowed to polymerize for 45 min. Then the acrylic comb was removed carefully not to distort the wells and resultant wells were cleaned with distilled water. The excess water was sucked out using a micro syringe and each well was cleaned carefully using bits of filter paper. The gel was then installed by removing the gasket in the electrophoresis apparatus to which electrode buffer was poured and pre-run for 10 min.

Preparation of seed sample: The 0.2 g of seed coat removed samples was ground using a pestle and mortar. The seed powder of each variety was taken in an eppendorf tube and l mL of defatting solution (solution D) was poured in each tube. After thorough shaking, the eppendorf tubes were

left for 3 h. The supernatant was decanted and this procedure was repeated three times. The samples were then kept overnight at a room temperature for drying. Next day, 1 mL of extraction buffer (solution A) was added and eppendorf tubes were kept overnight at 10°C (inside the refrigerator). The next day, the samples were centrifuged in a refrigerated centrifuge (4°C) at 12,000 rpm for 30 min.

Supernatant solution from each sample was taken into a separate eppendorf tube, to this equal volume of sample buffer (solution B) was added and mixed well. This was boiled in water for 2-3 min, then cooled and used for loading.

Electrophoresis: Electrophoresis was conducted using electrophoretic unit. The gel castle was then immersed in the electrode buffer solution (solution 1) and then upper reservoir was connected to the power pack fitting the electrodes in sockets identical colour. The voltage was fixed at 100 V till the tracking dye reached the bottom of the gel and then the electrophoresis was stopped.

Fixing and staining: The gel was removed after the run and fixed overnight in 15% Tri-Chloro Acetic acid (TCA). On the following day, the TCA was drained off and the gel was rinsed with distilled water and immersed in the stacking solution.

Staining solution

Comassie brilliant blue R. 250	:	0.1 g
Methanol	:	40 mL
Glacial acetic acid	:	10 mL
Distilled water	:	50 mL

Destaining solution

Methanol	:	40 mL
Glacial acetic acid	:	10 mL
Distilled water	:	50 mL

In the staining solution the gel was kept minimum of 6 h. Then using destaining solution the gel was destained up to background become clear. The gels were placed in a trans-illuminator and photographed.

Evaluation and documentation: The gel was documented using Biovis gel documentation software from which the Rf values of the bands were known. Based on Rf values dendrograms were prepared wherever necessary using Biovis gel match software. Apart from these, intensity, position, presence or absence of bands were critically observed and recorded to discriminate the cultivars.

RESULTS AND DISCUSSION

The total seed protein was extracted and separated by SDS-PAGE method. The detailed electrophoretic profile of nine mustard genotypes studied has been presented in Fig. 1.

The detailed electrophoregram of total soluble seed protein has been presented in Fig. 2. The entire protein was divided into five regions starting from A to E. This was in the order of increasing Rf values and decreasing molecular weight of proteins. Among the five regions B and E were most useful to distinguish cultivars due to clear banding appearance. The Rf value for all the bands

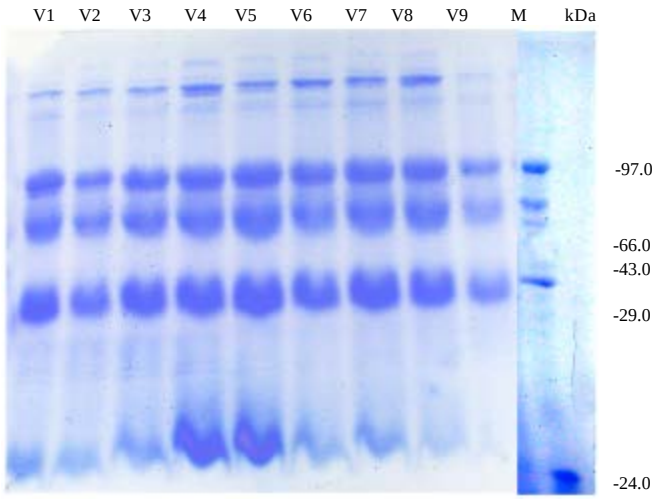

Fig. 1: Total seed protein profile of mustard genotype through SDS PAGE. V1: Rohini, V2: Bio 902, V3: Kranti, V4: Maya, V5: GM 2, V6: Varuna, V7: Pcr 7, V8: Pusa bold, V9: RN 393

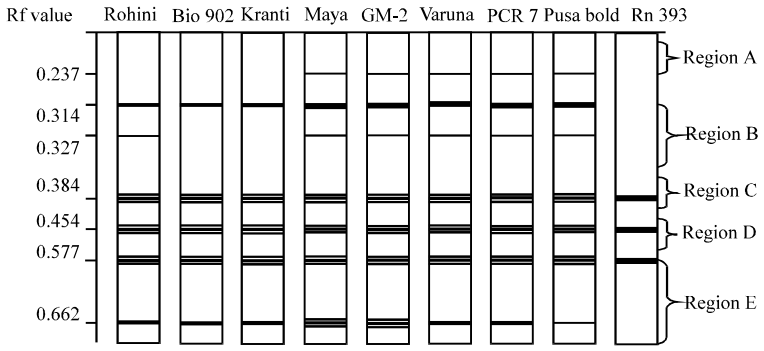

Fig. 2: Zymogram of total soluble seed protein of mustard genotypes through SDS PAGE

found in the entire profile have been presented in Table 1. The presence or absence of a band and the intensity of band were used for varietal characterization.

The band with low intensity of Rf value 0.237 was categorized as Region A (97.4 kDa). Region B (43.00 kDa) was characterized with two bands of low, medium and high intensity whose Rf values lies between 0.314 and 0.382. Only one band of high and very high intensity was present in Region C (29.0 kDa). In Region D (20.1 kDa) single band with high and very high intensity was appeared with the Rf value of 0.477 and Region E was characterized with one band of Rf value 0.662.

The present study revealed that there was no difference in the banding pattern in the region C and D. the difference in banding pattern was mainly confined only to A, B and E region (Fig. 2), these regions of the total seed protein gel profile obviously indicated the usefulness of the high and low molecular proteins than the medium range molecular weight proteins for varietal discrimination of the mustard genotypes.

Table 1: Rf values, intensity and position of protein bands for mustard genotypes using SDS PAGE

Band	Rf value	Rohini	Bio 902	Kranti	Maya	GM-2	Varuna	PCR 7	Pusa Bold	RN 393
1	0.205	-	-	-	+	+	+	+	+	-
2	0.229	++	++	++	+++	+++	+++	+++	+++	-
3	0.270	+	-	-	+	+	+	+	+	-
4	0.297	++++	++++	++++	++++	++++	++++	++++	++++	+++
5	0.377	++++	+++	++++	++++	++++	++++	++++	++++	+++
6	0.416	++++	++++	++++	++++	++++	++++	++++	++++	+++
7	0.486	++	++	++	++++	++++	++	++	+	-

+: Low intensity, ++: Medium intensity, +++: High intensity, ++++: Very high intensity

In the present investigation for total soluble seed proteins, three common bands (Rf value 0.384, 0.454 and 577) was detected for all the nine genotypes. The band at Rf value 0.237 was present in Maya, GM-2, Varuna, PCR 7 and Pusa Bold, while it was absent in Rohini, Bio 902 and RN 393. Similarly the band at Rf value 0.384 was present in all the genotypes except in Bio 902, Kranti and RN 393. The band at Rf value 0.662 was present in all the genotypes except RN 393 (Table 1). Thus, the absence of these bands was effectively used for discriminating the mustard genotypes.

The variation in number and intensity of the bands might be due to differential extraction or difference in solubility of protein or lack of separation of several proteins having similar migration rates (Ladizinsky and Hymowitz, 1979). Similar observations based on band intensity were reported by Chun *et al.* (1994), Asghar *et al.* (2003) and Varma *et al.* (2005) in maize genotypes, Devi (2000) in sunflower, Vijayan (2005) in rice, Paul and Datta (2006) in celery and ajowan, Nisha (2007) in wheat and Sumathi (2007) in oats.

CONCLUSION

Electrophoresis of seed proteins showed a total of 7 bands in Maya, GM-2, Varuna, PCR 7 and Pusa Bold whereas for rest of the genotypes some bands were absent. The critical bands for identification were the bands at Rf values 0.237, 0.327 and 0.667 which formed the 1st, 3rd and 7th band, respectively. The intensity of the band also varied among all the genotypes. Hence the present study obviously indicated the use of SDS page profile through electrophoresis for discrimination of mustard genotypes.

REFERENCES

Asghar, R., T. Siddique and M. Afzal, 2003. Inter and intra-specific variation in sds-page electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. Pak. J. Biol. Sci., 6: 1991-1995.

Chehregani, A. and F. Kavianpour, 2007. Efects of acid rain on the development stages of ovules and seed protein in bean plants (*Phaseolus vulgaris* L.). Am. J. Plant Physiol., 2: 367-372.

Chun, W., B. Ke, Z. Huaxiao, Z. Zhanming and W. Jian, 1994. Polyacrylamide gel electrophoresis of salt-soluble proteins for maize variety identification and genetic purity assessment. Seed Sci. Technol., 22: 51-57.

Devi, S.G., 2000. Varietal identification through electrophoresis in sunflower (*Helianthus annus* L.). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Hassan, A.E., S. Heneidak and S.M.H. Gowayed, 2007. Comparative studies of some triticum species by grain protein and amino acids analyses. J. Agron., 6: 286-293.

Kamel, E.A., H.Z. Hassan and S.M. Ahmed, 2003. Electrophoretic characterization and the relationship between some Egyptian cruciferae. J. Biol. Sci., 3: 834-842.

Kour, A. and S.P. Singh, 2004. Evaluation of genetic diversity in different genotypes of *Brassica juncea* by SDS-PAGE. Proceedings for the 4th International Crop Science Congress, Sept. 26-Oct. 1, The Regional Institute Ltd., Gosford, Australia, pp: 1129-1129.

Kumar, J., R.L. Agrawal, A. Kumar and G.K. Garg, 2001. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis for determination of genetic purity of sunflower hybrids. Seed Sci. Technol., 29: 647-652.

Ladizinsky, G. and D. Hymowitz, 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. Theoret. Applied Genet., 54: 145-151.

Milka Anjana, R., 2002. Varietal characterization of groundnut through morphological features, chemical tests and electrophoretic techniques. M.Sc. Thesis, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.

Mukhlesur, R.M., Y. Hirata and Shah-E-Alam, 2004. Genetic variation within *Brassica rapa* cultivars using SDS-PAGE for seed protein and isozyme analysis. J. Biological Sci., 4: 239-242.

Nisha, C., 2007. Assessment of seed quality in wheat (*Triticum aestivum* L.) varieties as influenced by different alien rust resistant genes under varying production conditions. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Paul, R. and A.K. Datta, 2006. Seed protein profiles in celery (*Apium graveolens* L.) and Ajowan (*Trachyospermum amni* L.) plant types. Int. J. Plant. Sci., 1: 36-38.

Roy, M., N. Mandal and P.K. Das, 2001. Seed protein characterization and isozyme diversity for cultivar identification in grass pea (*Lathyrus sativus* L.). Indian J. Genet. Plant Breed., 61: 246-249.

Shete, D.M., 2004. Seed production techniques in castor (*Ricinus communis* L.) hybrid TMVCH I. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Sumathi, S., 2007. Varietal identification in oats (*Avena sativa* L.). M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Varier, A.M., Robert and J. Cooke, 1992. Identification of sunflower (*Helianthus annus* L.) hybrids and inbreds by SDS- PAGE of seed proteins. Seed Res., 20: 138-141.

Varma, V.L.K., N.M. Reddy, K. Keshavulu and R. Ankaiah, 2005. Characteriztion of maize (*Zea mays* L.) genotypes through seed protein electrophoresis (SDS-PAGE). Crop. Res., 30: 124-127.

Vijayan, R., 2005. Organic seed production in rice cv. ADT 43. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.