ELECTROPHORETIC STUDIES ON PROTEINS OF SOME COMMONLY GROWN VARIETIES OF WHEAT SEEDS IN RELATION TO LEVELS OF AFLATOXIN PRODUCTION

RAKESH KUMAR & K. K. SINHA
University Department of Botany, T. M. Bhagalpur University, Bhagalpur, Bihar, India

ABSTRACT

Seeds of nine varieties of wheat were studied by using the technique of polyacrylamide gel electrophoresis (PAGE) and the banding pattern was correlated with the levels of aflatoxin production. PAGE was run both under native and reduced conditions (SDS PAGE). It was observed that those varieties which showed more diverse kinds of proteins showed more resistance to aflatoxin production. On the other hand those varieties which showed lesser kinds of proteins showed lesser resistance to aflatoxin production.

KEY WORDS: PAGE, SDS- PAGE, Aflatoxin, Resistance

INTRODUCTION

Protein is considered the most important nutrient for humans and animals, as manifested by the origin of its name, from the Greek proteios for primary. The protein content of wheat grains vary between 10%-18% of the total dry matter. Wheat proteins are classified according to their extractability and solubility in various solvents. Classification is based on the classic work of T. B. Osborne at the turn of the last century. In his procedure, sequential extraction of ground wheat grains result in the following protein fractions:

1. Albumins, which are soluble in water;
2. Globulins, which are insoluble in pure water, but soluble in dilute NaCl solution, and insoluble at high NaCl concentration;
3. Gladiins, which are soluble in 70% ethyl alcohol, and;
4. Glutenins, which are soluble in dilute acid or sodium hydroxide solutions.

Albumins are the smallest wheat proteins, followed in size by globulins. Gladiins and glutenins are complicated high molecular weight proteins. Most of the physiologically active proteins (enzymes) in wheat grains are found in the albumin and globulin groups. In cereals, the albumins and globulins are concentrated in the seed coats, the aleurone cells and the germ, with a somewhat lower concentration in the mealy endosperm. The albumin and globulin fractions cover about 25% of the total grain protein.

Gladiins and glutenins are storage proteins and cover about 75% fo the total protein content. The wheat plant stores proteins in this form for future use by the seedling. Gladiins and glutenins are mainly located in the mealy endosperm and are not found in the seed coat layers nor in the germ. Storage proteins in wheat are unique because they are technologically active. They have no enzyme activity, but they have a function in the formation of dough as they retain gas, producing spongy baked products.

In an earlier study we have observed that wheat seeds of UP 262 variety was found to be most susceptible to aflatoxin production whereas Lokmaya variety was found to be most resistant to aflatoxin production. We have also observed that higher the quantity of protein, lower the level of aflatoxin production and vice versa. In the present study we have
analyzed the quality of protein of nine varieties of wheat seeds in relation to aflatoxin production by using the technique of polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE.

**MATERIALS AND METHODS**

**A.** Following buffers and solutions were prepared

1. **Acrylamide solution (30% acrylamide, 0.8% bisacrylamide):** 30 g of acrylamide and 0.8 g of N, N’-methylene bisacrylamide were dissolved in 100 ml of double distilled water, the solution was filtered through a Whatman filter paper no.1 and stored in a brown coloured bottle. Utmost precautions were taken while preparing the solution since nonpolymerized acrylamide is a neurotoxin and is easily absorbed by the skin.

2. **Resolving gel buffer (1.5 M Tris-Cl, pH 8.8):** 9.705 g of Tris-hydroxymethyl aminomethane was dissolved in 30 ml of double distilled water and the pH was adjusted to 8.8 using conc. HCl. The final volume of the solution was made 50 ml using double distilled water and the buffer was stored in a refrigerator.

3. **Stacking gel buffer (0.5 M Tris-Cl pH 6.8):** 1.5 g of Tris-hydroxymethyl aminomethane was dissolved in 15 ml of distilled water and the final pH was adjusted to 6.8 and the final volume to 25 ml using double distilled water.

4. **10% Sodium dodecyl sulfate (SDS):** 10 g of SDS was added to 100 ml of double distilled water.

5. **10% Ammonium persulfate (APS):** 0.2 g of APS was added to 20 ml of double distilled water.

6. **Promoter:** Commercially available TEMED (N, N, N’, N’-Tetramethylene diamine) was used.

7. **Tank buffer (0.025 M Tris pH 8.3, 0.192 M Glycine, and 0.1% SDS):** 3 g Tris, 14.4 g glycine, 10 ml of 10% SDS were added in 500 ml of double distilled water and the final volume was adjusted to 1 liter after adjusting the final pH to 8.3 using conc. HCl. Freshly prepared tank buffer was used for each run.

8. **2X sample treatment buffer:** (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol and bromophenol blue): 5 ml of stacking gel buffer, 8 ml of 10% SDS, 4 ml of glycerol, 0.8 ml of 1% aqueous Bromophenol blue and 0.06 ml of mercaptoethanol were mixed and the final volume was adjusted to 20 ml.

9. **Staining solution:** 50 mg Coomassie Brilliant Blue R250 was dissolved in 100 ml of a mixture of distilled water-methanol-acetic acid in the ratio of 46 ml: 45 ml: 9 ml.

10. **Destain solution:** 1 litre of destain solution was prepared by mixing distilled water, methanol, and acetic acid in the ratio of 87:5:8.

**B.** For running vertical slab gel electrophoresis, following items were procured

- A set of two glass plates especially designed for slab gel electrophoresis.
- Three spacers, a comb with a suitable teeth size and clips.
- A vertical slab gel electrophoretic tank with upper and lower reservoirs.
- Electrophoretic leads and a power pack of 0-500 volts.

**C.** Pouring and setting the resolving gel

Glass plates were cleaned with ethanol to free them from grease. Three spacers were placed on the three edges of the intact glass plate and the other wedged plate was placed on it in such a way that the wedged side is away from the spacers. The assembly was hold in place using bulldog clips and the three sides were sealed using 1% molten agarose. The assembly was placed vertically using clamps and the separating/resolving gel mixture was poured in the pocket created between the glass plates up to about two thirds of the area and was allowed to polymerize at room temperature.
D. **Preparation of discontinuous gel** Once the resolving gel was set, the isobutanol layer was carefully poured off carefully. Stacking gel mixture was prepared and immediately poured on the top of the resolving gel. Then the comb was inserted and the assembly was left undisturbed to polymerize.

E. **Arranging the gel assembly for electrophoresis** The comb and the spacer present at the bottom of the gel were gently removed. The gel-plate assembly was mounted into the electrophoretic chamber in such a manner that the wedged plate was positioned towards inside. Both the upper and lower reservoirs were filled with the tank buffer so that the wells were immersed in the buffer.

F. **Sample preparation (under native condition)** The 5mg wheat seed powder was mixed with 100ml of phosphate buffer (pH=6.2) and then centrifuged at 3000rpm for 20 minutes.

G. **Sample preparation (under denaturing conditions)** Before loading the sample on the SDS-PAGE gel, 100µl of the sample buffer was mixed with 10 mg of wheat seed powder. The sample was then heated at 100°C for 3 minutes and centrifuged at 3000 rpm for 20 minutes.

H. **Electrophoresis** Once the samples were loaded, the buffer levels were checked in the upper and lower chambers and the terminals were connected to the power pack. The current was switched on and the gel was run initially at 15mA for 15 minutes and then at 25 mA till the front almost reached the bottom of the gel.

I. **Staining the gel** The power supply was switched off, the terminals were disconnected and the gel assembly was removed from the electrophoretic apparatus. The side spacers were removed, the wedge plates were lifted and the gel was slipped gently into the stain that was kept in a trough of a suitable size so as to immerse the gel into it. The gel was soaked in it for an hour with gentle shaking.

J. **Destaining the gel** The gel was transferred in a destaining solution and it was ensured that it was shaken mildly during destaining. The destaining solution was changed frequently and the process was continued till deep blue bands were clearly visible against a faded background.

**RESULTS AND DISCUSSIONS**

Electrophoretic analysis of proteins of different wheat varieties under native condition, revealed the presence of varied nature of bands in different varieties of wheat (Table 1 & Fig 1). The number of bands in healthy seeds ranged from three in UP 262 variety to five in Lokmanya. Of these bands, one band (band II) was common to all the nine varieties of wheat seeds. A unique band (band III) was present in the most resistant variety Lokmanya under study, which seems to be the protein which somehow inhibited the production of aflatoxin in this variety. On the other hand, band I was absent only in the most susceptible variety UP 262. The absence of this protein might be correlated with the high levels of aflatoxin production in that variety.

SDS-PAGE of the wheat seed of different varieties revealed a number of protein bands (Table 2 & Fig 2). Some of the features noted in the banding pattern are outlined here. Band no VII and XV were found to be present in all the varieties of wheat seeds whereas band no XI was present only in the UP 2003, PBW 343, Lokmanya, RR 21 and Kedar varieties. All 15 protein bands were visualized in the variety RR 21 which shows that proteins of the most diverse nature having different molecular weights affect adversely the production of aflatoxin because this variety showed maximum resistance to aflatoxin production. This variety was earlier reported to have the highest amount of protein too. Thus, quantitatively more protein and qualitatively diverse kinds of protein inhibit the production of aflatoxin. On the other extreme is UP 262 variety, which was found to facilitate maximum amount of aflatoxin under laboratory condition, is having less diverse kind of proteins (only 8 protein bands). Quantitatively also this variety is having less amount of
protein. Electrophoretic studies on protein of wheat seeds done earlier also depicted the varied nature of bands in different varieties.

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REFERENCES


Table 1: Protein Profile of Different Varieties of Wheat Seeds Showing Bands with their $R_m$ Values under Native Condition

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**Table 2: SDS PAGE Analysis of Proteins Different Varieties of Wheat Seeds Showing Bands with their \( R_m \) Values**

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**Fig. 1:** Polyacrylamide Gel Electrophoresis of Proteins of Nine Varieties of Wheat Seeds Under Native Condition  
(S1= Kundan; S2= HD 2285; S3=UP 2003; S4=PBW 343; S5= Lokmanya; S6= RR 21; S7=HD 2329; S8= Kedar; S9= UP 262)

**Fig. 2:** SDS PAGE of Nine Wheat Varieties Showing Banding Patterns.  
(S1= Kundan; S2= HD 2285; S3=UP 2003; S4=PBW 343; S5= Lokmanya; S6= RR 21; S7=HD 2329; S8= Kedar; S9= UP 262)
Electrophoretic Studies on Proteins of Some Commonly Grown Varieties of Wheat Seeds in Relation to Levels of Aflatoxin Production

A. K. Sinha and Rakesh Kumar

PAGE of nine varieties of wheat seeds revealed diverse banding patterns, which was then correlated with the levels of aflatoxin production in each variety. The greater the diversity in the banding pattern the greater the resistance against aflatoxin production.