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Variation at *Glu-1, Glu-3* and *Gli-B1* alleles and classification of landraces, old varieties and rust resistant sources in durum wheat

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Abstract

In durum wheat genotypes, analysis of seed storage protein profiles of 30 genetic stocks was done using Acid and SDS PAGE electrophoresis to find out the existing genetic diversity, and to assess its utility for improvement in grain yield along with quality traits. More alleles were observed at Glu-1 loci i.e., Glu A1 (2*) and Glu B1 (14+15, 7*+8 and new type allele) and band 20 is common in genetic stocks. Genetic stocks contain 12 different pattens at Glu-3 loci, indicating more diversity for this class of proteins. The rust resistance sources do not possess γ-45/ *Gli-1* alleles, so these lines can be used as donors to introduce disease resistance in the good quality recently released varieties, which are containing γ-45/*Gli-1* alleles. Six different *Gli-B*$_1$ alleles were found in land races, rust resistance sources and old released varieties. From hierarchical analysis, it was found that landraces, old released varieties and rust resistance sources are genetically distinct. Significant differences were observed for protein content and sedimentation value between the land races, old released varieties and rust resistant sources. The presence of new LMW –B glutenin and γ-gliadin patterns is interesting in rust resistance sources and need to be investigated for their role in pasta making as well as overall technological quality of durum wheat.

Key words : Durum wheat, hierarchical analysis, allelic variation, gliadins

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Introduction

Wheat is one of the world's most important food crops, which provides over 20 per cent of the calories and protein in human nutrition. In India, about five per cent of the total wheat area is under durum wheat (*Triticum turgidum* var. *durum*) cultivation and approximately 2.5 million tons of durum wheat is produced annually. Durum wheat is unique in that it is generally considered the hardest of all wheats and has high protein content and especially suited for production of pasta products with high yellow pigment content required for attractive appearance of pasta in dry and cooked form. Till recently, wheat breeding efforts in India were mainly directed towards the improvement and stabilization of production and productivity. With changing consumer demands, improvement in processing quality of durum wheat, (mainly gliadin and gluten profiles) is receiving attention. The gluten complex is composed of two main groups of proteins, gliadins and glutenins. The gliadins are responsible for the dough's cohesiveness, whereas glutenins for the property of resistance to extension to give good pasta products. Variation in gluten strength and elasticity depends on the quality and quantity of gluten proteins i.e., glutenins and gliadins (Pogna *et al*., 1990).

The usefulness of gliadin γ-45 and γ-42 encoded at *Gli-B1* locus (Joppa *et al*., 1983) is known as protein markers of good and poor pasta quality, respectively. The genes coding for most γ and ω gliadins have been mapped on short arms of chromosomes *1A, 1B* & *1D* at the *Gli-A1, Gli-B1* and *Gli-D1* loci respectively, whereas the genes coding for most α and β gliadins occur in short arms of group 6 chromosomes at *Gli A-2, Gli B-2* and *Gli D-2* loci. (Payne, 1984).

In the present study, analysis of seed storage protein profiles was done using A-PAGE using Indian durum wheat genetic stocks, containing land races, old released varieties and rust resistant sources, to generate information on the direction in crossing as well as in selection programmes for improvement of durum wheat varieties.

Materials and methods

Thirty durum wheat genotypes from the collections at Indian Agricultural Research Institute-Regional Station, Indore including land races, old varieties and rust resistance sources were analysed with three replications in the present study. The lines viz., 'Edmore', 'Langdon', 'Kalyansona', 'MACS 2496' and 'Marquis' were used as standards for glutenin and gliadin subunits.

Gliadin analysis: Single seed or half seed was crushed into fine powder using metal crusher or 20 mg flour was used for extraction. 100 µl of 70 per cent ethanol was added to the powder and incubated at 37°C for 30 minutes with brief vortexing at the time interval of 10 minutes. The tubes were centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. The residue left was used for glutenin extraction. 62.5 µl of the dilution buffer was added to 50 µl of the supernatant obtained. 20 µl of the sample was loaded into the well of the gel for A-PAGE separation of gliadins.

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The electrophorograms of the gliadin proteins are conventionally evaluated according to Bushuk and Zillman (1978) based on relative mobility. The relative mobility (Rm) values are calculated with reference to a specific band, designated 50, of the variety 'Marquis' (a Canadian hard red spring wheat variety). The Rm value of a given band is obtained by dividing the distance the band migrated from the origin by the distance band 50 migrated from the origin and then multiplying this value by 50. The value obtained is the Rm for that band.

Glutenin analysis : 500 µl of 50 per cent (v/v) Propon-1-ol was added to the residue left after the extraction of gliadin and incubated for 30 minutes at 65 °C with brief vortexing. The tubes were centrifuged at 600 rpm for 2 min and supernatant was discarded. The same process was repeated for 3 times. 100 µl of glutenin extraction buffer (pH = 8.0) containing 1.4 per cent of freshly mixed 4-Vinyl pyridine was added and incubated for 15 minutes for protein alkylation. The sample was then centrifuged at 10,000 rpm for 2 minutes. An aliquot of 100 µl of the supernatant was transferred to a new tube containing 100 µl of dilution buffer, vortexed briefly and incubated at 65 °C for 15 minutes before loading 10 – 20 µl of extract in the individual slots of the SDS – PAGE gel for glutenin fractionation.

Genetic distances : were calculated based on presence or absence of bands taking band 50 as reference, using Hierarchical cluster analysis of SPSS programme and dendrograms were drawn using average linkage between groups and Euclidean distance method.

Results and discussion

Allelic variation at Glu-1 loci: Eight different HMW banding patterns were observed (Fig. 1a) in the genetic stocks. At Glu-A1, two alleles were observed i.e., Glu-A1c (null) and Glu A1b (2*), whereas, at Glu-B1 loci, seven different alleles were observed viz., 6+8 (G), 7+8 (H), 20 (C), 13+16 (F), 14+15 (D), 7*+8 (I) and a new type (two bands (E) were observed below 14+15). Band 20 is common in the genetics stocks and 2* at *Glu-A1* and 14+15, 7*+8 and a new type of allele (E) at *Glu B1* (Line 1172) were present, indicating good extent of diversity.

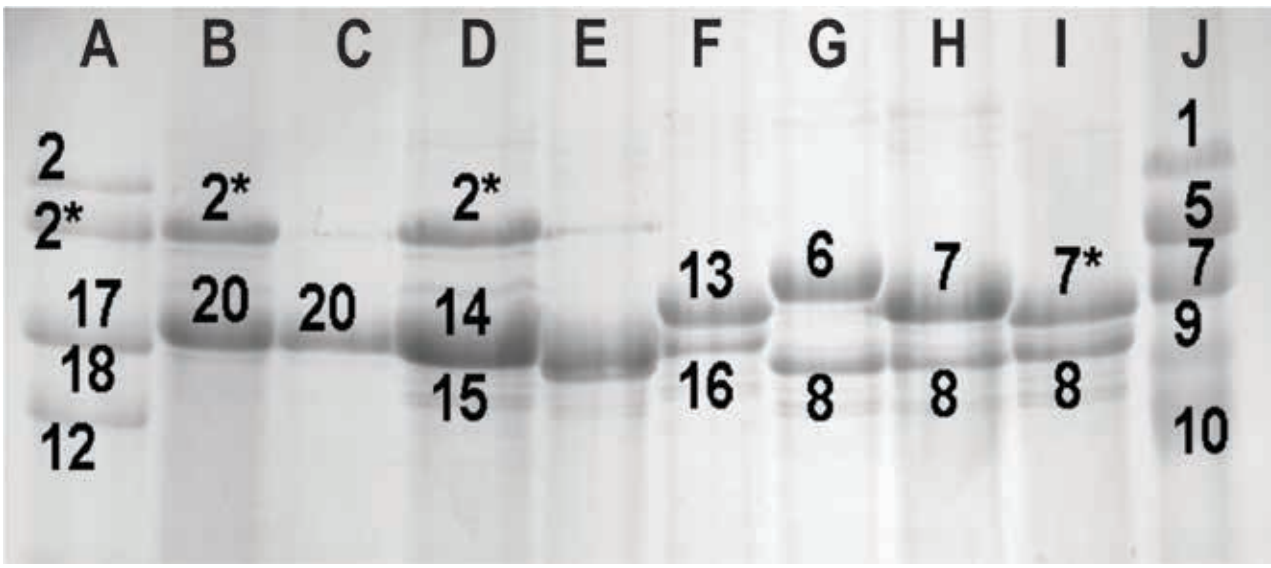

Fig 1a. Variation at *Glu 1* loci in genetic stocks

A. Kalyana Sona, B. Mandsaur Local, C. Malvi Local, D. Sarangpur Local, E. Line 1172, F. Kathia 25, G. NIDW 15, H. IWP 5019, I. ED 2398-A, J. MACS 2496.

Note: HMW glutenin subunits are numbered according to Payne and Lawrence (1983) and Branlard *et al.*(1989).

Allelic variation at Glu-3 loci: In genetic stocks, SDS-PAGE profiles of LMW glutenins showed 11 different banding patterns considering only B sub units (Fig 1b). Each genotype had generally 3 to 6 LMW glutenin bands except B 662 (Fig 1b, lane H arrow), which showed only one LMW glutenin band. LMW –1 and -2 were named as reported by Pogna *et al.*, (1990) and the other eight variants of LMW B glutenin patterns (only showed in Fig. 1b) were arbitrarily named as LMW-3 to 10. Genotypes showing different LMW B glutenin patterns other than LMW-1 and –2 are to studied for their effects on pasta making and overall quality. The presence of different patterns of LMW bands in genetic stocks indicate more diversity for this class of proteins. It had been proved that LMW-2 / γ-45 is good for pasta making, while LMW-1 / γ-42 is poor (Anindya Roy *et al.*, 2002, Tanaka *et al.*, 2005, Izadi-Darbandi *et al.*, 2010). Hence, care should be taken to select for the presence of LMW-2 / γ-45 to ensure better quality durum wheat.

Allelic variation at Gli-B1 loci: A total of six different *Gli-B1* alleles i.e., γ-45 (50%), γ-44 (23.3 %), γ-43.5 (10 %), γ-42 (6.7 %), γ-47 (6.7 %) and null (3.3 %) were found (Fig. 1c) among the land races, old released varieties and rust resistance sources. Most of the land races showed γ-45, γ-43.5 and γ-44, and only two genotypes 'Yuk' and 'NIDW 15' showed γ-42 i.e., where 'Yuk' is exotic and 'NIDW 15' is a developed variety. None of the land races showed presence of γ-42 gliadin band. Two genotypes 'ED 2398-

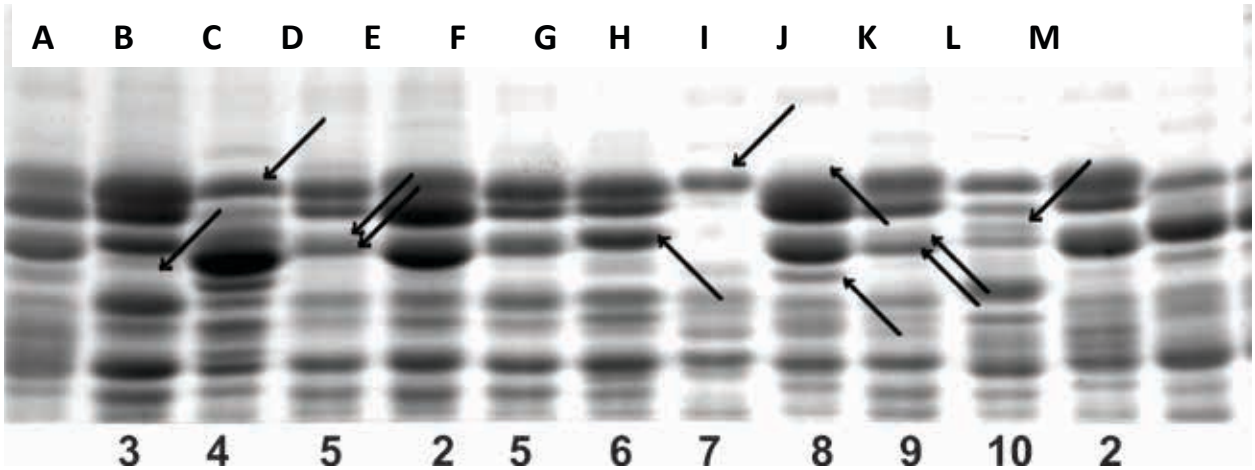

Fig 1b. Variation at ***Glu-3*** loci in genetic stocks

A. Edmore, **B**. Sarangpur Local, **C**. Karnataka Local, **D**. Malvi Local, **E**. Trinakaria, **F**. Kathia Red, **G**. NP 404, **H**. B 662, **I**. IWP 5019, **J**. ED 2398-A, **K**. Line 1172, **L**. IWP 5004-1, **M**. Langdon.

Note: The numbers below the lane indicate different LMW B glutenin patterns.

A' and 'Line 1172' showed γ-47 band and one genotype 'B 662' did not show any γ gliadin band (Null). 'MACS 1967', 'N 59', 'NP 404', 'JU 12', 'Meghdoot', 'Bijaga Red' and 'Kathia 25' showed γ-44 gliadin band, which are all tall and grown in rainfed conditions. In this group, only 'Kathia 25' is land race type and rest of them are old released varieties, which are having Gaza as one of the parents in their pedigree (or parents derived from Gaza x local cross). 'Trinakaria', the known donor for quality is also having γ-45 band (Flaete and Uhlen., 2003).

Genotypes showing γ-47 and null type are having high disease resistance potential, which are derived from inter-specific crosses. All these disease resistant genotypes did not show γ-gliadin 45, which is good for pasta making, and showed γ-47 and null loci instead. So it is necessary thereby to study γ-47 and null linked LMW B glutenins and their pasta making potential, or transfer of disease resistance to γ-45 containing genotypes. Three genotypes 'A 206', 'Karnataka local' and 'A 9-30-1' showed γ-43.5. Of these, 'A 206' and 'Karnataka local' are local types and 'A 9-30-1' is an old released variety, derived from a cross in which A 206 is one of the parent.

Genetic distances : Hierarchical classification was done based on the presence or absence of the bands of gliadin upto γ-50. Three distinct groups were observed among the land races, old released varieties and rust resistance sources, of which the land races formed one group, old released varieties ie., 'NP 404', 'A 9-30-1', 'MACS 1967', 'IWP 5004-1', 'Bijaga Yellow' and 'Meghdoot' in the second group, and rust resistance sources like 'B 662', 'ED 2398A' 'IWP 5019' and 'Line 1172' formed another group along with some old released varieties. 'Marquis' formed an entirely separate group from the tetraploid lines (Fig 1d).

The rust resistance sources do not possess γ-45/*Gli-1* alleles, so these lines can be used as donors to introduce disease resistance in the good quality recently released varieties, which are possessing γ-45/*Gli-1* alleles. By observing the allelic frequencies of Glu-1, Gli-1 and Glu-3, genetic stocks were found to have more diverse alleles at all three loci. From hierarchical analysis, it was found that land races, released varieties and rust resistance sources are genetically distinct. The presence of new γ-gliadin patterns are interesting in rust resistance sources and need to be investigated for their role in pasta making as well as overall technological quality of durum wheat. It is advisable to select high yielding agronomically superior genotypes for improvement of its rust resistance involving the resistant sources as well as old land races (LMW-2 / γ-45) based on their diversity to improve their rust resistance and quality traits, respectively. Based on above observations, a sound breeding strategy can be designed to exploit the existing variation in durum wheat to develop high yielding, rust resistant genotypes with good quality traits.

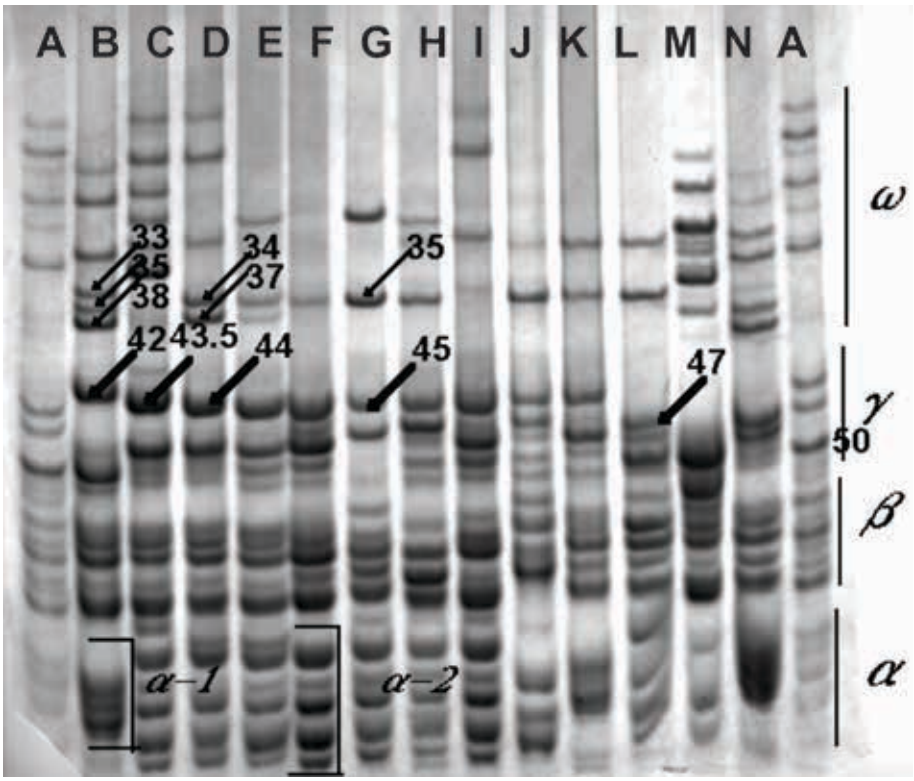

Fig 1c: Variation at *Gli B1* loci in genetic stocks

A. Marquis, B. Yuk, C. Karnataka Local, D. NP 404, E. MACS 1967, F. Sarangpur Local, G. Sawer Local, H. Dahod Local, I. Kathia Red, J. IWP 5019, K. B 146, L. ED 2398-A, M. B 662, N. Line 1172.

Note: Gliadin bands are numbered according to Bushuk and Zillman (1978)

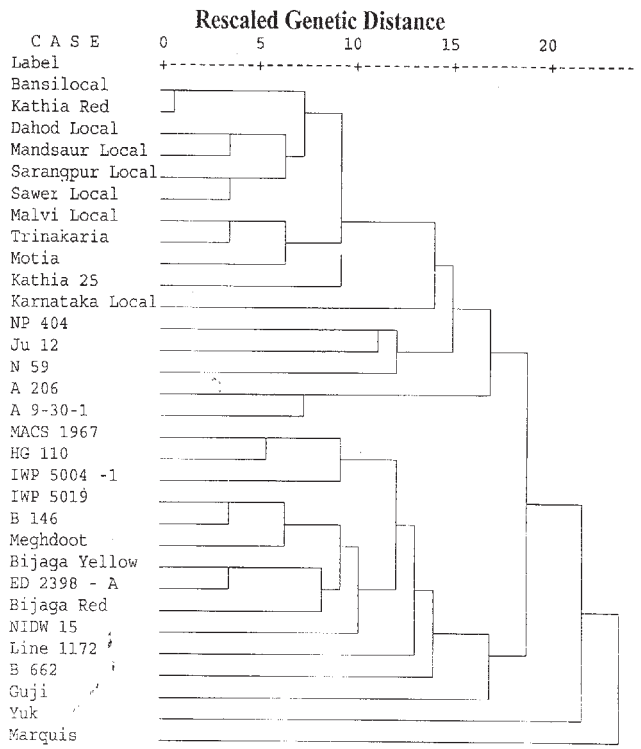

Fig 1d. Hierarchical classification dendogram of durum genetic stocks

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