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Characterisation of Pakistani Wheat Varieties for General Cultivation in the Mountaineous Regions of Azad Kashmir

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Abstract: The high molecular weight (HMW) glutenin subunits from seven Pakistani wheat genotypes were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), in order to characterise the plant material and test the variability within species. The mitotic results indicated that the chromosomal count in six genotypes was $2n = 42$. The number of chromosomes in variety Sargab-92 were somewhat irregular, showing $2n = 40$ as well as 41 instead of 42 chromosomes. The banding patterns of HMW glutenin subunits of 6 Pakistani wheat genotypes were proved to be similar and a genetic variability was shown by the genotype Sargab-92, in which 4 extra bands had been identified.

Key words: High molecular weight (HMW), SDS-PAGE, wheat cultivars, protein, glutenin

Introduction

Proteins occur in all organisms, where they participate in a wide variety of biological functions. Proteins provide the structure, rigidity and flexibility associated with external body parts such as skin, hair and the exoskeletons of arthropods. By changing their structure they provide the basis for biological motions, such as muscle contraction. Large classes of proteins, the enzymes, serve as biological catalysts, enhancing greatly the rates of chemical reactions that are vital to life.

The endosperm of the wheat grain usually contains between 7 and 15% by weight of protein and just less than one half of this glutenin. Cereal grains synthesize and accumulate large amounts of storage proteins that is deposited in protein bodies during the course of seed development. Gliadins and glutenins are the major storage proteins of wheat (*Triticum aestivum* L.) endosperm. Together they constitute 80% of the total protein in the grain (Osborne, 1907). Glutenin is a large, heterogeneous protein complex ranging in molecular weight from several hundred thousands to several millions (Huebner and Wall, 1976). It is build up of 15 or more different sub-units. The advent of SDS-polyacrylamide gel electrophoresis has now enabled the high-molecular-weight sub-units (90,000-150,000) to be well separated, although the smaller sub-units (30,000-51,000) are still rather poorly resolved (Payne and Corfield, 1979). Glutenin plays an important role in the bread-making process, adding strength and elasticity to a dough (Bietz *et al.*, 1973). Payne *et al.* (1981a) showed that the differences in baking quality between several wheat cultivars could be related to the presence or absence of specific HMW sub-units, although the exact contribution made by individual components could not be directly assessed. Because of the importance of the HMW glutenin sub-units their genetics has been studied extensively (Payne *et al.*, 1982).

The pioneering studies of Shepherd (1968) and Wrigley and Shepherd (1973) used the aneuploids of Sears (1954) and 1- and 2-dimensional methods of gel electrophoresis to demonstrate that all gliadin protein components of hexaploid common wheat endosperm are coded by genes located on chromosomes of homologous groups 1 and 6 (Lafindra *et al.*, 1984). Allelic variation of high molecular weight (HMW) sub-units of glutenin in 185 cultivars of bread wheat has been described by Payne *et al.* (1981b), where about 20 different major sub-units were distinguished by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). Over the past few years, more efforts have been focused on analysis at the molecular level. Some cDNA clones have been isolated that encode LMW glutenin components (Bartels and Thompson, 1983).

In present paper Seven Pakistani wheat varieties has been characterized by using the technique of SDS-PAGE.

Materials and Methods

This study was conducted at the Department of Plant Breeding and Genetics, Martin-luther University, Federal Republic of

Germany during six months visit programme, sponsored by German Academic Exchange Service (DAAD).

Plant material: The wheat varieties used in this study consisted of a collection of 7-genotypes, provided by the National Agricultural Research Council (NARC), Islamabad, Pakistan for general cultivation in the mountainous region of Azad Kashmir. The material comprised of: Local (Rawalakot), Inqalab-91, Bakhtawar, Chakwal, Kohistan-97, Rawat-87 and Sargab-92.

Cytology: For the confirmation of the chromosome numbers all the varieties were tested cytologically. Preparations were made from the young seedlings grown inside the laboratory. Chromosomes were pre-treated with ice for 24 h and fixed in 3:1 (Alcohol: acetic acid). The fixed root tips were hydrolysed in 1 N HCl at 60°C for 12 minutes and stained with fuchsin for 1h and squashed in 2% aceto-carmine.

SDS-PAGE: In addition to other characters, the variability of seed reserve protein was also analysed in order to characterize the seven Pakistani summer wheat varieties.

Single grains were ground to fine flour by using a hammer mill. Tubes with flour were labelled. Extraction solution was prepared by mixing together 5 ml glycerol (20%), 3.12 ml 1 M Tris pH 6.8, 6.87 ml distilled water, 2 g SDS (7.06%), 500 μ l and bromphenol blue (0.1%).

For the extraction of 35 specimens, 5 ml extraction solution, 4500 μ l double distilled water and 500 μ l 2-Mercaptoethanol were mixed together. From this material, 10 μ l 1 mg⁻¹ material was used.

A vortex mixer mixed combination, and material was kept overnight (16 h) at 40°C. Next day tubes were cooked in water bath for 2.5 minutes. Samples were then transferred to a centrifuge at 3000 rpm for 10 minutes.

The uppermost phase was transferred to a new tube; material is now ready for the experiment purposes, which could be kept for a longer time at 4°C.

For the preparation of 3-different gels, the following combination was used:

Lower gel: Acrylsolution 10 ml, Temed 25 μ l and APS 70 μ l.

Separating gel: Double distilled water 24.2 ml, Acrylsolution 20.2 ml, lower Tris-buffer 15.0 ml, Temed 30.2 μ l and APS 302 μ l.

Collecting gel or stacking: Double distilled water 4.6 ml, Acrylsolution 980l, upper Tris-buffer 1860 μ l, Temed 7.6 μ l and APS 37.8 μ l.

Gel preparation: Glass plates were cleaned with methanol and using plastic tape and clips made a glass cabinet. Lower gel was added to the glass cabinet. After an hour, separating gel was

added to lower gel, followed by butanol for 45 minutes. Butanol was replaced by distilled water for 45 minutes. Gel was dried with the help of a filter paper and collecting gel was added. Comb was fixed inside the collecting gel in such a way that there must be no bubbles at all (if present must be removed by moving the comb left-right). Glass cabinet was fixed with the electrophoresis apparatus; fill the electrophoretic compartment of the electrophoretic tray with electrode buffer (100 ml 10 x Tankbuffer and 900 ml distilled water), while the tray was added with ice water. Experimental material was loaded on the gel connected to the power supply, no more than 200 Volts.

Staining: Gel was then transferred to electric shaker. Pour the stain into the developing tray, stain for 45 minutes, followed by destaining for a longer time (in between stain was replaced). Mark the top left hand corner of the gel with a small cut for the identification. A care should be taken that a high dose of current will increase heating of gel, which may affect the proteins. At the end a picture was taken with a video camera, connected to a computer programme.

Results

Cytological observations: Seven Pakistani summer wheat varieties were tested cytologically, in order to confirm their chromosome number and ploidy level. For this purpose a mitotic study was arranged, in which 10 plants from each variety were examined under the microscope. The chromosome number was confirmed to be $2n = 42$. The first 6 wheat varieties were proved to be hexaploid (Fig. 1), but in case of variety Sargab-92 the chromosomes with $2n = 40$ or 41 were observed, which clearly indicated the variability within the genotype.

During this course of examination the wheat genotypes were tested only for their chromosome numbers (Mitosis), and a behaviour of metaphase I chromosome could not be observed due to the non-availability of time. Therefore, it was suggested that a meiosis study should be conducted for more critical screening of the material under observation.

SDS-PAGE: During this course of investigation sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyse the gliadin patterns of extracts from seeds of seven Pakistani wheat varieties, where they were executed for their homogeneity. The technique followed was described previously by Payne *et al.* (1981a). Electrophoretic patterns of gliadins are shown in Fig. 2, in which variety Local, Inqalab-91 and Bakhtawar are having two slots and Chakwal-86, Kohistan-97,



Fig. 1: Variety Chakwal with $2n = 42$ chromosomes

Rawat and Sargab-92 having three slots for comparison. During this course of investigation the banding patterns of first 6 genotypes were found to be identical, while a great deviation was observed within the variety Sargab-92. Slot 1 of variety Sargab-92 indicated some extra high molecular weight sub-units as compared to slot 2 and 3. The diagonal arrows mark the extra bands on slot 1 of variety Sargab-92. Slot 2 and 3 seems to be similar, but a clear definition was difficult, due to low staining intensity.

In order to confirm this statement the whole procedure was repeated. In the second experiment the variety Rawat, Kohistan and Chakwal having 2 slots and variety Local, Inqalab-91, Bakhtawar and Sargab-92 having 3 slots for comparison (Fig. 3). The same picture was obtained during the second observation also.

In order to define the materials more precisely, a third experiment was arranged. In this experiment an extra lane was added to varieties local, Inqalab-91 and Bakhtawar, while variety Rawat-87 and Kohistan-97 and Chakwal having two lanes and variety Sargab-92 having three lanes comparison. Here the lane arrangement of variety Sargab was changed to 3, 2 and 1, instead of lane 1, 2 and 3. Results of the third experiment also confirmed the findings of the 1st and 2nd experiments. Hence the variation in high molecular weight (HMW) gliadin sub-unit composition of variety Sargab-92 was confirmed (Fig. 4).

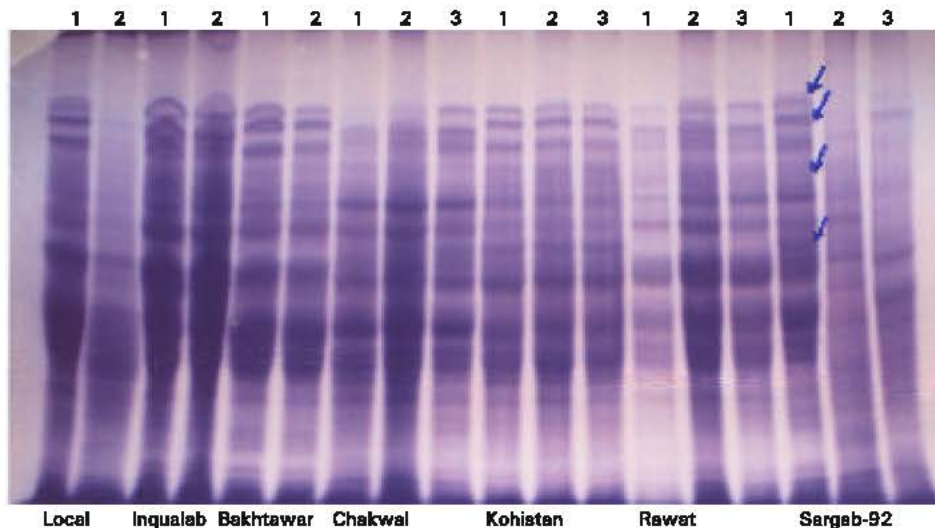


Fig. 2: SDS-PAGE banding patterns of 7 Pakistani wheat varieties

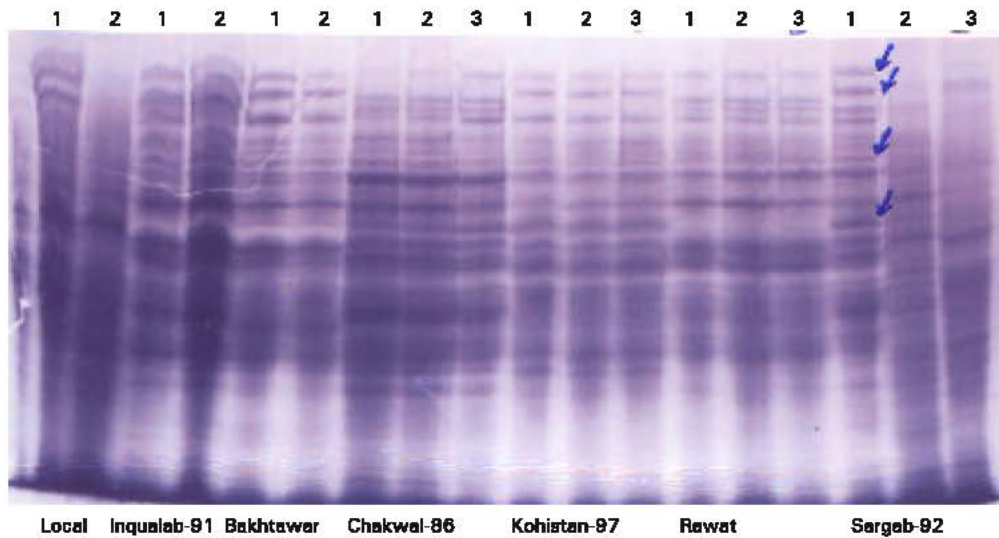


Fig. 3: SAD-PAGE banding patterns of 7 Pakistani wheat varieties

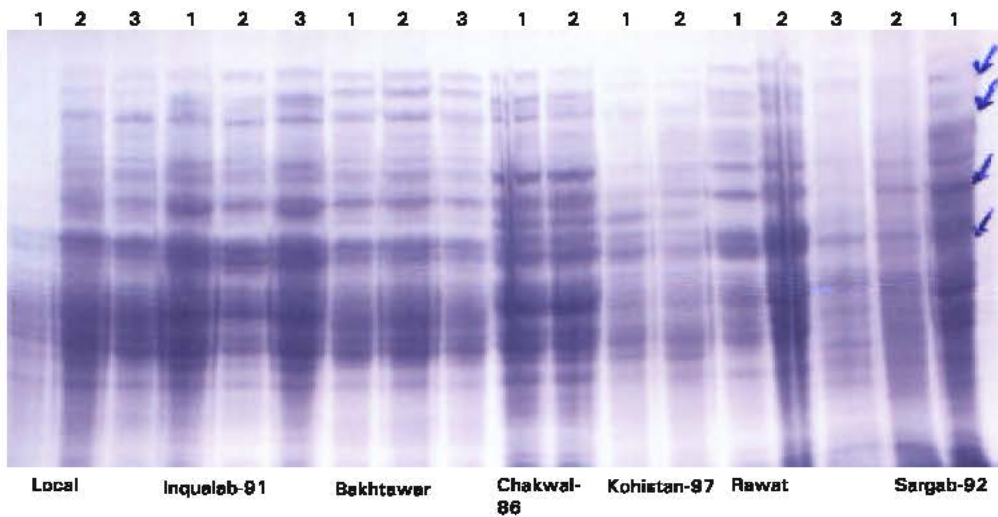


Fig. 4: SDS-page banding patterns of 7 Pakistani wheat varieties

Discussion

A total of 7 wheat genotypes were extracted from single seeds and fractionated by SDS-PAGE. As a result 6 genotypes were proved to be identical and a genetic variability was observed by the genotype Sargab-92, in which some extra bands had been reported (Fig. 2-4).

The electrophoretic patterns of the 2nd and 3rd experiment indicating the same banding patterns, where the lane 1 of variety Sargab-92 again shows 4 extra bands quite similar to the previous experiments. Similar type of results have been reported by Waines and Payne (1987) by analysing glutenin through SDS-PAGE in the A genome of 497 diploid wheats and in 851 landraces of bread wheat, in which 4 races with HMW sub-units were discovered. Similarly 3 extra glutenin sub-units in case of Alcedo and 1 extra sub-unit in variety Apollo have been reported by Edossa (1996). Gliadin variations in HMW sub-units in durum wheat cultivars Creso and Duramba have also been reported by Ciaffi *et al.* (1993) by studying 315 populations, in which total 44 different banding patterns were identified. Polymorphism of high molecular weight

(HMW) glutenin sub-units in 446 accessions of the wild tetraploid wheat in Israel was characterised by Levy and Feldman (1987) with regard to the ecogeographical distribution of the HMW glutenin alleles, both between and within 22 populations. Intrapopulation variability was interpreted by geographical distribution, while marginal population tended to be more uniform than those at the center of distribution. According to vavilov's theory " the centers of distributions are also centers of variation. The narrow variation of marginal populations might result from a double founder effect:

- a) The founder effect of the first settler that colonized this new location and
- b) A recurrent founder effect i.e. marginal populations, having a non-optimal level of adaptation, fluctuating much stronger than central populations.

This type of variations may be due to intermixing of seeds or due to cross-pollination or altitude, average temperature and rainfall

Khan *et al.*: High molecular weight (HMW), SDS-PAGE, wheat cultivars, protein, glutenin

associated with micro-environmental differences in soil type (Payne *et al.*, 1981a; Bietz *et al.*, 1973; Lawrence and Shepherd, 1981).

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