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ORIGINAL ARTICLE

Rheological evaluations and molecular marker analysis of cultivated bread wheat varieties of India

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Abstract The aim of this study was to screen Indian cultivated wheat varieties and list out the parameters/genes required to be improved for an end-product. Therefore, 30 Indian wheat varieties under cultivation by farmers were screened for 14 physico-chemical and rheological parameters, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for high molecular weight glutenin subunits (HMW-GS), DNA based molecular markers for low molecular weight glutenin subunits (LMW-GS) and puroindolines (*Pin*) genes. Based on grain texture, sedimentation value, farinographic, alveographic, HMW-GS and LMW-GS and biscuit making parameters, HS490 was found to be a highly suited for biscuit and soft wheat products. HI1563 and DBW14 were also found to possess characteristics such as low protein, low to medium SDS-sedimentation value and combination of 2*, 7+8 and 2+12 (HMW-GS). DBW14 also had LMW alleles desirable for biscuit quality. DBW14 needs to be improved for grain softness to make it suitable for biscuit quality while both grain softness and LMW alleles need to be improved for HI1563 to improve its biscuit spread factor and alveographic indices for extensible gluten. Rest varieties showed moderate to very strong gluten but the gluten lacked extensibility. Only four varieties K307, DBW39, NI5439 and DBW17 possessed high flour protein and moderately strong gluten. They had more balanced deformation energy (W) and configuration ratio (P/L) combination suggestive of strong and extensible gluten needed for raised bread making. Marker assisted backcross breeding is suggested as solution to produce end-use specific varieties where appropriate alleles at only a few loci need to be incorporated.

Keywords Wheat grain quality · Gluten · Grain texture · High molecular weight (HMW) · Low molecular weight (LMW) glutenins

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Introduction

The wheat grain is milled before processing into edible products. Common or bread wheat is utilized for the production of several baked products such as breads of various types, biscuits, cakes, cookies, noodles etc. Durum wheat is mainly used for making extruded products like pasta and macaroni. In India flat breads, leavened breads, noodles and pasta made from both aestivum and durum wheat are consumed. Dicoccum (khapli) wheat semolina and flour is used for local preparations in niche areas of the country.

Grain texture, endosperm storage proteins and carbohydrates are the primary determinants of the quality of wheat. The grain of a genotype can be hard, medium hard or soft. Greater force is required to mill a hard and medium hard grain than the soft grain. Consequently, hard wheat suffers greater starch damage and hence absorbs more water than soft wheat (Greer and Stewart 1959). Therefore hard wheat is more suitable for bread and other leavened product while soft wheats are preferred for unleavened, low water absorbing products such as biscuits, cakes, cookies, pretzels etc. The Grain hardness is controlled by two extremely closely linked genes (*Pina-D1* and *Pinb-D1*) located on chromosome 5DS. The wild-type allele of both the genes together produces soft genotype (*Pina-D1aPinb-D1a*) while mutation in any one of the genes produces hard wheat (Giroux and Morris 1997). Molecular markers for alleles of the genes have been produced and are used in breeding programmes.

Another important biomolecule in wheat grain is the endosperm storage protein that was termed gluten by Beccari (1745) due to its glue-like property. Gluten is a major and complex protein of wheat grain. When flour is kneaded with water, a viscoelastic network of polypeptides is formed. These polypeptides are the constituents of the gluten. The amount of gluten is known to influence the quality of the end product. As a thumb rule, high ($\geq$ 11%) protein content for bread making and low ($\leq$ 11%) protein content for cakes and cookies is preferred (Payne 1987). During the 80's, several studies showed that in addition to gluten content, it's quality or type also played a key role in determining the end-use quality of wheat. Rheological studies have established that strong and extensible flour dough is desirable for pizza, bread and other leavened products whereas weak and extensible dough is suitable for cake, cookies and other short textured products. Medium strong and extensible gluten is indicated for good chapatti making quantity. Several tests that measure the quantity and quality of gluten have been developed. Total protein and wet and dry gluten can be measured to know the quantity of gluten in the grain. Pelshenke and sodium dodecyl sulfate (SDS) sedimentation test provide a measure of the gluten content and strength. A high positive correlation between the SDS-sedimentation value and gluten strength has been demonstrated (Katyal et al. 2016). To determine the strength and extensibility, more elaborate tests such as the faringograph, the extensograph and alveograph have been developed that can help to predict the end useability of flour without the need of an actual baking test (AbuHammad et al. 2012; Singh et al. 2018). Gluten consists of polymeric high molecular weight proteins (80–130 kDa) called the high molecular weight glutenin subunits (HMW-GS), the predominantly monomeric, low molecular weight (10–70 kDa) proteins called the low molecular weight glutenin subunits (LMW-GS) and the monomeric fraction called the gliadins (kDa) (Shewry 2009). The genes coding for HMW-GS, LMW-GS and gliadins have been locatedon six chromosomes (Payne 1987). The polypeptides of each fraction show variations in their structure which arise due to changes in the corresponding genes giving rising to different forms or alleles. Molecular markers for discerning the presence of these alleles and their corresponding polypeptides have been developed (Zhang et al. 2004; Wang et al. 2009, 2010; Feng et al. 2013). The allelic differences for HMW-GS and LMW-GS have been correlated with rheological parameters (Appelbee 2007). Databases on HMW and LMW-GS genes are now available for more than 8000 wheat varieties from different parts of the world (Békés and Wrigley 2013) from which the end-use potential of the genotype can be predicted.

In India, 75–80% of wheat produced is consumed as chapatti and its related culinary forms. However, in recent years, the market for ready to eat products has expanded phenomenally. Wheat based baking industry is expanding and is growing at 14% per annum. The bakers need product specific flours but there is scanty information on the end-use potential of wheat varieties currently cultivated in India. In the recent years, protein profiling, rheological, pasting and baking properties of some hard, medium hard and soft bread wheat (Katyal et al. 2017, 2018a) and noodle making potential of durum wheats have been studied in greater detail (Kaur et al. 2016; Katyal et al. 2018b). Therefore, in the present study, a set of Indian wheat varieties under cultivation by farmers, as judged by the breeder seed indent of various state agriculture departments, were selected and subjected to grain, flour and dough quality evaluations and molecular marker analysis to classify them according to their overall quality profiles.

Materials and methods

Plant materials

Thirty five bread wheat varieties were grown in randomized complete block design during the crop season 2014–2015. Of these, thirty varieties have been released during last decades for different agroclimatic zones in India and five wheat varieties were released in Australia for different end-uses. The varieties Barham, Longreach Orion, EGA 2248 and Yenda fall in Australian soft wheat class and possess weak and extensible gluten (Cornish et al. 2007; Matthews and McCaffery 2011) while Yitpi is classified as Australian hard wheat possessing very strong and extensible gluten (Cornish et al. 2007). These were imported in 2010 in collaborative Indo-Australian Programme on Marker Assisted Wheat Breeding and were used as reference varieties for *Pin*, *Glu-1* and *Glu-3* genes and for their end-use quality traits. The parentage of the varieties is given in Online Resource 4.

Quality traits

The varieties were evaluated for 14 traits viz. grain hardness index (GHI), thousand kernel weight (TKW), test weight (TW), flour protein percent (P%), wet gluten (WG), dry gluten (DG), gluten index (GI), SDS-sedimentation value of flour (SDS-SV), water absorption (WA), dough development time (DDT), degree of softening (DOS), farinograph quality number (FQN), deformation energy (W), configuration ratio (P/L). All the quality traits were analyzed using internationally accepted AACC (2000) methods. Random samples from grain of each of the field replicates were drawn and analyzed in replicate and averaged. The mean values of two averaged replicates are given in results.

Grain characteristics

The parameters GHI and TKW (g) were analyzed with Single Kernel characterization system (SKCS) 4100 (Perten Instruments, Australia) using the procedure described in AACC (2000) method 55-31. TW (kg/hl) was measured using hectoliter weight machine provided by IIWBR, Karnal, India.

Protein content

Total flour protein (%) was measured using Diode Array NIR Analysis Systems 7200 (Perten Instruments).

Gluten content

WG (%), DG (%) and GI was analyzed using Glutomatic 2200 (Perten Instruments, Australia) according to AACC (2000) method 38-12A.

SDS-sedimentation value test

The SDS-SV (ml) of wheat flours is based on the principle that the strands of glutenin absorb water and swell on treatment with lactic acid. SDS-SV was analyzed as per Axford et al. (1979).

Farinograph indices

The dough mixing properties were analyzed on a 10 g (14% moisture basis) Farinograph (Barbender OHG, Germany) using the AACC method 54-21 (2000). Farinograph parameters: WA (%), DDT (min), DOS and FQN were obtained by the software of the farinograph manufacturer.

Alveograph

The Alveograph parameters were determined on Chopin alveoconsistograph using the AACC (2000) method 54-30. The following parameters were obtained: P/L determines the dough strength and extensibility; W, determines the deformation energy or baking strength, P/L, a combination of dough strength (P value) and extensibility (L value) expressed in terms of joules.

Biscuit baking

Biscuit baking was performed using the AACC (2000) method 10-50D. Biscuit diameter (cm), thickness (mm), spread factor, hardness (N), fracturability and texture analysis were analyzed with texture analyzer, stable micro systems and evaluated statistically.

Bread baking

The bread loaves were prepared from 100 g flour using the AACC (2000) method 10-11. Loaf volume was measured using rapseed displacement method.

SDS-PAGE analysis

Protein from five seeds of each variety was extracted and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was carried out as per protocol described by Sarkar et al. (2015). Reference cultivars with previously known and published subunits of HMW-GS were used as controls and are given in Table 1.

DNA extraction and PCR parameters

Ten seeds of each of the genotypes were germinated in the dark in a medium containing sterilized peat and 12–15 day old seedlings were used for the genomic DNA extraction by cetyltrimethyl ammonium bromide procedure (Doyle and Doyle 1987). Molecular typing of LMW-GS genes at *Glu 3*, grain texture genes at *Pina-D1* and *Pinb-D1* loci was carried out using allele specific PCR (AS-PCR) primers (Online Resource 5). PCR was performed using 0.3 µl Taq DNA polymerase in 15 µl reaction volumes containing approximately 50 ng of genomic DNA, 1 × PCR buffer with 1.5 $MgCl_2$, 10 pmol of each PCR primer and 100 µM of each of dNTPs. PCR cycling conditions were 94 °C for 5 min following 35 cycles of 94 °C for 35 s, 57-60 °C for 35 s, 72 °C for 90 s, and a final extension at 72 °C for 8 min. The PCR products were separated by electrophoresis in 1.5% agarose and visualized by ethidium bromide staining.

Table 1 Molecular genotyping of Indian genotypes for alleles of the *Glu-1*, *Glu-3*, puroindoline genes

Variety	Alleles of the genes for HMW-GS			Glu-1 quality score	Alleles of the genes for LMW-GS		Alleles of the puroindoline gene		
	Glu A1	*Glu B1*	*Glu D1*		*Glu A3*	*Glu B3*	*Pina-D1a*	*Pina-D1b*	*Pinb-D1a*
DPW 621-50	2* (*b*)	17+18 (*i*)	5+10 (*d*)	10	*b*	*b*	−	+	+
HI 1563	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*b*	*Na*	−	+	+
WH1080	1 (*a*)	7 (*a*)	5+10 (*d*)	8	*d*	*g*	−	+	+
HD2967	2* (*b*)	17+18 (*i*)	5+10 (*d*)	10	*c*	*g*	−	+	+
HD3043	1 (*a*)	13+19	5+10 (*d*)	7	*b*	*j*	−	+	+
DBW14	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*c*	*b*	−	+	+
HD3086	1 (*a*)	17+18 (*i*)	5+10 (*d*)	10	*c*	*g*	−	+	+
HD2329	2* (*b*)	7+9 (*c*)	2+12 (*a*)	7	*b*	*h*, *g*	−	+	+
PBW343	1 (*a*)	7+9 (*c*)	5+10 (*d*)	9	*b*, *c*	*j*	−	+	+
C306	2* (*b*)	20 (*e*)	2+12 (*a*)	6	*c*	*i*	−	+	+
HD2985	2* (*b*)	17+18 (*i*)	5+10 (*d*)	10	*c*	*b*, *i*	−	+	+
HD2987	2* (*b*)	7+8 (*u*)	5+10 (*d*)	10	*c*	*i*	−	+	+
HS490	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*c*	*d*	+	−	+
HUW234	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*c*	*j*	−	+	+
HUW468	2* (*b*)	17+18 (*i*)	2+12 (*a*)	8	*b*, *i*	*i*	−	+	+
HW 2004	2* (*b*)	13+16 (*f*)	2+12 (*a*)	8	*c*	*i*	−	+	+
HW 2045	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*f*	*h*	−	+	+
K 307	2* (*b*)	17+18 (*i*)	2+12 (*a*)	8	*b*	*h*	−	+	+
NW2036	1 (*a*)	7+9 (*c*)	5+10 (*d*)	9	*c*	*j*	−	+	+
PBW373	2* (*a*)	7+9 (*c*)	5+10 (*d*)	9	*c*	*j*	−	+	+
PBW550	2* (*b*)	7+9 (*c*)	5+10 (*d*)	9	*b*	*j*	−	+	+
PBW590	null (*c*)	7 (*a*)	5+10 (*d*)	6	*b*	*d*	−	+	+
RAJ 3765	2* (*b*)	7+8 (*u*)	2+12 (*a*)	8	*c*	*c*	−	+	+
WH 542	2* (*b*)	7+8 (*u*)	5+10 (*d*)	10	*c*	*b*, *j*	−	+	+
DBW17	2* (*b*)	7 (*a*)	5+10 (d)	8	*c*	*j*	−	+	+
WH1021	2* (*b*)	7+8 (*u*)	2+12 (a)	8	*c*	*j*	−	+	+
DBW39	2* (*b*)	13+16 (*f*)	5+10 (*d*)	10	*c*	*b*, *j*	−	+	+
HD2733	null (*c*)	7+9 (*c*)	5+10 (*d*)	6	*c*	*j*	−	+	+
NI5439	null (*c*)	7+8 (*u*)	2+12 (*a*)	6	*b*	*f*, *g*	−	+	+
HD2888	null (*c*)	20 (*e*)	2+12(*a*)	4	*c*	*i*	−	+	+
Barham	1 (*a*)	7+9 (*c*)	2+12 (*a*)	6	*c*	*b*	+	−	+
Long Orion	1 (*a*)	7+8 (*u*)	2+12 (*a*)	8	*f_c*	*b*	+	−	+
Yenda	2* (*b*)	7+9_17+1 (*c_i*)	2+12 (*a*)	7/8	*b_c*	*b_h*	+	−	+
Yitpi	1 (*a*)	7+8 (*u*)	5+10 (*d*)	10	*c*	*h*	+	−	−*
EGA 2248	2* (*b*)	17+18 (*i*)	2+12 (*a*)	8	*b*	*b*	+	−	+

+ = present; − = absent; Na = no amplification; * = Pinb-D1(serine) marker amplified in "this line"

Result and discussion

Analysis of quality traits

In a multi-environment analysis of quality traits, Delhi was found to be a good location for the optimum expression of most quality traits included in the present study (Sarkar et al. 2014). Therefore, material was grown at ICAR-IARI Delhi farms and was evaluated for quality.

Grain characteristics

GHI is the indicative feature of the hardness of grain and is measured as resistance to fracture of the grain. GHI of HS490 among the Indian wheat varieties was only 12.73 whereas others ranged between 65.67 and 95.22 (Table 2). DBW14, HD3086, PBW343, DBW17, DBW39, NI5439 showed GHI between 66 and 72 while rest of Indian wheats were above 72. Among the Australian wheat varieties, only

Table 2 Grain, Gluten Index and protein characteristics of flour from different wheat varieties

Variety	GHI	TKW (g)	PRO. (flour) (14%)	Test wt. (kg/hl)	SDS-SV (Flour) (ml)	Wet gluten (%)	Dry gluten (%)	Gluten Index (%)
DPW621-50	89.04^{d}	34.38^{j}	12.77^{def}	76.50^{lmn}	55^{b}	34.7^{g}	13.6^{d}	100^{a}
HI1563	74.07^{o}	42.17^{c}	9.64^{rs}	81.60^{b}	43^{gh}	27.4^{rs}	9.4^{mn}	63^{f}
WH1080	80.35^{kl}	31.94^{l}	12.55^{efg}	75.90^{n}	64^{a}	30.4^{lm}	12.0^{e}	100^{a}
HD2967	79.81^{l}	34.27^{jk}	11.95^{fghik}	76.00^{n}	52^{c}	29.40^{nop}	10.54^{hijk}	96^{b}
HD3043	89.19^{cd}	31.56^{l}	12.35^{efghi}	77.70^{jk}	45^{f}	35.8^{ef}	11.9^{ef}	58^{gh}
DBW14-	72.29^{p}	36.21^{hi}	10.19^{pqr}	78.70^{ghi}	42^{hi}	29.8^{mno}	9.3^{n}	56^{hi}
HD3086	72.00^{p}	38.20^{f}	12.96^{de}	77.00^{klm}	52^{c}	27.8^{qrs}	9.4^{mn}	76^{d}
HD2329	89.09^{d}	38.89^{f}	10.38^{nopqr}	81.10^{bc}	45^{f}	29.3^{nop}	10.1^{klm}	71^{e}
PBW343	69.84^{r}	43.60^{b}	11.63^{hijkl}	78.60^{hi}	38^{k}	29.13^{nop}	9.92^{klmn}	99^{a}
C306	90.01^{bc}	36.16^{hi}	12.02^{fghi}	82.60^{a}	45^{f}	40.09^{c}	13.32^{d}	29^{n}
HD2985	74.31^{o}	40.84^{d}	10.21^{opqr}	76.30^{nm}	50^{d}	24.40^{u}	8.38^{o}	91^{c}
HD2987	76.35^{n}	39.90^{e}	10.63^{mnopq}	81.30^{bc}	48^{e}	22.20^{v}	8.07^{o}	100^{a}
HS490	12.73^{x}	45.13^{a}	13.63^{d}	77.00^{klm}	34^{n}	17.44^{w}	5.48^{p}	91^{c}
HUW234	82.27^{ij}	33.37^{k}	11.00^{lmno}	77.70^{jk}	42^{hi}	29.3^{nop}	10.1^{klm}	71^{e}
HUW468	83.68^{g}	31.75^{l}	11.46^{ijklm}	77.30^{k}	52^{c}	31.9^{ijk}	10.9^{hij}	100^{a}
HW2004	90.66^{b}	38.68^{f}	10.84^{lmnopq}	77.20^{kl}	44^{fg}	32.6^{hi}	10.9^{hij}	27^{n}
HW2045	84.68^{f}	40.14^{de}	10.12^{pqrs}	81.70^{b}	41^{ij}	28.5^{pq}	9.8^{lmn}	75^{d}
K307	95.22^{a}	30.61^{m}	11.36^{jklm}	74.90^{o}	34^{n}	34.9^{fg}	11.2^{fgh}	27^{n}
NW2036	77.74^{m}	36.62^{gh}	11.09^{klmno}	79.50^{ef}	40^{j}	29.3^{nop}	9.9^{klmn}	77^{d}
PBW373	83.22^{g}	38.32^{f}	11.15^{jklmn}	80.60^{cd}	35^{mn}	31.1^{kl}	10.4^{ijkl}	42^{l}
PBW550	83.03^{ghi}	37.14^{g}	10.31^{nopqr}	78.30^{hij}	44^{fg}	27.9^{qr}	9.7^{lmn}	100^{a}
PBW590	81.46^{j}	37.22^{g}	10.94^{lmnop}	79.10^{fgh}	43^{gh}	30.03^{mn}	10.21^{jkl}	96b
RAJ3765	80.87^{k}	43.15^{b}	9.60^{rs}	81.70^{b}	37^{kl}	28.9^{op}	9.4^{mn}	32^{m}
WH542	87.89^{e}	32.34^{l}	10.93^{lmnop}	78.30^{ij}	40^{j}	32.8^{hi}	11.0^{ghi}	59^{g}
DBW17	70.94^{q}	40.69^{de}	11.16^{jklmn}	80.20^{de}	36^{lm}	26.92^{s}	9.27^{n}	99^{a}
WH1021	77.90^{m}	38.21^{f}	10.93^{lmnop}	79.40^{efg}	36^{lm}	35.3^{fg}	12.1^{e}	59^{g}
DBW39	72.46^{p}	42.14^{c}	11.38^{jklm}	77.20^{kl}	41^{ij}	32.83^{h}	10.80^{hij}	55^{i}
HD2733	83.36^{gh}	35.34^{i}	12.48^{efgh}	77.30^{k}	41^{ij}	36.4^{de}	11.7^{efg}	70^{e}
NI5439	65.67^{s}	45.70^{a}	9.24^{s}	76.20^{nm}	47^{e}	25.46^{t}	8.30^{o}	50^{j}
HD2888	82.54^{ijh}	43.23^{b}	11.71^{ghijkl}	79.80^{def}	41^{ij}	32.35^{hij}	10.81^{hij}	48^{j}
Barham	26^{w}	26^{n}	16^{b}	57^{s}	64^{a}	40.49^{c}	17.23^{a}	56^{hi}
Longreach Orion	38^{t}	24^{p}	10^{qrs}	63^{r}	56^{b}	31.65^{jk}	9.91^{klmn}	42^{l}
Yenda	29^{v}	25^{o}	15^{c}	63^{r}	52^{c}	43.68^{a}	15.92^{b}	44^{kl}
Yitpi	69^{r}	21^{q}	16^{b}	66^{q}	65^{a}	37.04^{d}	13.49^{d}	94^{b}
EGA2248	37^{u}	25^{o}	17^{a}	68^{p}	56^{b}	41.96^{b}	14.63^{c}	45^{k}

GHI Grain Hardness Index, *TKW* thousand kernel weight, *PRO. (Flour)* protein flour content, *SDS-SV (Flour)* sedimentation flour

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$)

Yitpi had a GHI of 69 while that of the other four varieties ranged between 26 and 38. Various classifications have been suggested with regard to GHI by different workers. Morris et al. (2001) suggested a classification: hard grain (GHI: $\geq$ 60), medium hard grain (GHI: 47–59), medium soft grain (GHI: 34–46) and soft grain (GHI: $\leq$ 33). Following this, in the tested Indian wheat varieties, only one variety HS490 is classified as soft wheat and rest 29 wheat varieties are classified as hard. Whereas, among the Australian wheats, the four varieties Longreach Orion, Barham, Yenda and EJA2248 were classified as soft wheat and Yitipi as medium hard. The molecular analysis with *PinaD1* and *PinbD1* AS-PCR markers showed that all hard and medium hard varieties of India had *Pina-D1b* and *Pinb-D1a* alleles ('*ba*'). Yitpi, the medium hard variety of Australia, showed '*ab*' allelic constitution. The Indian soft variety HS490 and Australian soft varieties all had '*aa*' constitution. Some more soft grained genotypes have been developed at ICAR-IARI due to their superiority for biscuit making and have been studied in detail for their protein profile and starch and pasting properties (Katyal et al. 2017, 2018a, b). It has been shown that the '*ab*' types have greater flour recovery than the '*ba*' types but such varieties are not present in our cultivated wheats (Feiz 2008).

The TKW and TW of the Indian varieties ranged from (30.61–45.70 g and 74.92–82.62 kg/hl respectively) (Table 2). C306, a best chapatti quality variety had the highest TW of 82 kg/hl. The Australian varieties on the other hand had very low TKW (21–26 g) as well as TW (57–68 kg/hl).

SDS-sedimentation value

SDS-SV of the tested Indian wheat varieties ranged between 34 and 65 ml (Table 2). Significant differences between wheat varieties were obvious indicating genotype effects on sedimentation trait. Sedimentation value is an indication of the gluten strength of a genotype. AbuHammad et al. (2012) proposed a classification of gluten strength based on the sedimentation value. Weak gluten strength: < 30 ml, moderate gluten strength: 30–40 ml predict, strong gluten strength cultivars: above 45 ml. While most of the Indian wheat varieties were classified as medium strong and only one as strong, three Australian varieties were medium to strong and the other two strong as per this classification. In several studies, it is validated that the GI and SDS-SV are highly correlated, indicating that they are measuring similar aspects of gluten strength (Katyal et al. 2016). A strong positive correlation was found in our study between SV with the protein content of the flour (r = 0.546, $p \leq 0.05$) and FQN (r = 0.373, $p \leq 0.05$) (Online Resource 7).

Protein content

Protein content is key specification for the wheat and flour purchasers since it is related to several processing properties. Flour with low protein content is desired for the cookies/biscuits and cakes etc. (Payne 1987). Higher protein content enhances WA and a longer mixing time to achieve optimum dough consistency, whereas low protein obviates the need for high WA. In the present study, the protein content of the Indian varieties ranged between 9.24 (NI5439) and 13.63% (HS 490) (Table 2). HI1563, DBW14, HW2045, RAJ 3765 and NI5439 showed the low protein content ≤ 10%. The protein content of Indian varieties can be considered realistic as their TKW and TW are in normal and acceptable range. The protein content of the four Australian varieties was very high i.e. between 15 and 17%. Their TKW and TW was very low and therefore, the starch proportion in these varieties would be lower resulting in false impression of higher than actual protein content. Longreach Orion had 10% protein with 24 g TKW and 63 kg/hl TW so that the actual protein in Longreach Orion would be lesser. The Australian varieties are adapted to rainfed, dry conditions and their protein content in Australian climate ranges between (8 and 10%) for soft wheat and 12–14% for Yitpi (Cornish et al. 2007).

Gluten characteristics

The Indian wheat cultivars WG yield ranged between 17.44 (HS490) and 40.9% (C306). GI explains grade of elasticity and extensibility of the flour. Horvat et al. (2002) found a strong correlation between GI, SDS-SV and P% and concluded that GI can be accepted as a fast and reliable tool for describing gluten strength of Croatian wheat flour. The Australian varieties WG yield ranged between 31.65 (Longreach Orion) and 41.96% (EJA 2248). The DG yield of the Indian wheat varieties was observed between 5.48 (HS490) and 13.32% (C306). Australian cultivars DG yield was observed highest for Barham and lowest for Longreach Orion with 17.23 and 9.91% respectively. DG of 10% and above is desirable for bread making while below this, the flour is preferred for biscuit making. 50% of the varieties studied here had DG exceeding 10%. Thus, Indian wheat varieties with low gluten and high gluten are available. In our study, a positive correlation is observed between GI and SV (r = 0.221, $p \leq 0.05$) and protein content (r = 0.035, $p \leq 0.05$) (Online Resource 7). The higher GI is the indicative of strong gluten. Wheat varieties on the basis of gluten index can be grouped as: 91–100 GI designates very strong gluten, 71–90 strong gluten, 31–70 moderately strong gluten and less than 31 indicate weak gluten (AbuHammad et al. 2012). The GI of the Indian wheat varieties ranged between 27 (K307, HW2004) and 100% for (DPW 621-50, WH 1080, HD 2987, HUW 468, PBW550) (Table 2). The Australian cultivars GI ranged from 42 to 94%. Thus, the Indian wheat varieties showed quite a wide range of variation from very strong gluten to weak gluten.

Alveograph

W and P/L were obtained through alveograph analysis of the flour. W refers to the energy necessary to inflate the dough bubble to the point of rupture. It appears to be the best test to differentiate the varieties for their gluten strength and extensibility. The P/L ratio below 0.2 is desirable for short textured products while a ratio nearing 1.0 is preferred for raised bread making (Preston and Williams 2003). Among the Australian varieties (Table 3), a balanced combination of W and P/L was observed. The biscuit varieties Longreach Orion, Barham, EJA2248 and Yenda had W ranging between 98 and 146 (all below 150) and P/L between 0.22 and 0.33. These values indicate weak

Table 3 Farinographic and alveographic properties of flour from different wheat varieties

Variety	WA (%)	DDT (min)	DOS	FQN	P/L	W
HS490	51.8^{o}	0.9^{t}	133^{a}	$37^{a'}$	0.74^{no}	$56^{a'}$
PBW373	62.86^{gh}	3.2^{nop}	79^{f}	56^{t}	3.60^{b}	101^{y}
PBW590	68.1^{b}	11^{d}	$10^{a'}$	200a	3.89b	128^{w}
DBW17	63.8^{fg}	4.7^{l}	43^{p}	52^{w}	1.84^{gh}	143^{u}
DBW14	60.39^{kl}	3.65^{mn}	66^{j}	59^{s}	1.12^{klm}	131^{v}
K307	62.9^{gh}	3.2^{nop}	60^{k}	55^{u}	1.00^{lmno}	160^{r}
WH1021	64.4^{ef}	3.9^{m}	84^{d}	52^{w}	0.91^{mno}	185^{o}
DBW39	64.2^{ef}	6.0^{hi}	40^{q}	91^{o}	0.93^{lmno}	199^{n}
NI5439	60.78^{kl}	4.48^{l}	17^{x}	136^{e}	0.66^{op}	227^{j}
C306	66.43^{c}	2.87^{pqr}	72^{h}	55^{u}	3.05^{c}	159^{r}
HD2888	66.11^{c}	2.42^{rs}	55^{m}	64^{r}	2.40^{ef}	160^{r}
HD2987	63.76^{fg}	3.18^{nop}	11^{z}	136^{e}	2.97^{cd}	166^{q}
HI1563	62.89^{gh}	4.7^{l}	29^{t}	98^{m}	1.54^{hij}	179^{p}
HW2004	66.2^{c}	3.9^{m}	45^{o}	69^{q}	3.16^{c}	180^{p}
HUW234	60.7^{kl}	5.5^{ijk}	26^{u}	105^{k}	1.78^{h}	204^{m}
HW2045	66.1^{c}	5^{kl}	36^{r}	80^{p}	2.67^{de}	218^{l}
HD2733	64.8^{e}	6.5^{h}	30^{s}	93^{n}	1.85^{gh}	221^{k}
PBW550	61.84^{ij}	18.2^{a}	48^{n}	200^{a}	4.91^{a}	226^{j}
NW2036	61.3^{jk}	7.9^{f}	16^{y}	117^{h}	1.24^{jklm}	231^{i}
WH542	66.1^{c}	6.5^{h}	30^{s}	99^{l}	1.25^{jklm}	235^{h}
HD2967	60.06^{l}	5.68^{ij}	25^{v}	107^{j}	1.04^{klmn}	245^{g}
HD2985	61.78^{ij}	5.32^{jk}	$6^{d'}$	160^{c}	1.23^{jklm}	257^{f}
HD2329	70.3^{a}	6.8^{g}	25^{v}	111^{i}	3.24^{c}	269^{e}
WH1080	63.8^{fg}	12.7^{c}	24^{w}	200^{a}	2.19^{fg}	269^{e}
HD3043	68.1^{b}	7.9^{f}	16^{o}	134^{f}	2.50^{ef}	286^{d}
HD3086	64.8^{e}	9.3^{e}	$9^{b'}$	119^{g}	1.20^{jklm}	326^{b}
HUW468	65^{de}	9.7^{e}	$4^{e'}$	194^{b}	1.38^{ijk}	312^{c}
DPW621-50	66.8^{c}	15.9^{b}	57^{l}	200^{a}	1.27^{jkl}	397^{a}
PBW343	60.96^{jkl}	3.17^{nop}	70^{i}	54^{v}	1.74^{hi}	129^{w}
RAJ3765	65.9^{cd}	3.5^{mno}	79^{f}	52^{w}	2.28f	155^{s}
Barham	56.1^{m}	3^{opq}	81^{e}	44^{x}	0.22^{q}	146^{t}
Longreach Orion	64.9^{e}	2.5^{qrs}	128^{b}	39^{z}	0.26^{q}	98^{z}
Yenda	54.8^{n}	2^{s}	77^{g}	43^{y}	0.29^{q}	101^{y}
EGA2248	57^{m}	3.4^{mnop}	91^{c}	44^{x}	0.33^{pq}	123^{x}
Yitpi	62.6^{hi}	7.9^{f}	$8^{c'}$	150^{d}	0.78^{no}	327^{b}

WA water absorption, *DDT* dough development time, *DS* degree of softening, *FQN* farinograph quality number, *P/L* configuration ratio, *W* Deformation energy

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$)

and highly extensible gluten. Flours have been classified on the basis of a strong correlation between W and gluten strength. The wheat varieties were grouped into five classes based on W-value: < 100 J indicate weak, W of 101–150 J indicates moderately weak, moderately strong is indicated by W between 151 and 200 J, while a W between 201 and 250 J strong gluten. W of 250 J and above is seen in case of very strong gluten wheat flours (AbuHammad et al. 2012). Varieties DBW14, PBW343, HS490, PBW590 and DBW17 were observed to have W value < 150 J which accounts them to have weak to moderately weak dough/gluten strength. The Indian varieties showed a wide variation in the W values ranging from as low as 5.6 J (HS490) to as high as 397 J (DPW621-50). There were six varieties in the weak, 17 varieties in moderate to strong and seven varieties in very strong category.

The P/L similarly showed a wide variation ranging from 0.66 (NI5439) to 4.91 (PBW590). Values exceeding 2 are considered to indicate poor extensible gluten and the flour tends to be ‘Bucky’ (Preston and Williams 2003) and not

Table 4 Quality parameters of biscuits in different wheat varieties

Variety	Biscuit thickness (cm)	Biscuit diameter (mm)	Biscuit spread factor	Hardness (N)	Fracturability (mm)
Barham	0.79[b]	8.11[b]	10.29[b]	64.01[b]	7.35[e]
Longreach Orion	0.78[b]	7.92[c]	10.05[c]	72.23[a]	7.96[c]
DBW14	1.05[a]	7.65[d]	7.19[d]	63.53[c]	10.47[a]
HI1563	1.08[a]	7.34[e]	6.77[e]	56.28[e]	9.63[b]
HS490	0.77[b]	8.36[a]	10.85[a]	62.03[d]	7.36[d]

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$)

useful for bread making. Incidentally, C306 considered the best chapatti quality also had a high P/L (3.05).

On the basis of grain quality parameters as well as Farinograph and alveograph study, it was found that the variety HS490 was ideal variety for biscuit making as it possessed soft grain, lower protein, lower WA, low DS and high DOS. However, its W value and P/L ratio need correction by reducing P/L and increasing W somewhat. The varieties PBW373, PBW590, DBW17 and DBW14 also possess weak gluten but with poor extensibility.

The biscuit traits of the varieties HS490, DBW14 and HI1563 were compared with those of Australian varieties Longreach Orion and Barham (Table 4, Online Resource 3). Biscuit spread factor of HS490 was superior to Longreach Orion and Barham and the biscuits were comparable for cookie hardness and fracturability. The other varieties DBW14 and HI1563 on the other hand showed lower spread factor, lower hardness and high fracturability. The higher spread factor, higher hardness and low fracturability makes HS490 highly suitable for cakes, cookies, choco-pies and similar unleavened products.

Four wheat varieties K307, WH1021, DBW39 and NI5439 had a more balanced W and P/L combination suggestive of strong and extensible flour needed for raised bread making. These varieties have W & P/L as 160 J & 1; 185 J & 0.91; 199 J & 0.93; and 227 J & 0.66 respectively. The breads of all 30 Indian varieties were prepared and bread score was calculated. The bread score of varieties K307, DBW39, HD 2967 and HW 2045 was found to be above 7.5 while rest other varieties values were below 7.5 (Online Resource 6). DBW14 also had bread score of 7.8. The lowest score was recorded for RAJ3765 (6.2). These varieties also possess 11% or more flour protein which is another desirable trait for bread making. The rest of the varieties either had higher W values or higher P/L or both. W and P/L were high and unsuitable for either raised breads or soft products. Lack of extensibility was found to be the major lacuna followed by overstrong gluten in Indian wheat cultivars. Varieties with W values above 250 J (HD2985, HD2329, WH1080, HD3043, HUW468, HD3086 and DPW621-50) may be suitable for European type breads like baguette etc. provided they can be improved for their gluten extensibility.

Farinograph

Farinograph measures the water absorption capacity of a flour to give dough that can be processed and the time needed for development of dough and its breakdown (AACC 2000). The farinograph parameters (WA, DDT, DOS and FQN) of different wheat varieties are shown in Table 3 and Fig. 1a–c. WA ranged from 51.8 to 70.3%. HS490, HD2967, DBW14 showed lower WA of 51.8%, 60.06% and 60.39% respectively, while HD2329, HD3043 and PBW590 showed higher WA of 70.3%, 68.1% and 68.1% respectively. For the Australian varieties the WA ranged from 54.8 to 64.9%. Yenda with the lowest WA of 54.8% and Yitpi with the highest of 64.9%.

DDT ranged from 0.9 to 18.2. PBW590 showed exceptionally higher DDTof 18.2 min, while that of HS490 was lowest with 0.9 min. DDT range for the Australian varieties were observed between 2 to 7.9 min. A positive correlation was observed between DDT and SDS-SV of the flour (r = 0.295, $p \leq 0.05$) and GI (r = 0.221, $p \leq 0.05$) (Online Resource 7). The earlier study by Singh et al. (2016) also described the positive correlations between the DDT and SDS-SV parameters and the extractable and unextractable polymeric proteins (which represent the glutenins). DOS of different wheat varieties was observed in the range of 4–133 BU. HS490 and WH1021 were shown to have maximum DOS of 133 and 84 respectively. In Australian varieties, DOS ranged between 8 and 128 BFU. In the present study, FQN ranged from 37 to 200 and was found to be strongly correlated to the gluten strength (r = 0.644, $p \leq 0.05$) (Online Resource 7). Other studies (Barak et al. 2013; Singh et al. 2016) also illustrated the highly significant positive correlation between FQN and gluten strength.

A bread making flour typically has higher WA ($\geq$ 65%), a DDT $\geq$ 3 min and DS $\geq$ 8 min. DOS values of < 60 are highly desirable (Gabriela et al. 2016). Majority of Indian varieties possessed one or more of these characteristics and

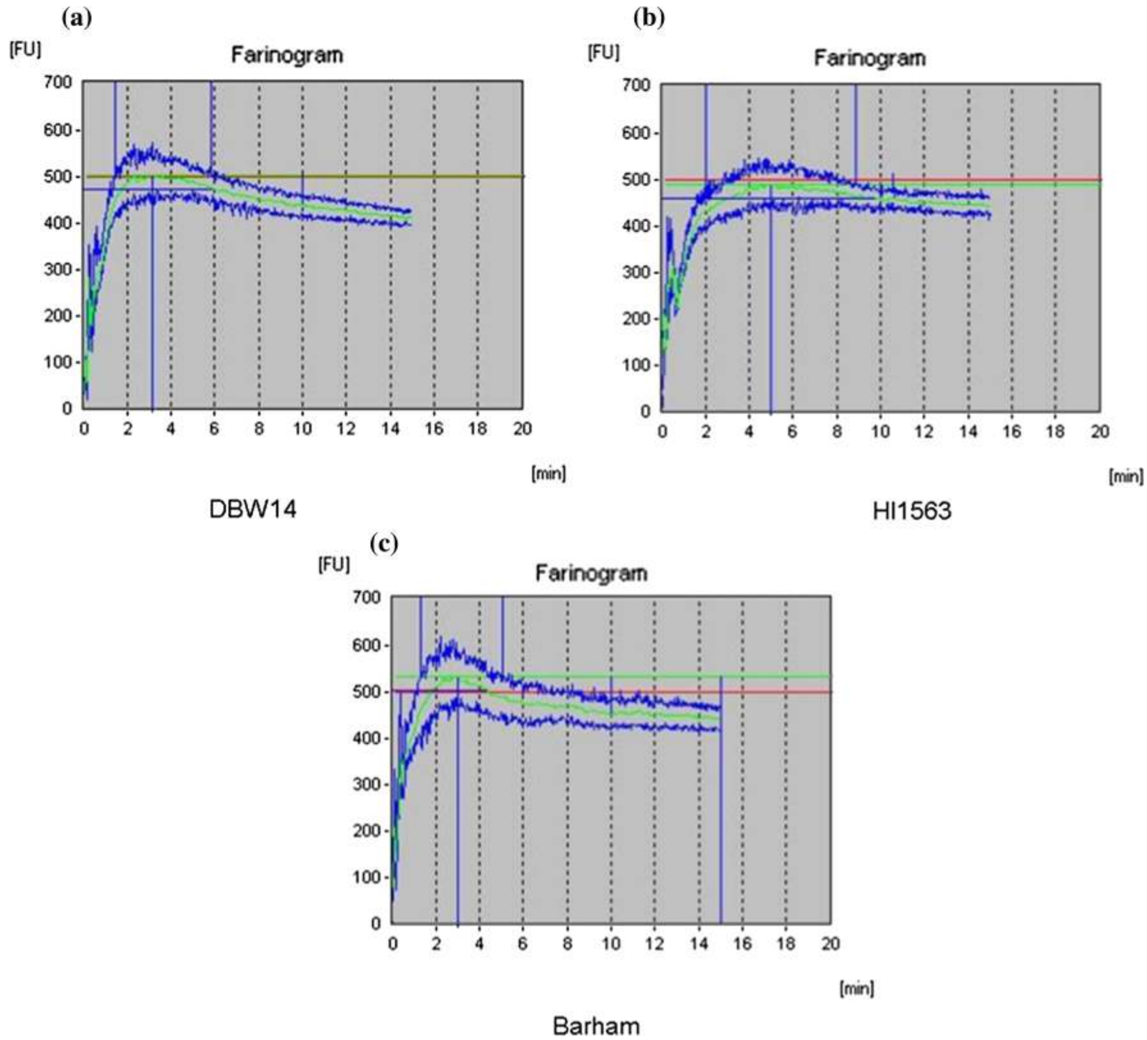

Fig. 1 Farinograph curve of samples. **a** Farinograph curve of sample DBW14 with water absorption 60%. **b** Farinograph curve of sample HI1563 with water absorption 63%. **c** Farinograph curve of sample Barham

only seven of the varieties failed to meet these flour criteria. Thus most of the cultivated wheat showed a high degree of tolerance to mixing indicating good gluten strength. On the other hand, the variety HS 490 possessed a low WA (51.8%) coupled with low DDT and high DOS suggested it to have a low tolerance to mixing and was also found to be soft grained. All its characteristics are similar or even better than the Australian biscuit wheat cultivars. Thus, HS490 is highly suitable for making soft wheat products.

SDS-PAGE and molecular marker analysis for HMW-GS

In the present study, HMW-GS were visualized on the SDS-PAGE gel for different varieties. Five single seeds per variety were used so as to find out the level of off types/mixture in the varieties. Two Indian (HD2967 and NI5439) and one Australian varieties (Yenda) had a mixture. The HMW combination that was same in majority of seeds was taken as the HMW composition of that variety. The results are given in Table 1 and representative gel is shown in Fig. 2.

HMW-GS have been studied in greater detail in the early years as these can be easily resolved on SDS-PAGE, being fewer in number than LMW-GS and gliadins. The combination of the low and high molecular weight glutenin subunits in the grain imparts the major rheological properties to the wheat flour. Several Indian varieties showed the HMW-GS *null* at *GluA1*. This allele is not suitable for bread or biscuit as it affects the extensibility negatively (Kolster et al. 1991) and the subunits 7+8 at *GluB1* and 2+12 at *GluD1* are considered most appropriate for producing weak dough (Payne et al. 1981) while the subunit

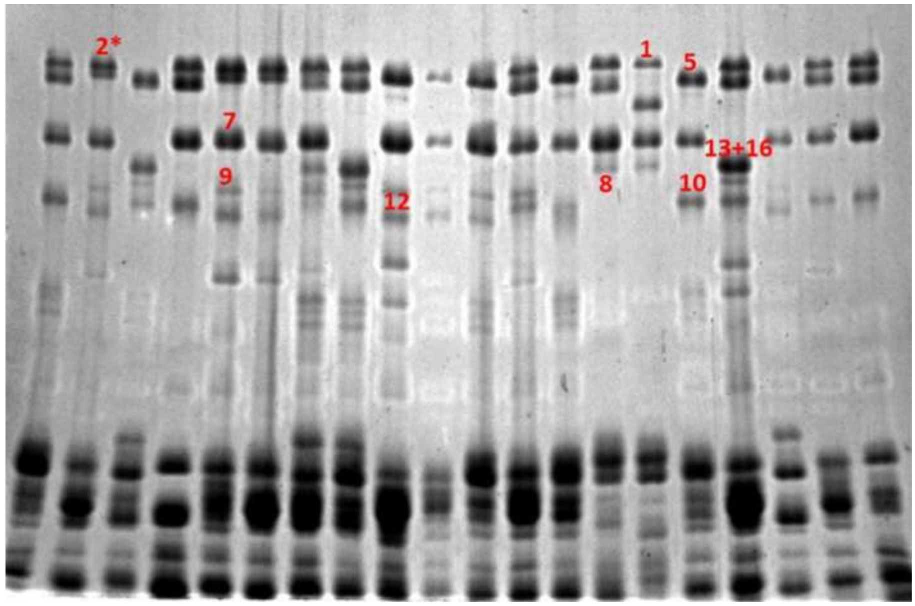

Fig. 2 Protein samples of plants in lane 1–20 run on 10% SDS PAGE gel

5+10 imparts appropriate strength to the dough (Payne et al. 1981). The Australian varieties Longreach Orion and Barham had the subunits 1 at *GluA1*, 7+8 or 7 + 9 at *GluB1* and 2+12 at *GluD1*. While Yenda appeared to be a mixture, Yitpi had 5+10 at *GluD1* and EGA2248 had 17+18 at *GluB1*. Among the Indian varieties analyzed, seven of them had the combination 2*, 7+8, 2+12. The varieties HI1563, DBW14, HS490 had this combination besides lower protein content and weak to moderately strong gluten.

The LMW-GS alleles at *GluA3* and *GluB3* loci were analyzed in this study. The allelic constitution at these two loci in the varieties is listed in Table 1 and representative Online Resource 2. LMW-GS represent about one-third of total seed storage proteins and 60% of glutenins and are also very closely linked with gliadin gene loci (gli-1 and gli-2). Being similar in molecular weight, the LMW and gliadins tend to migrate together in PAGE and hence appear difficult to identify (Liu et al. 2010). However, several workers (Zhang et al. 2004; Wang et al. 2009, 2010) have developed, through varietal and Nils's analysis, AS-PCR markers that can be conveniently used singly and in combination as multiplex PCRs to identify the alleles of the LMW-GS genes in wheat genotypes. There has also been a flood of information in the last decade on the effects of these alleles on the end-use quality and their relation with rheological tests that predict the gluten strength, extensibility and other quality traits (Appelbee 2007; Jin et al. 2013). LMW-GS have been shown to contribute significantly to dough strength and extensibility. At *Glu-A3*, the ranking of alleles for dough strength is proposed as *Glu-A3d* > *GluA3b* > *GluA3c* > *GluA3e*. Ranking for dough extensibility is slightly different, viz., *Glu-A3c* > *GluA3b* ≥ *GluA3f* > *GluA3e* (reviewed in Rasheed et al. 2014). *GluA3b* is an allele that provides both strength and extensibility and hence is preferred for bread making while alleles such as *GluA3c* are preferred where weak but extensible dough is desired (H Eagles, pers. commun.). At *Glu-B3*, alleles were ranked *GluB3b* = *GluB3d* = *GluB3g* > *GluB3h* > *GluB3a* ≥ *GluB3b* > *GluB3d* It is generally agreed that *Glu-A3* and *Glu-B3* alleles effect on the wheat flour processing qualities is highly significant in comparison to the *Glu-D3* alleles (Rasheed et al. 2014).

Australian breeders have developed several soft grained varieties with *GluA1a* or *b*, *GluB1u* or *c* and *GluD1a* at *Glu-1* loci and *GluA3c*, *GluB3b* and *GluD3c* combinations as these have been found to develop flour with weak and extensible dough favorable for cookies/biscuits, pan cakes etc. The bread making quality varieties frequently have the allele *b* at both *Glu-A3* and *GluB3* (H Eagles, pers commun.).

LMW-GS composition of Australian and Indian bread wheat varieties was analyzed in the present study with molecular markers for *GluA3* and *GluB3* alleles. Australian varieties Longreach Orion, Barham and EGA 2248 had *c* and *b* at *GluA3* and *GluB3* respectively. Longreach Orion also showed the allele '*f*' at *GluA3* locus. Yitpi had *c* and *h* at *Glu-A3* and *Glu-B3* respectively. Yenda had more than one allele at *GluA3*, *GluB3* and also at *Glu-1* loci. In the Indian lines, *GluA3c* was the most frequent allele at *GluA3* followed by *b*. At *GluB3*, the allele's 'a' and 'k' were typically absent. The soft grained variety HS 490 had *c* and *d* at *GluA3* and *B3* respectively. DBW14 was found to be the only variety with *GluA3c and GluB3b* similar to Australian biscuit lines but is hard grained. In case of HI 1563, none of the primers of *Glu-B3* amplified, it was inferred that HI1563 may harbor a novel allele.

The farino- and alveographic indices were analyzed in relation to HMW and LMW combination of the varieties. Four varieties K307, WH 1021, DBW 39 and NI5439 had near desirable W and P/L values. These lines had 2+12 subunit at *GluD1* locus. 2+12 imparts lower gluten strength than 5+10. This was reflected in the lower W values of these lines compared to the higher W values of varieties that possessed subunit 5+10. However, the W values were further increased for those varieties where 17+18 at *GluB1*and 1 or 2* at *GluA1* combined with 5+10 whereas the subunits null or 7 reduced both the Glu score and the W value. The lines with a P/L around 1 also had the *c* or *b* alleles at *GluA3* and *b* or *j* allele more frequently at the *GluB3* locus indicating their desirable effect on P/L ratio for bread making quality. The HMW-GS and the LMW-GS of most other varieties and the distribution of physico-chemical and rheological parameters indicated that the varieties have a haphazard distribution of different traits required for an end-use and hardly any varieties qualified highly suitable for the end-use. HS 490 qualified for biscuit quality. DBW 14 had the desirable protein, sedimentation, farinograph, HMW-GS and LMW-GS traits but lacked grain softness and had a higher P/L ratio for a biscuit quality variety. Given the availability of molecular markers for grain softness, the hard grain of DBW 14 can be converted to soft grain by marker assisted backcross breeding to get a new essentially derived variety (EDV) for biscuit quality. The variety HI 1563 has a desirable HMW-GS, low protein but possesses higher P/L and also is hard grained. The higher P/L ratio appears to be due to the LMW-GS composition and may also be affected by grain hardness. Therefore, marker assisted backcross breeding if carried out for the relevant alleles at these loci may render the EDV of HI1563 wheat more suitable for biscuit quality.

Conclusion

The exhaustive analysis of the grain quality traits, rheological indices and HMW-GS and LMW-GS in thirty cultivated wheat varieties popular among the Indian farmers has been carried out in this study. It was found that though there is a diversity for almost all the quality traits among the varieties, the combination of desirable traits required to produce a flour highly suitable for a specific end-use is present in very few of the tested varieties. In the present study, HS490 is classified as soft wheat of high quality. DBW14 is found suitable for conversion into soft wheat as it has most other traits required for biscuit quality. The varieties K307, WH1021 and DBW39 having strong gluten flour can be further improved for their LMW-GS genes to impart extensibility for raised breads. Wheat quality breeding has progressed from the breeding based on, hybridizations between high quality parents, carrying the bulked progenies till they are fixed and detailed quality evaluation of fixed progenies to a stage where molecular markers technology can be used to select desirable plants in the early generations obviating the need of carrying the entire progenies further thus saving space and labor. Considering the increasing demand for product specific varieties, Indian quality wheat breeders should now focus on targeted breeding programmes employing molecular markers. However, a parallel change in the segregated procurement and premium to the farmers has to be ensured by all the stakeholders in this endeavor for making such varieties available in the market.

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