Indian J. Plant Physiol., Vol. 8, No. 2 (N.S.) pp. 120-124 (April-June, 2003)

BIOCHEMICAL CHARACTERIZATION OF HMW GLUTENINS IN WHEAT VARIETIES DIFFERING IN *CHAPATI* CHARACTERISTICS

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Received on 20 March, 2003, Revised on 10 July, 2003

SUMMARY

Five wheat (*Triticum aestivum*) varieties differing in their *chapati* characteristics (C-306, K 68, HD 2745 and HD 2735 – good, Sonalika – Poor) were selected for HMW glutenin characterization. SDS-PAGE of HMW glutenins revealed that polypeptides of MW's 112 kD and 83 kD corresponding to HMW subunits 5 and 10 may be responsible for good *chapati* characteristics. On reduction and alkylation with urea and acrylonitrile, HMW glutenins gave a very clear distinction between good and poor *chapati* quality wheat varieties. A 98 kD polypeptide was observed only in good wheat varieties. Peptide mapping of HMW glutenins was carried out using chymotrypsin, trypsin and pepsin enzymes. Chymotrypsin digest showed the presence of 68 and 22 kD polypeptide, trypsin digests also showed the presence of 22 kD and peptic digest showed presence of a 45 kD polypeptide in the good *chapati* quality wheat varieties.

***Key words*: *Chapati* characteristics, HMW glutenin, peptide mapping, subunits.**

INTRODUCTION

Storage proteins (gliadins and glutenins) of wheat play important role in determining the rheological properties of wheat (Shewry and Lazzeri 1997). Glutenins are considered to be the major determinants of bread quality of wheat and provide elasticity to the wheat dough. Allelic variations in the HMW glutenin subunits have been associated with variation in bread making property (Payne *et al.* 1981). The HMW subunits are coded by genes at three genetically unlinked loci, glu-A1, glu-B1, glu-D1, which are present on the long arm of chromosomes 1A, 1B and 1D, respectively (Payne *et al.* 1980). Each locus consists of 2 genes encoding x and y type subunits and exhibit extensive allelic variation. The subunit Dx5 and Dy 10 encoded by glu-D1 locus have been shown to be responsible for good bread making quality and these allelic glutenin subunits can be easily distinguished by SDS-PAGE (Payne and Lawrence 1983). Gupta *et al.* (1995) showed that deletion of HMW subunits resulted in great reduction in the amount of HMW polymers and gluten elasticity. HMW subunit variation in Portuguese wheat was studied in selected accessions and the subunits 2*, 13+16, 2+12 and null were found to be most frequently present (Nascimento *et al.* 1998).

Various genetic approaches have demonstrated that the differences in protein quality for bread making between wheat varieties arise mainly by different combinations of both HMW and LMW gluten subunits thus allowing the possibility for tailoring of wheat for good quality alleles (Payne 1987, Payne *et al.* 1987). In India, though a large part of wheat is consumed mainly in the form of *chapati*, practically very little information is available about the role of glutenins in imparting good chapati characteristics in wheat. The present investigation was therefore, undertaken to characterize HMW glutenins in wheat varieties differing in chapati characteristics.

MATERIALS AND METHODS

Seed materials of five wheat cultivars (C-306, K 68, HD 2745, HD 2735 and Sonalika) were used for the present investigation. Cv. C 306, K 68, HD 2745 and HD

2735 are good, whereas, Sonalika is poor in chapati characteristics.

Extraction of HMW glutenins:

Extraction was done with isopropanol at 60°C (Melas *et al.* 1994). Extraction solution 'A' consisted of 50 per cent (v/v) isopropanol and extraction solution 'B' consisted of 50 per cent (v/v) isopropanol containing 0.08 M Tris-HCl pH 8. Albumin, globulin and gliadin fractions were eliminated by suspending 300 mg flour in 15 ml of solution 'A'. Extraction was carried out for 30 minutes with intermittent vortexing and supernatant was discarded by centrifugation at 40,000xg. Residue was resuspended in 15 ml of solution 'A'. Extraction was repeated and the residue was finally washed with 7.5 ml sol 'A' and resuspended in 1.5 ml of solution 'B' containing 1 per cent (w/v) dithiothreitol. Incubation was done for 30 min at 60°C and centrifuged. To the supernatant, 1.5 ml of solution 'B' and 2 ml of acetone was added and incubated for 10 min and centrifuged. The residue obtained was mainly HMW glutenin subunits. Electrophoresis was done as described previously (Lammeli 1970). HMW glutenins were then reduced by adding 0.05 M Tris HCl, pH 7.5 containg 8M urea and 0.1 per cent dithiothreitol and incubated for 2 hrs. at room temperature with intermittent shaking. Alkylation was done by adding acrylonitrile to a final concentration of 0.5 M and incubated for 2 hrs. at room temperature with intermittent vortexing. Alkylation was terminated by adding 500 µl of glacial acetic acid, centrifuged at 35.000 xg for 15 min and the supernatant was electrophoresed on 10 per cent SDS polyacrylamide gel (Laemmli 1970).

Peptide mapping of HMW glutenins:

Glutenin was digested with chymotrypsin, trypsin and pepsin (Ewarts 1966). For chymotrypsin and trypsin hydrolysis, 20 mg of glutenin extract and 0.4 mg of each enzyme in 0.1 M ammonium bicarbonate (pH 7.9) was incubated for 24 hrs at 37°C. For pepsin hydrolysis, 20 mg of protein in 5 ml of 0.05 M HCl (pH 1.8) was incubated with 1 mg of enzyme for 24 hrs at 37°C. The digestion was stopped by boiling in a water bath and the digested samples were then electrophoresed on 17.3 per cent SDS polyacrylamide gels. After electrophoresis was complete the gel was stained in staining solution for 30 min to prevent further acid hydrolysis and destained for 1 hr.

RESULTS AND DISCUSSION

HMW glutenins isolated from five wheat varieties were analysed on 10 per cent SDS polyacrylamide gel. The molecular weights of the polypeptides were calculated by comparing them with the subunit molecular weight as described previously (Ng and Bushuk 1989). On comparing the banding pattern (Fig. 1) it was observed that the polypeptide bands with MW's 112 and 83 kD were present in all the good varieties. The molecular weight of these polypeptides corresponded to Dx5 and Dy 10 HMW glutenin subunits. Two polypeptides of MW's 116 kD and 82 kD were present only in Sonalika and these corresponded to Dx2 and Dy 12 HMW glutenin subunit. A 98 kD (subunit

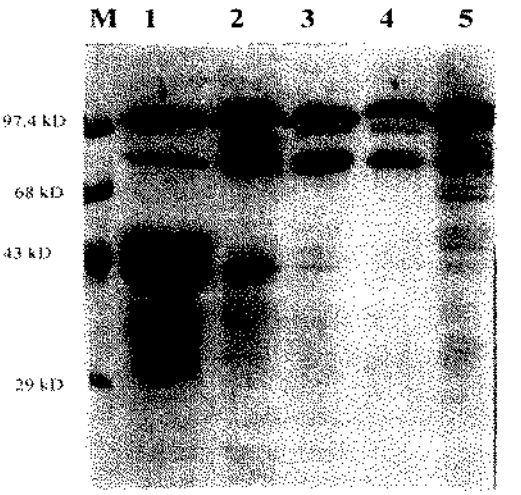

Fig.1. SDS-PAGE of HMW glutenins. (1-C306, 2-K68, 3-HD2748, 4-Sonalika, 5-HD2735)

7) polypeptide was present in all varieties except K 68. All 81 kD polypeptide (subunit 9) was observed in K 68, HD 2745 and HD 2735 while a 95 kD polypeptide (subunit 14) was present in K 68 and HD 2735. Allelic variations in the HMW glutenin subunits have been associated with the variation in bread making quality (Payne *et al.* 1981). The present investigation was done to study the allelic variation of HMW glutenin in relation to variation in *chapati* characteristics in wheat varieties. The SDS-PAGE of HMW glutenins in the present study revealed that polypeptides of MW's 112 and 83 KD may be responsible for the good *chapati* characteristics. These polypeptides correspond to Dx5 and Dy 10 HMW subunits (Payne and Lawrence 1983), which have been reported to be responsible for good bread making quality. Sonalika being poor in

chapati characteristics showed the presence of 116 and 82 kD polypeptides which correspond to Dx2 and Dy12 HMW glutenin subunits. Thus, the presence of subunit 2 and 12 in Sonalika may be responsible for its poor quality. Presence of Dx5 HMW glutenin subunit in the good wheat varieties has also been proven by PCR amplification using gene specific primer (Bhatnagar *et al.* 2002), thereby, suggesting that good bread making and *chapati* making characteristics could be as a result of similar causal factors.

Glutenins consists entirely of disulphide stabilized polymers (Shewry and Tathan 1990). On treatment with SDS, HMW glutenins are partially reduced *i.e.* they are to some extent, still involved in intra and inter chain bonding. Thus total reduction and alkylation would lead to a clearer picture of the HMW glutenins subunits. HMW glutenins on reduction and alkylation showed more number of polypeptides, as compared to the unreduced and unalkylated glutenins. The banding pattern of reduced and alkylated HMW glutenins (Fig. 2) showed that a 98 kD polypeptide was present in all the good quality wheat varieties while

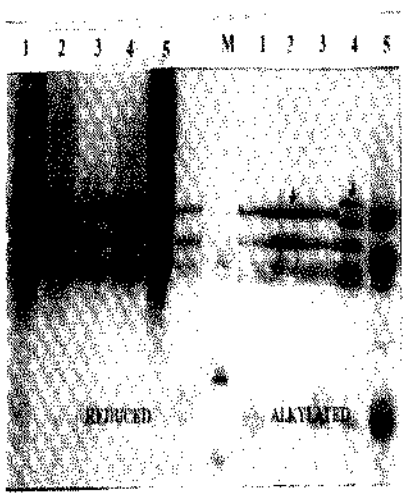

Fig.2. SDS-PAGE of alkylated and reduced HMW glutenins. (1-C306, 2-K68, 3-HD2748, 4-Sonalika, 5-HD2735)

Sonalika showed the presence of two polypeptides with MW's of 125 and 95 kD. In HD 2735, 98 kD as well as 95 kD polypeptide were present. Other minor differences within the varieties were also observed in both the reduced and alkylated HMW glutenins. Thus the reduced and alkylated glutenins pattern page a very clear difference between the good and poor quality wheat varieties showing that 98 kD polypeptides may be responsible for good *chapati* characteristics and the absence of this polypeptide results in poor *chapati* characteristics. Alternatively, the presence of 125 and 95 kD polypeptides may be responsible for the poor *chapati* characteristics in Sonalika.

Peptide mapping study involving enzymatic digestion of glutenins have been directed towards elucidating reasons for the unique physical properties of wheat flour proteins (Biserte and Han 1965). Peptide mapping of hordeins of barley showed a primary structural homology between different polypeptides (Holder and Ingverson 1978). While comparative study of fingerprints of pepsin digests of gliadins and glutenins have suggested that they are not likely to have common peptides (Ram and Nigam 1986). In the present investigation, peptide mapping of HMW glutenins provided a good distinction between good and poor wheat varieties.

Chymotryptic digestion of HMW glutenins (Fig. 3) revealed that polypeptides with MW's 68 and 22 kD were present in all the good wheat varieties but absent in Sonalika (poor quality wheat). Sonalika showed the presence of a 23 kD polypeptide, which was not present in the good wheat varieties. The undigested glutenin pattern showed 4-5 polypeptides in HMW glutenin range *i.e.* between 81-124 kD. Tryptic digestion (Fig. 4) revealed 5-6 polypeptides in good quality wheat varieties whereas 4 polypeptides were present in poor quality wheat. The good quality wheat varieties showed the presence of a distinct 22 kD polypeptides which was absent in Sonalika

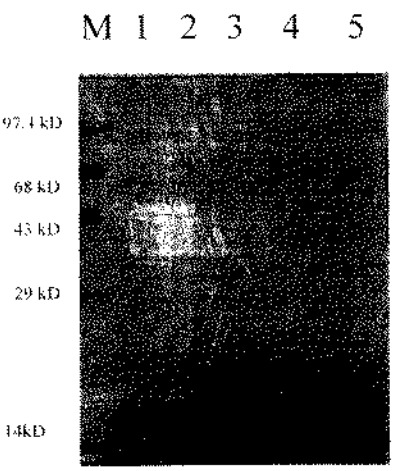

Fig. 3. SDS-PAGE of chymotrypsin digested HMW glutenins. (1-C306, 2-K68, 3-HD2748, 4-Sonalika, 5-HD2735)

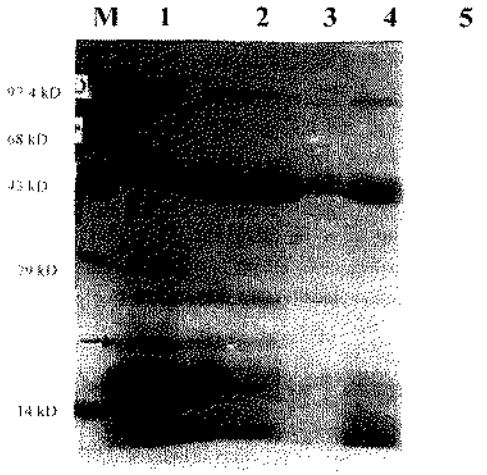

Fig. 4. SDS-PAGE of trypsin digested HMW glutenins. (1-C306, 2-K68, 3-HD2748, 4-Sonalika, 5-HD2735)

and also in HD 2735. Other differences in the polypeptide pattern were present within the good varieties and were thus not taken into account for the present study. Peptic digestion of HMW glutenins (Fig. 5) showed that the polypeptides of MW 40, 35 and 30 kD were present in all varieties. A 45 kD polypeptide was present in only the good wheat varieties. Thus in the chymotrypsin digests polypeptides of MW's 68 and 22 kD could be used to distinguish good wheat varieties. Alternatively, 73 and 23 kD polypeptides may be used for distinction of poor quality wheat varieties. Trypsin digestion gave a 22 kD

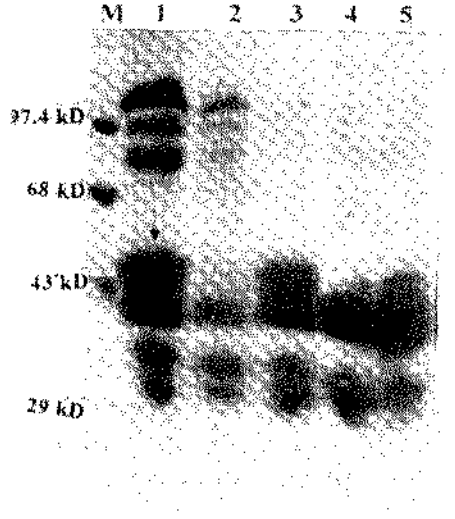

Fig. 5. SDS-PAGE of pepsin digested HMW glutenins. (1-C306, 2-K68, 3-HD2748, 4-Sonalika, 5-HD2735)

marker for good wheat varieties while peptic digestion revealed a 45 kD polypeptide which was present in the good wheat varieties.

The results of the present investigation thus showed some significant qualitative differences in the polypeptide pattern of HMW glutenins. The good *chapati* characteristics of wheat varieties may be attributed to the presence of polypeptides with MW's 112 and 83 kD which correspond to subunit Dx5 and Dy10 of HMW glutenins. Alternatively, it is possible that the absence of these polypeptides may be responsible for poor *chapati* characteristics in Sonalika. Besides this, the polypeptide pattern of Sonalika indicated that the polypeptides with MW's 116 and 82 kD corresponding to Dx2 and Dy12 HMW glutenin subunits may also be responsible for its poor *chapati* characteristics. Peptide mapping of HMW glutenins pinpoint certain biochemical markers that could help in differentiating good and poor *chapati* characteristics of wheat varieties. However, further confirmation of exact differences between good and poor *chapati* characteristics can be made known after screening of larger and more varied wheat varieties.

ACKNOWLEDGEMENT

The senior author is thankful to the Director, Indian Agricultural Research Institute, New Delhi for the award of Senior Research Fellowship during the course of this study.

REFERENCES

Bhatnagar, T., Sachdeva, A., Johari, R.P. (2002). Molecular characterization of HMW glutenins in wheat varieties differing in *chapati* characteristics. *J. Plant Biochem. & Biotech.* **11:** 33-36.

Biserte, G. and Han, K. (1965). Residual peptides in the enzymic hydrolysis of wheat gluten. *Bull Soc. Chem. Biol.* **47:** 597-600.

Ewarts, J.A.D. (1966). Fingerprinting of glutenin and gliadins. *J. Sci. Food Agric.* **17:** 30-33.

Gupta, R.B., Popineau, Y., Lefeburet, J., Cornec, M., Lawrence, G.J. and MacRitchie, N. (1995). Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low and high MW glutenin subunits. *J. Cereal Sci.* **21:** 103-116.

Holder, A.A. and Ingverson, J. (1978). Peptide mapping of the major components of the *in vitro* synthesized barley hordien: Evidence of structural homology. *J. Carlsberg Res. Comm.* **43:** 177-184.

Laemmli, V.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T_4. *Nature*, **227:** 681-685.

Melas, V., Morel, M.H., Autran, J.C. and Feillet, P. (1994). Simple and rapid method for purifying low molecular weight subunits of glutenin from wheat. *Cereal Chem.* **71-3:** 234-237.

Nascimento, M.P., Carnide, V.P., Igregas, G., Gwdes-Pinto, H. (1998) High molecular weight glutenin subunit variation in the Portugese wheat Land race Barbela. *Cereal Res. Comm.* **26-2:** 21-216.

Ng, P.K.W. and Bushuk, W. (1989). Concerning the nomenclature of HMW glutenin subunits. *J. Cereal Sci.* **9:** 53-60.

Payne, P.I. (1987). The genetical basis of bread making quality. *Aspects Appl. Biol.* **15:** 79-90.

Payne, P.I., Holt, L.M. and Law, C.N. (1981). Structural and genetical studies on the high molecular weight subunits of wheat glutenin. *Theor. Appl. Genet.* **60:** 229-236.

Payne, P.I., Law, C.N. and Mudd, E.E. (1980). Control by homologus group 1 chromosomes of the HMW subunits of glutenin, a major protein of wheat endosperm. *Theor. Appl. Genet.* **58:** 113-120.

Payne, P.I. and Lawrence, G.J. (1983). Catalogue of alleles for the complex gene loci, gln-A1, gln-B1, gln-D1 which code for high molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Comm.* **11:** 29-33.

Payne, P.I., Nightingale, M.A., Krattiger, A.F. and Holt, L.M. (1987). The relationship between HMW glutenin subunit composition and the breadmaking quality of British grown wheat varieties. *J. Sci. Food Agric.* **40:** 51-65.

Ram, B.P. and Nigam, S.N. (1986). A comparative study of the fingerprints of pepsin digests of gliadins and glutenins non identidy of comparable ninhydrin positive spots. *J. Food Sci. Technol.* **23:** 117-184.

Shewry, P.R. and Lazzeri, P. (1997). Molecular approaches to wheat quality improvement. *Chem. Indust.* **21:** 559-562.

Shewry, P.R. and Tatham, A.S. (1990). The prolamin storage proteins of cereals seeds: Structure and evolution. *Biochem. J.* **267:** 1-12.