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BIOCHEMICAL CHARACTERISATION OF INDIAN DURUM WHEATS

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SUMMARY

Eight varieties of durum wheat-PDW 233, MACS-9, HI-8498, A-9-30-1, Raj 911, Raj 1555 having good quality characteristics and Bijaga yellow, NIDW-15 having poor quality characteristics have been characterized for their gluten components which govern their dough properties. The protein content in these varieties varied from 11.1% to 13.8% and the β-carotene content from 4.1 to 5.85 ppm with maximum in PDW-233 and minimum in A-9-30-1. A 45 kD polypeptide was present only in the good quality durum varieties. PCR amplification of the genomic DNA from the selected varieties using LMW glutenin gene specific primers, however, showed no polymorphism between the good and poor quality varieties under study.

Key words **: Gliadins, glutenin, LMW glutenin gene, storage proteins, *T. durum*.**

INTRODUCTION

Wheat is one of the most important crops in the world. More than 35% of the world's population subsists on wheat (FAO 1995). Nutritional importance of wheat is due to its storage protein gluten. Its visco-elasticity allows wheat to be used for a wide variety of food products including leavened and non leavened breads, cakes, biscuits, pastas and noodles. The technological properties of the dough and the protein quality are largely governed by the functional properties of the two major gluten components: gliadins and glutenins (Kasarda *et al.* 1976). Durum wheat or macaroni wheat (*Triticum durum*) is an important crop for production of pasta, macaroni and various types of breads which are currently in demand due to increased standards of living. In durums too gluten strength is recognized to be one of the most important quality criteria controlled by gluten proteins. At present there is very little information available regarding storage protein characteristics of Indian durum wheat varieties, at botn biochemical as well as molecular level. Such information is essential for plant breeders for crop improvement. Therefore, studies on storage proteins of durum wheat in relation to pasta making quality were undertaken in some varieties varying in technological characters.

MATERIALS AND METHODS

Seeds of the durum wheat varieties viz. Bijaga yellow, A-9-30-1, MACS-9, PDW-233, Raj 1555, Raj 911, NIDW-15, were obtained from Divison of Genetics, Indian Agricultural Research Institute, New Delhi and seeds of the varieties HI 8498 and *T. timopheevi* were provided by Regional Research Station, I.A.R.I., Indore (M.P.). Chemicals and reagents used were of Analar or G.R. grade from Glaxo Lab (India) Ltd., E. Merck, S.R.L. and Sigma Chemical Company, USA. PCR primers were custom synthesized from Genset Singapore Biotech Pvt. Ltd. Taq polymerase and $MgCl_2$ were obtained from Genei, Bangalore.

Total nitrogen content of seeds was determined by micro-Kjeldhal method (A.O.A.C. 1965). The values obtained were multiplied by the factor 5.75 to obtain the

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percentage of protein. Storage proteins were extracted from single grains of each variety of wheat by the modified method of Singh and Shephard (1985). Each grain was ground using a mortar and pestle in 0.5 ml of solvent which comprised of 50 mM KH_2PO_4-NaOH (pH 6.8) containing 8 M urea, 4%SDS, 20% glycerin and 5% β-mercaptoethanol. The homogenate was sonicated for a few minutes and then centrifuged at 15000 rpm for 4 min. in Sorvall RC 5C centrifuge using SS-34 rotor. The supernatant was used for SDS-PAGE.

Gliadins were electrophoresed on a 12% SDS-polyacrylamide gel using 0.025 M Tris-glycine buffer (pH 8.3) containing 0.1% (w/v) SDS (Laemmli 1970). LMW sub-units of glutenins were extracted and electrophoresed on 12% SDS-polyacrylamide gel as described by Singh *et al.* (1991). β-Carotene content was determined according to the method of American Association of Cereal Chemist (AACC 1962).

Genomic DNA from seven days old seedlings of all the eight varieties of durum was isolated by CTAB method (Murray and Thompson 1980) and purified using the Maniatis protocol (Sambrook *et al.* 1989). PCR analysis of eight durum wheat varieties was conducted using a set of LMW glutenin gene specific primers, custom synthesised from Gen Set Oligos, Singapore.

RESULTS

Protein content

The protein percentage in different durum wheat varieties varied from 11.1% to 13.8% (Table 1). A good quality durum variety HI 8498 showed the maximum protein percentage and NIDW-15 the minimum. The absolute amount of protein on mg/grain basis followed a similar pattern with Bijaga yellow and NIDW-15 showing relatively lower protein content i.e. 5.95 and 5.55 mg of protein/grain respectively as compared to the rest of the varieties in which the protein content was in the range of 6.65 to 6.90 mg/grain.

Table 1. Protein content in durum wheat varieties

Varieties	Protein	
	Percentage	mg/grain
Bijaga yellow	11.9±0.02	5.95±0.010
A-9-30-1	13.3±0.02	6.65±0.010
MACS-9	13.1±0.01	6.55±0.005
PDW 233	13.5±0.01	6.75±0.005
Raj 1555	13.5±0.02	6.75±0.010
HI 8498	13.8±0.01	6.90±0.005
Raj 911	13.6±0.01	6.80±0.005
NIDW-15	11.1±0.01	5.55±0.005

β-Carotene content

β-Carotene content varied from 4.10 to 5.85 ppm (Table 2) PDW-233 showed the highest β-carotene content whereas A-9-30-1 the lowest.

Table 2. β-Carotene content in durum wheat varieties

Varieties	β-Carotene content (in ppm)
Bijaga yellow	4.94±0.03
A-9-30-1	4.08±0.02
MACS-9	4.71±0.05
PDW 233	5.80±0.04
Raj 1555	4.82±0.01
HI 8498	4.21±0.03
Raj 911	4.90±0.03
NIDW-15	4.63±0.04

SDS-PAGE of soluble proteins

Total soluble proteins isolated from different varieties were analysed on a 12% SDS-PAGE, the banding pattern within the MW range of 11kD to 98kD. No significant qualitative or quantitative differences were observed in the banding pattern of these proteins.

SDS-PAGE of total storage proteins

Total storage proteins isolated from different durum varieties were analysed on a 12% SDS-PAGE. Protein band pattern is presented in Plate 1. SDS-PAGE analysis revealed a MW range of 14kD to 110 kD of the various polypeptides. The polypeptides with MWs 72kD, 67kD, 58kD, 56kD, 36kD, and 34kD were common in all the varieties. Less intense bands with MWs 98kD, 90kD, 18kD, 17kD, 16kD, and 14kD were also common in all the varieties. No major qualitative or quantitative differences were observed in the MW range of 20 to 30kD

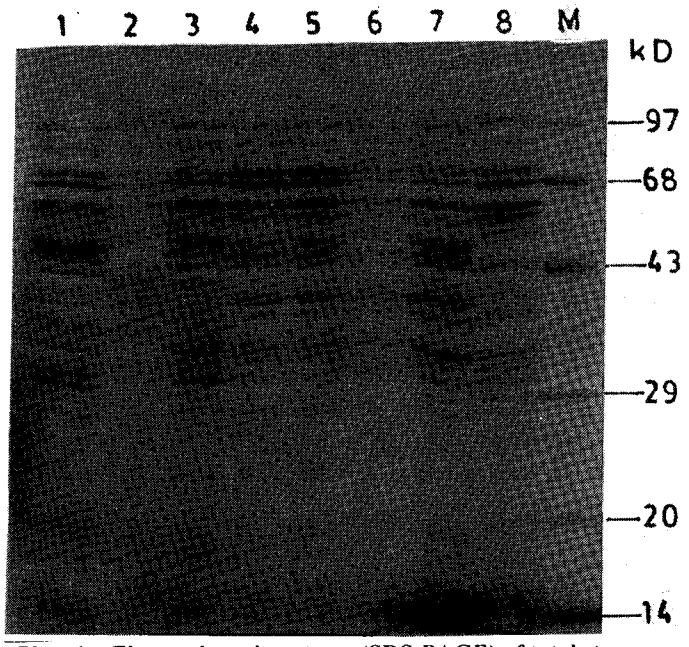

Plate 1. Electrophoretic pattern (SDS-PAGE) of total storage proteins of different durum wheat varieties. Lane 1, Raj 911; lane 2, Bijaga yellow; lane 3, Raj 1555; lane 4, A-9-30-1; lane 5, MACS-9; lane 6, PDW-233; lane 7, HI-8498; lane 8, NIDW-15; lane M, Molecular weight marker

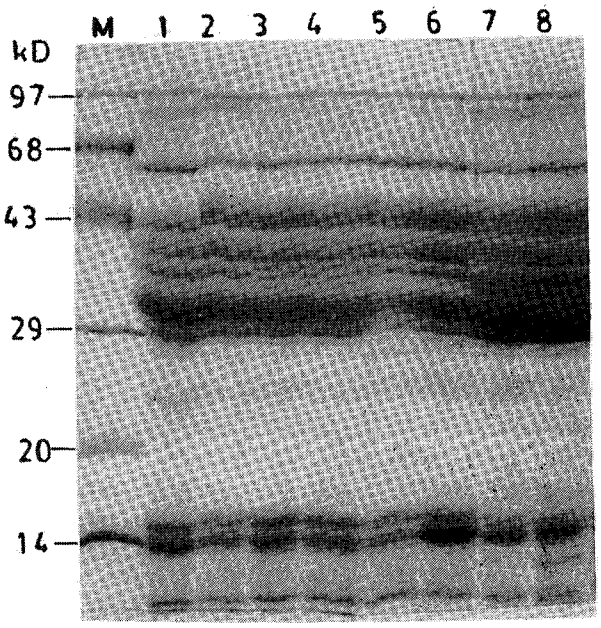

Plate 2. Electrophoretic pattern (SDS-PAGE) of gliadins of different durum wheat varieties. Lane 1, Bijaga yellow; lane 2, MACS-9; lane 3, PDW-233; lane 4, Raj 911; lane 5, Raj 1555; lane 6, A-9-30-1; lane 7, HI 8498; lane 8, NIDW-15; lane M, Molecular weight marker

in all the eight varieties. Comparative polypeptide pattern analysis showed the presence of 45 and 48 kD polypeptides in only the good quality durum varieties and a HMW polypeptide of 110kD in only the poor quality durums under study.

SDS-PAGE of gliadins

Total gliadins isolated from different durum varieties were analysed on a 12% SDS-PAGE. The pattern of protein bands is shown in Plate 2. Polypeptides of MWs 12kD, 14kD, 15kD, 27kD, 29kD, 30kD 31kD, 34kD and 36kD were common in all the varieties. Amongst the high MW polypeptides the pattern was almost similar except a 98 kD polypeptide which was common in all the varieties other than poor quality variety Bijaga yellow. A 63kD polypeptide in the ω-gliadin region was also common in all the varieties. A polypeptide of MW 45 kD was observed in all the varieties under study except the poor quality Bijaga yellow whereas a 39kD polypeptide was observed only in Bijaga yellow. A 35 kD band of medium intensity was observed only in PDW-233 and Raj 911 and an intense band of 32 kD was observed mainly in NIDW-15, HI 8498 and A-9-30-1. Amongst the low MW polypetptides a 15kD polypeptide was distinctly absent in A-9-30-1, HI 8498 and NIDW-15, whereas, a 14kD polypeptide was observed only in the above three varieties. A 13kD band was observed only in HI 8498 and NIDW-15.

SDS-PAGE of glutenins

Glutenins extracted from the selected varieties were analysed on a 10% SDS-PAGE. The pattern of protein bands is shown in Plate 3. SDS-PAGE analysis revealed polypeptides of both HMW glutenins sub-units (HMW-GS) and LMW glutenin sub-units (LMW-GS) in the range of 90 to 110 kD and 36 to 46 kD respectively. Amongst the HMW-GS the 98 kD and 90 kD polypeptides were common to almost all the selected varieties except MACS-9.

A 110 kD polypeptide was observed in the poor quality varieties, Bijaga yellow and NIDW-15. A low intensity band of 84 kD was common in three varieties namely Raj 1555, Raj 911 and Bijaga yellow. A 63kD minor peptide was found in all the varieties. In the LMW-GS region a 40kD and a low intensity 36 kD polypeptide were common in all varieties. A 45 kD polypeptide was distinctly absent in Bijaga yellow and its intensity in

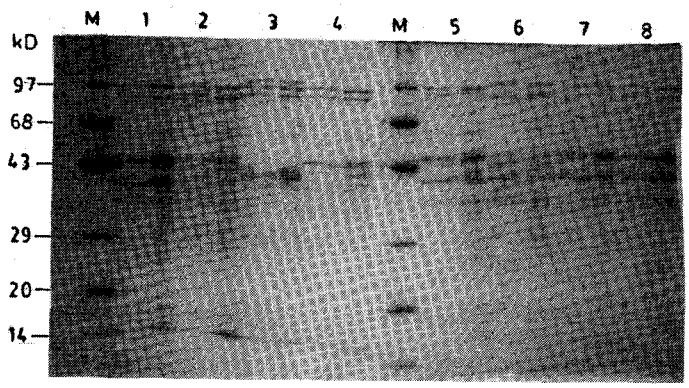

Plate 3. Electrophoretic pattern (SDS-PAGE) of glutenins of different durum wheat varieties. Lane 1, Raj 1555; lane 2, Raj 911; lane 3, Bijaga yellow; lane 4, HI 8498; lane 5, MACS-9; lane 6, NIDW-15; lane 7, A-9-30-1; lane 8, PDW-233; lane M, Molecular weight marker

NIDW-15 was also low as compared to the good quality durum varieties, whereas, a high intensity 39 kD polypeptide was observed only in'Bijaga yellow.

PCR amplification of LMW glutenin genes

In order to amplify the coding region of LMW glutenin sub-unit gene, a set of primers was custom synthesized using published sequences (Cassidy and D' Vorak 1991). The amplified products are shown in Plate 4. It was observed that the LMW glutenin gene specific primers resulted in an amplified product of ~1450 bps in all the varieties under study. No major polymorphism was, however, observed in the banding pattern between the good and poor quality durums. In the control lane where genomic DNA of a tetraploid wild species of wheat, *Triticum timopheevi*, lacking "B" genome was used, no amplification was observed.

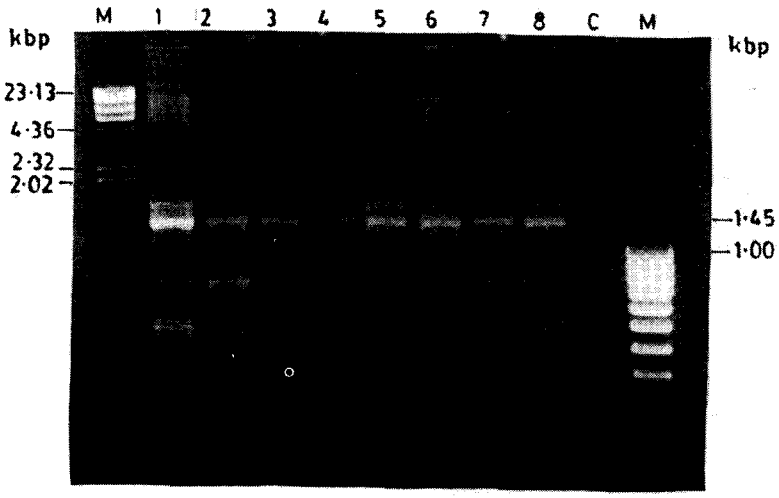

Plate 4. Amplified LMW glutenin gene (PCR). Lane 1, A-9-30-1; lane 2, MCS-9; lane 3, RAJ 911; lane 4, NIDW-15; lane 5, Raj 1555; lane 6, HI 8498; lane 7, Bijaga yellow; lane 8, PDW-233; lane M, Molecular weight marker.

DISCUSSION

Very little information is available on the quality of durums in India with reference to their use in the preparation of macaroni, spaghetti, noodles etc. Such information is important as durums of certain quality attributes have export potential at a premium price to countries where those products are consumed on large scale. Gluten the major storage protein imparting quality to wheat consists of two components namely gliadins and glutenins. The glutenin polymers are largely responsible for dough visco-elasticity while gliadins ineract non-convalenly with each other and with glutenin polymers to plasticise the gluten mass (Shewry *et al.* 1992).

Preliminary study on the storage protein composition of six good quality and two poor quality durums was done. Protein content varied from 11.1% to 13.8% with relatively low percentage in the poor quality durum wheat varieties i.e. Bijaga yellow and NIDW-15. These results were coherent with reports of Matveef (1966) indicating that a protein content lower than 11% results in poorly processed products. Yellow colour in pasta imparts an attractive appearance to the pasta products therefore, majority of pasta consumers prefer the yellow pigment in pasta. β-carotene contributes to the colour production in semolina. The durum kernels/endosperms contain about twice the concentration of β-carotene than that in hexaploid wheats (Sims and Lepage 1968). In the present study β-carotene content among the varieties ranged between 4 ppm (A-9-30-1) to 6 ppm (PDW 233) which correlates with the finding of Prabhavathi *et al.* (1976) that Indian durums are rich in β-carotene. However, no correlation was observed in the quality of the durum wheat varieties and β-carotene content.

In the present study SDS-PAGE of gliadins showed no major qualitative or quantitative difference in the ω-gliadin range, as a single polypeptide of 63kD was observed in all the selected durum wheat varieties. Notable differences were, however, observed in the α, β and γ components of gliadins as a polypeptide of 45kD was common to all the varieties except the poor quality Bijaga yellow and a 39kD polypeptide was observed only in Bijaga yellow. The low molecular weight polypeptides (12-15 kD) observed in the gliadins exhibited an almost similar mobility pattern as the major polypeptides of albumins and globulins. It was possible that these proteins may have been tightly bound to the gluten matrix during the gliadin extraction and thus appeared as major contaminants of gliadins. The occurrence of such low molecular weight proteins has been reported earlier by Beitz and Wall (1972). High molecular weight polypeptides of glutenins in the range of 98-110 kD were also observed in the SDS-polyacrylamide gel electrophoretogram of gliadins which indicate their co-separation in the native state during gliadin extraction. Such co-separation has been reported earlier by Shewry *et al.* (1983). Overall analysis of the polypetide profile revealed that most of the gliadin components were well within the molecular range of 30-44 kD which agrees with the reported values of 30-50 kD (Benerdin *et al.* 1967). Analysis of total storage protein electrophoretogram also showed the absence of the 45kD polypeptide in the poor quality varieties: Bijaga yellow and NIDW-15. SDS-PAGE profile of the glutenin polypeptides too indicated the difference in the same position. The presence of 45kD polypeptide in the glutenins extracted subsequent to gliadins indicated this polypeptide to be a low MW glutenin component, which could serve as a marker during selection of good quality durum varieties. However, more information is required to establish relationship between the different LMW glutenin sub-unit specific alleles and the technological qualities of the wheat flours. LMW glutenin polypeptides of durum wheat have been reported to be highly correlated with gluten strength (du Cross 1987). Allelic differences in LMW glutenin subunits are significantly related to flour quality in durum wheat (Autran and Feillet 1987). They actually determine the viscoelastic properties of durum wheat dough, whereas γ-gliadins are merely genetic markers (Pogna *et al.* 1990). Another important difference observed in these two poor quality varieties was the presence of a HMW glutenin subunits of 110 kD which was absent in other varieties. The poor resolution of the HMW glutenin polypeptides observed in the SDS-PAGE of both the total storage proteins as well as of the gliadins may be attributed to its lower amount (~11% of the total protein) and its disulphide linked polymeric structure as compared to the easliy soluble LMW subunits which account for ~27% of the total protein in durum wheat. Carrilo *et al.* (1990) reported a weak correlation of HMW-GS with the rheological and technological qualities of durum wheats. Pasta making quality has been associated with the presence of specific LMW-GS encoded by the Glu-B3 locus. Despite their influence on qualitative properties of durum wheat, only a limited number of LMW GS genes have been characterized so far. In the present investigation primers specific to the LMW glutenin gene sequences were custom synthesised on the basis of published sequences and were used for the PCR analysis of the good and poor durum varieties. The electrophoretic separation of the PCR products using genomic DNA of the selected varieties showed a single amplification product of about 1450 bps. This band length coincides with the reported gene length between the primers. No polymorphism was observed between the poor and good quality durum varieties. However, to identify the locus specificity of the amplification product a simulatneous PCR assay was performed on the genomic DNA of the *Triticum timopheevi* which served as a control. The absence of the amplification product in this species lacking the 'B' genome confirmed the locus specificity of this PCR product. Similar studies were performed by D'Ovidio *et al.* (1997) using LMW glutenin gene specific primers to further isolate and characterize the LMW-GS genes encoded at the *Glu-B3* locus.

The results of the present investigation thus showed some significant qualitative differences in the polypeptide (45 kD) pattern in the SDS-PAGE of glutenins of the selected poor and good quality durum varieties. The poor technological characteristics of the two poor quality varieties may be attributed to the absence of this polypeptide.

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