SDS PAGE Electrophoresis in Mustard Cultivars

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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 F1 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 its parental lines and Hassan et al. (2007) in triticum. 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

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Gel concentration (10%) for 15 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide solution (C)</td>
<td>5.0 mL</td>
</tr>
<tr>
<td>Separating gel buffer</td>
<td>3.75 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>6.25 mL</td>
</tr>
<tr>
<td>10% SDS (G)</td>
<td>100 μL</td>
</tr>
<tr>
<td>10% APS (H)</td>
<td>100 μL</td>
</tr>
<tr>
<td>Tetramethyl Ethylene Diamide (TEMED)</td>
<td>20 μL</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Stock acrylamide solution (C)</th>
<th>1.3 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stacking gel buffer (F)</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>10% SDS (G)</td>
<td>100 mL</td>
</tr>
<tr>
<td>10% APS (H)</td>
<td>50 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>6.1 mL</td>
</tr>
<tr>
<td>Tetramethyl Ethylene Diamide (TEMED)</td>
<td>10 μL</td>
</tr>
</tbody>
</table>

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 1 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**

<table>
<thead>
<tr>
<th>Comassie brilliant blue R. 250</th>
<th>0.1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>40 mL</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>10 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>50 mL</td>
</tr>
</tbody>
</table>

**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: Per 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

<table>
<thead>
<tr>
<th>Band</th>
<th>Rf value</th>
<th>Rohini</th>
<th>Bio 902</th>
<th>Kranti</th>
<th>Maya</th>
<th>GM-2</th>
<th>Varuna</th>
<th>PCR 7</th>
<th>Pusa Bold</th>
<th>RN 393</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.205</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.229</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.270</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.297</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>0.377</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>0.416</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>0.486</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+: 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 May, 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.


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


