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Understanding dynamics of gluten harvest in augmenting bread quality in high yielding Indian wheats

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Abstract

Ten years data of bread wheat entries evaluated under different environments in India was utilized to understand the factors influencing wet gluten content in high-yield genotypes. Study based on 503 entries revealed that besides genetic factors; agro-climatic variations, production conditions, protein-gluten ratio, water soaking capacity of gluten and HMWGS at *Glu D1* also influenced harvest of wet gluten. Regional specificity was noticed in protein-gluten ratio, which was low in cooler climate and high under hot and dry conditions. Protein-gluten ratio and water retention emerged as characteristic features of a zone and were hardly influenced by varying crop duration of genotypes tested within the zone. While collating gluten characteristics with bread making quality, it was observed that high wet gluten, derived from better gluten protein-gluten ratio and water soaking capacity, made a crucial difference.

Key words: Gluten content, HMWGS, Indian wheats, protein content, protein-gluten ratio, wet-dry gluten ratio

Introduction

Gluten content in bread wheat (*Triticum aestivum* L.) is crucial to articulate quality of baked products. It determines end-use quality by forming storage protein conferring dough its visco-elastic properties (Pena et al. 2002) and regarded as most prominent quality component in loaf volume of Indian wheats (Mohan and Gupta 2013). Gluten is a grain protein component representing 85% of the total protein in the flour; therefore its dependence upon grain protein content is obvious. All India Coordinated Wheat & Barley Improvement Project (AICW&BIP) evaluates high-yield derivatives developed for cultivation in different agro-climatic conditions. Whether protein-gluten relationship remains similar under all environments is always an anxiety in wheat researchers. Just like protein content (8.8 to 14.5% at 14% grain moisture), wide range of wet gluten content (17.1 to 40.4%) had been recorded in released and pre- released wheat varieties evaluated for irrigated conditions in AICW&BIP. Such a wide range of gluten content indicates that not only genetic factors; but certain issues related to grain and non-grain parameters also matter for such expressions in Indian wheats. This study investigated grain and non-grain factors crucial for gluten harvest in high yielding wheat genotypes developed and tested under different agro-climatic conditions prevailing in India. Bread quality of bread wheat varieties differs in five mega-environments of the country as every zone is characterized with different climate, yield potential, disease pattern and grain quality features (Mohan et al. 2011; Mohan et al. 2013). The present investigation also tries to assess how crucial are the gluten properties in articulating bread quality under Indian conditions.

Materials and methods

The material included released and pre-released bread wheat varieties tested under irrigated trials of AICW&BIP in five agro-climatically diverse zones in India i.e. northern hills zone (NHZ) north-western plains zone (NWPZ), north-eastern plains zone (NEPZ), central zone (CZ) and peninsular zone (PZ). Southern hills zone (SHZ) was excluded in this investigations as samples for grain analysis are received only from one location whereas other five zones have 3-5 testing sites for quality analysis. In total, 503 entries were evaluated during the period 2005-14 at ISO 9001-2008

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certified laboratory of the institute. For HMWGS divergence, electrophoresis was performed on vertical gel according to SDS-PAGE protocol. Wet gluten content (WGC) and bread loaf volume was recorded as per international standards (AACC 200). Grain protein content (GPC) measured by infra-red transmittance based instrument Infra-tec 1125 is presented at 14% grain moisture. Wet gluten per unit protein was described as protein-gluten ratio (PGR). Ratio between wet and dry gluten contents was derived to know harvest of wet gluten from per unit of dry gluten. Difference in mean value was statistically analyzed by Student t-test. Diversity in each component was gauged through coefficient of variability (CV). Relationship between protein and gluten contents was examined by plotting polynomial trend-line of order 2 utilizing data analysis tool of the excel programme.

Results and discussion

Environment effects

In this investigation, the environment for wheat growth was different in each zone. NHZ that covers hills and foothills of Himalayas has long winter with low temperature. NWPZ and NEPZ represent the Indo-Gangetic plains where NWPZ considered as most productive wheat land has the most congenial environment for wheat growth whereas adjoined NEPZ has humid climate with short winter. Crop in CZ often faces soil moisture stress as climate is hot and dry. Peninsula in down south i.e., PZ has similar temperature and soil moisture conditions but climate is not that dry as relative humidity is higher. Zone-wise assortment of these entries reveals that just like GPC, differences in WGC also prevail among the wheat zones. Average WGC in each zone was distinctly different ($P < 0.05$) from rest of the zones (Table 1). WGC was minimum in NHZ and maximum in PZ. Though gluten content appeared zone specific, but range in wet gluten suggested that genotypes with WGC = 34% were available in each zone. It ascertains that genotypes of good WGC can be traced in each zone although their frequency can vary zone-wise. It was observed that diversity goes high in the zones where WGC remains low. NHZ had lowest gluten content amongst all zones but CV was highest in that hill region. The trend was same in NEPZ; another zone with average WGC (<30%). It was evident that gluten content in Indian wheats varied according to the agro-climatic conditions prevailing in different zones. Variations occurring in wheat quality of different zones are in reports from India (Mohan et al. 2011) and China (Zhang et al. 2004).

Under irrigated conditions, wheat is grown under two production conditions in India i.e. timely-sown (TS) and late-sown (LS) and the study material involved genotypes from both trial series. The late-sown wheat is planted 15-20 days after the sowing schedule of timely-sown wheat. Since late planted wheats get shorter time to mature, only short duration genotypes mature in time under such condition. Certain wheat grain quality attributes vary under such conditions, too (Mohan et al. 2011). Heat stress during post-anthesis period is higher in the late-sown wheat, consequently grain ripening period reduces. The grain weight of late-sown wheats therefore remains lower in comparison to timely-sown wheats. Since there is

Table 1. Zonal difference in grain quality parameters

Characteristics	Statistic	NHZ	NWPZ	NEPZ	CZ	PZ
Protein content	Mean	10.8	12.1	11.5	11.7	12.3
	Range	8.8-13.2	10.5-14.2	9.5-13.3	9.9-13.6	10.1-14.5
	CV	8.72	6.07	8.15	7.64	7.27
Wet gluten	Mean	26.9	30.8	29.4	31.6	32.7
	Range	17.7-34.8	25.8-36.2	19.3-37.4	25.7-36.6	27.1-40.4
	CV	15.1	7.48	10.4	8.02	8.51
Protein wet-gluten ratio	Mean	2.49	2.55	2.56	2.70	2.66
	Range	1.83-2.92	2.14-3.03	1.92-2.96	2.33-3.05	2.20-3.18
	CV	10.4	6.84	7.59	5.58	5.69
Wet-dry gluten ratio	Mean	2.96	2.99	3.04	3.04	3.06
	Range	2.25-3.33	2.49-3.22	2.58-3.34	2.81-3.30	2.76-3.27
	CV	7.73	4.41	4.71	3.41	3.34

adverse relationship between grain weight and GPC (Nagarajan et al. 2007); grain protein is generally higher in late-sown wheats. Enhancement in WGC is therefore obvious under late-sown condition as gluten is a grain protein component. In high-yield genotypes of India, this trend was apparent in four zones of Indian plains (Table 2). Study asserts that not only agro-climates but production conditions or crop duration also influence WGC.

Water absorption capacity

Gluten content is expressed in two ways i.e. wet gluten and dry gluten. Content of both types of gluten varied in different zones but the pattern of increase or decrease was similar. Further investigations revealed that quantity of wet gluten produced from per unit dry gluten was higher in certain zones like NEPZ, CZ and PZ. To substantiate it, ratio between wet and dry gluten was derived (Table 1). It was observed that ratio between wet and dry gluten was significantly higher in central-peninsular India and NEPZ (P 0.001) in comparison NWPZ and NHZ. Harvest of wet gluten per unit dry gluten was lowest in the hills. It shows gluten of PZ has higher water soaking capacity than NWPZ which ultimately enhance dough properties of PZ wheats. Results asserted that besides GPC, climate also influenced the water soaking capacity of gluten harvested in different genotypes. Plotting of gluten contents against protein, further demonstrated that dry gluten content did go higher with increase in protein content in both the zones as R^2 value matched in NWPZ (0.52) and PZ (0.60). Scenario however changed in case of wet-gluten, as the positive association between protein and wet-gluten was weakened in NWPZ (R^2: 0.23) as compared to PZ (R^2: 0.56). Though zonal differences were apparent in wet-dry gluten ratio, variation levels indicate that efficient genotypes exist in each zone. Opportunity therefore lies in each zone to pick up genotypes which can soak more water in the gluten to give high wet gluten content.

High molecular weight glutenin subunits

High molecular weight glutenin subunits (HMWGS) play important role in wheat dough quality (Payne et al. 1981; Pena 2008) and their relevance in bread quality has been illustrated in Indian wheats, too (Mohan and Gupta 2013). The genes controlling HMWGS are located on *Glu-A1, Glu-B1* and *Glu-D1* loci. There is differential quality effect linked to glutenin subunit composition as 1, 2* (*Glu A1*); 7+8, 17+18 (*Glu B1*); and 5+10 (*Glu D1*), generally contribute positively to high dough strength. Divergence for HMWGS in wheat has been highlighted in several studies (Horvat et al. 2002; Ram 2003). Relevance of 5+10 in wheat dough quality, especially to bake high quality breads, has been strongly advocated whereas other allelic subunits at *Glu D1* locus recognized as 2+12 are associated with poor bread making properties (Payne et al. 1987). Assortment of 503 lines in different HMWGS allelic subunits revealed that variations at *Glu B1* locus made no difference in gluten content (Fig. 1). At *Glu A1* locus however, gluten content at 2* subunits was statistically higher than 1 or N (*P 0.05*). Difference in gluten content was quite large (*P <0.001*) between two subunits of *Glu D1* locus i.e. 5+10 (30.7%) and 2+12 (20.8%). It illustrates that variations in HMWGS at locus *Glu D1* and *Glu A1* also amount to gluten content differences in bread wheat.

Protein-gluten ratio

Investigations asserted that harvest of wet gluten

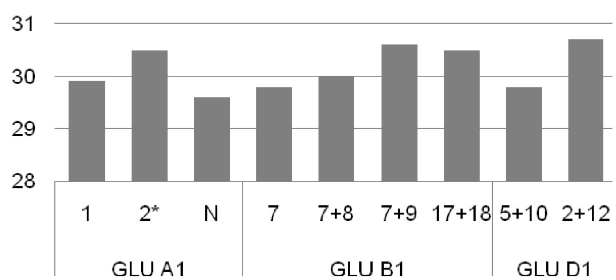

Fig. 1. Wet gluten content (%) in wheats of different glutenin subunits

Table 2. Production conditions and grain quality under different wheat zones

Parameters	NWPZ		NEPZ		CZ		PZ		NHZ	
	TS	LS	TS	LS	TS	LS	TS	LS	TS	LS
Grain protein (%)	12.0	12.2*	11.2	11.8**	10.9	12.6**	12.0	12.5**	10.8	10.8
Wet gluten (%)	30.4	31.3*	28.6	30.5**	29.5	33.0**	32.1	33.2*	26.9	27.0
Gluten-protein ratio	2.54	2.57	2.54	2.58	2.70	2.70	2.68	2.65	2.48	2.50

depends not only on protein and dry gluten contents but the ratio between wet and dry gluten and the HMWGS also matter. Since all these three factors had expressed differential impact under different climates, the harvest of wet gluten from per unit protein was certain to vary. Genotypic differences in protein-gluten ratio (PGR) had been reported in bread wheat [12]. PGR differences in Indian wheats had also been reported at *Glu D1* locus (Mohan and Gupta 2013a). This analysis illustrated high variations among the genotypes (PGR: 1.83 to 3.18) and environments (Table 1). The study made it amply clear that besides GPC; PGR also has great relevance in gluten harvest. It means that even when GPC is low, gluten content can go high provided PGR is good (2.5-2.6). In this investigation, average GPC in NWPZ was significantly higher than CZ (*P: 0.001*), still WGC in CZ was better than NWPZ (*P: 0.011*) because PGR in CZ was higher in comparison to NWPZ (*P: <0.001*). Under Indian conditions, mean PGR was lowest in the hills, highest in central-peninsular India (CZ and PZ) and moderate in the Indo-Gangetic plains (NWPZ and NEPZ). Statistically, there was no difference among the two zones of Indo-Gangetic plains and Central-Peninsular India. It illustrates that PGR is a climatically governed attribute. It goes high when climate is hot and drops under cooler environment. There was no difference in mean PGR between timely and late sown wheats in any zone (Table 2). It shows that although PGR is region specific, it remains unaltered by the crop duration.

Protein-gluten relationship

Though gluten depends heavily on protein, relationship pattern varied in different zones. In regression analysis, relationship gauged by coefficient of determination (R^2) was highly significant in each zone but the magnitude differed (Table 3). R^2 value was strikingly lower in NWPZ (0.24) in comparison to NHZ, NEPZ, CZ and PZ (0.47 to 0.55). It means that relationship between protein and gluten is altogether different in NWPZ as it is not as strong as observed in other parts of the country. Coefficient of variation in GPC (Table 1) was also much lower in NWPZ (6.07%) in comparison to rest of the zones (PZ: 7.27%, CZ: 7.64%, NEPZ: 8.15% and NHZ: 8.72%). Zonal differences were visible in the equation too (Table 3). It indicates that relationship pattern between WGC and GPC can differ in certain environments.

To study the relationship pattern between gluten and protein contents, polynomial trend line of order 2 was derived as indicated in Fig. 2. In certain zones, continuous increase in WGC was visible with advancing GPC levels. Such trend was noticed in the region where WGC was on the extreme i.e. either high as in PZ or low as in NHZ. In rest of the zones i.e., NWPZ, NEPZ and CZ; plateau occurred once GPC reached 13% level. PZ and NWPZ were chosen to give symptomatic demonstration of each type as protein range was almost similar in both the regions (NWPZ: 10.5 to 14.2%; PZ: 10.0 to 14.5%). This comparison revealed

Table 3. Statistics of linear trend in protein-gluten relationship

Zones	Direction	Coefficient of determination		Equation
		R^2 value	P value	
NWPZ	Positive	0.236	<0.0001	y = 1.528x + 12.348
NEPZ	Positive	0.472	<0.0001	y = 2.246x + 03.608
CZ	Positive	0.538	<0.0001	y = 2.075x + 07.271
PZ	Positive	0.544	<0.0001	y = 2.294x + 04.489
NHZ	Positive	0.549	<0.0001	y = 3.194x - 07.574

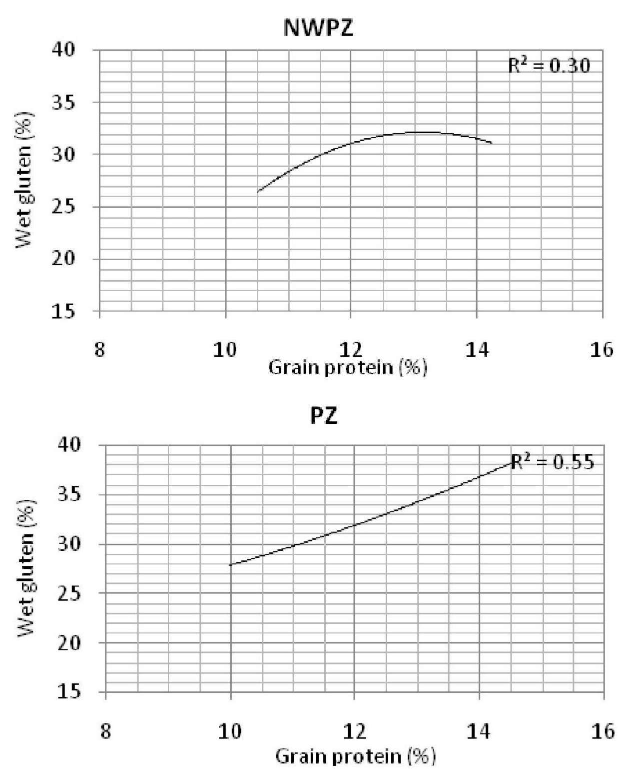

Fig. 2. Relationship (polynomial trend-line of order 2) between gluten and protein contents

that maximum WGC in NWPZ (32%) was harvested at 13% GPC and at the same GPC level; 2% extra WGC (34%) was harnessed in PZ. It points that protein-gluten ratio could be the root cause of differential pattern in diverse environments.

This study further illustrated that wet-gluten content under PZ condition kept on rising under advancing protein levels. Under such conditions, even 38% wet-gluten could also be harvested in PZ. The trend however changed in NWPZ as maximum wet-gluten (32.3%) was achieved between the protein range 12.5 to 13.5% only.

Influence on bread quality

Under Indian conditions, protein and gluten contents have been reported essential contributes for good quality bread [2]. Although gluten harvest is good in CZ, low gluten quality (index) and gluten strength (sedimentation value) make the bread quality poor (Table 3). The hill zone varieties suffer mainly because of low grain hardness and gluten content. Grain quality characteristics of the wheats grown in peninsular region had traditionally been rated high in India. However focused efforts on wheat quality improvement in NWPZ have brought this productive zone to the level where high-yield genotypes match PZ in terms of protein content, sedimentation value, gluten index and grain hardness index. Good bread quality cultivars still elude wheat researchers of NWPZ although majority of the developed material had 5+10 at *Glu D1* locus. This study amply demonstrates that higher gluten-protein ratio and better water soaking capacity of the gluten harvested in PZ make that region distinct in comparison to NWPZ. Study also suggests that unlike PZ, enhancement of wet-gluten through protein can only be achieved up-to a certain limit in NWPZ (Fig. 2). Mohan and Gupta had reported that such ceiling on wet gluten content occurs in 5+10 wheats [13, 14]. High gluten content therefore is a big handicap in improving bread quality under north-western conditions.

The study targeted to assess gluten properties of high yielding Indian wheats has amply demonstrated that wet gluten content, a key component to articulate bread making quality, is highly influenced by grain and non-grain variations. Although it is hard to overcome the influence of environment or climate at a given research centre, this analysis shows that opportunities to exercise desired selection still prevail as tremendous variability exists at the genotypic level in each zone.

Table 4. Important quality characteristics in relation to bread quality

Parameter	NHZ	NWPZ	NEPZ	CZ	PZ
Bread loaf volume (cc)	539.00	560.00	566.00	556.00	580.00
Protein content (%)	10.80	12.10	11.50	11.70	12.30
Wet gluten content (%)	26.90	31.20	29.40	31.60	32.70
Protein wet-gluten ratio	2.49	2.55	2.56	2.70	2.66
Wet-dry gluten ratio	2.96	2.99	3.04	3.04	3.06
Sedimentation value (ml)	42.00	43.00	44.00	41.00	44.00
Gluten index (%)	60.00	61.00	62.00	53.00	60.00
Grain hardness index	68.00	77.00	75.00	75.00	76.00
Percentage of 5+10 types	42.00	83.90	47.30	17.00	40.70

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