Australian Journal of Crop Science AJCS
AJCS 4(1):63-67(2010) ISSN:1835-2707

Differential accumulation of gliadin proteins in wheat grain of RILs grown at two different agroclimatic conditions and their effect on loaf volume

Elangovan M.[1], Dholakia B.B.[1], Gaikwad S.M.[2], Gupta V.S.[1*]

[1]Plant Molecular Biology Unit, Division of Biochemical Sciences, National Chemical Laboratory, Pune 411008, India
[2]Division of Biochemical Sciences, National Chemical Laboratory, Pune 411008, India

*** Corresponding author: vs.gupta@ncl.res.in**

Abstract

The end use of wheat depends on the quality and quantity of protein accumulated in the grain during the grain filling stage. Gliadin families of proteins are proved to affect the bread making quality (BMQ) by affecting the rheology and functionality of dough. The gliadin accumulation was analysed using Indian genotypes grown in two diverse agro climatic zones. About 16 RILs along with their parents HI977 and HD2329 revealed significant difference in accumulation of all gliadin fractions. High accumulation of α and β gliadin with low level of γ gliadin was observed for Kota as compared to Pune location. However, this significant difference in gliadin accumulation due to environment, did not affect the loaf volume performance of the RILs at statistically significant level.

Keywords: Wheat protein, RP-HPLC, loaf volume (Lv).

Introduction

Wheat proteins impart the visco-elastic property of the dough and gliadins are most abundant protein in the wheat grain. Gliadins constitute about 40% of the total endosperm protein and are a heterogenous mixture of single-chain polypeptides of molecular weight ranging from 28-70 kDa (Payne et al., 1982). They are further divided into four groups as α, β, γ and ω gliadins based on their electrophoretic mobility at low pH. Some of them such as α, β and γ gliadins show interchain disulfide bond, which lacks in case of ω gliadins. Most of the genes coding for ω and γ gliadins are tightly clustered at three homologous loci such as *Gli-A1*, *Gli-B1* and *Gli-D1* and as a result, the gliadin polypeptides coded by each locus are inherited strictly as a block, which is referred as gliadin allele (Pogna et al., 1994). Several researchers have observed significant correlations bet- ween gliadin components and gluten quality. The expression of gliadin genes and accumulation of gliadin is often affected by the environment in which it is grown (Triboi et al., 2000). Though gliadin and glutenin forms the functional proteins for controlling BMQ, their differential accumulation and impact on BMQ is not yet investigated in detail. A systematic attempt was made to decipher the differential accumulation of gliadin proteins in wheat grains using a set of recombinant inbred lines, along with their parents using RP-HPLC and their effect on loaf volume was also recorded.

Materials and methods

The HI977 x HD2329 population was grown at Pune and Kota in the year 2003-04, using Augmented Randomised Complete Block Design (ARCBD) as described by Hessler et al., (2002) without any replications. About 16 RILs were randomly selected from the cross HI977 x HD2329, whose loaf volume (Lv) ranged between 500–600 cc. The parents (HI977 and HD2329) along with 3 varieties were used as checks and replicated in 8 blocks. The statistical analysis was performed as described in our earlier report (Elangovan et al., 2008). The parents HI977 and HD2329 from every alternate block (4 samples) were taken for Reverse Phase High Performance Liquid Chromatography (RPHPLC) analysis. The analyses of variance on these parents (replicated) for gliadin fractions were performed and the interpretation was extended to the RILs (unreplicated). The gliadin proteins were extracted from 100 mg of flour prepared by pooling and grinding 5 seeds of each genotype. The wheat flour was incubated with 50% 1-propanol and the slurry was mixed and incubated at 65°C for 30 min. The gliadin was separ-

Table 1. ANOVA of gliadin fractions extracted from HI977 and HD2329 grown at two locations

Source of Variation	*df*	ω gliadin			α+β gliadin			γ gliadin		
		MS	*F*	*P-value*	*MS*	*F*	*P-value*	*MS*	*F*	*P-value*
Location	1	0.008	123.623	0.000***	0.396	208.637	0.000***	15.108	25.301	0.000***
Genotype	1	0.000	4.429	0.057^{ns}	0.041	21.636	0.001**	6.586	11.029	0.006**
Location x Genotype	1	0.003	45.072	0.000***	0.004	2.240	0.160^{ns}	5.694	9.535	0.009**
Error	12	0.000			0.002			0.597		
Total	15									

*** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05 and ns-not significant, MS- Mean Sum of Square

Table 2. Accumulation of various gliadin proteins in the parents HI977 and HD2329

	ω gliadin				α+β gliadin				γ gliadin			
	Pune		Kota		Pune		Kota		Pune		Kota	
	HI977	HD2329	HI977	HD2329	HI977	HD2329	HI977	HD2329	HI977	HD2329	HI977	HD2329
SD	0.00904	0.007195	0.000943	0.013622	0.047661	0.055521	0.008156	0.046658	0.316028	0.341198	0.466367	1.39813
Cv	0.00226	0.001799	0.000236	0.003406	0.011915	0.01388	0.002039	0.011664	0.079007	0.085299	0.116592	0.349532
Average	0.149443	0.133423	0.169643	0.205075	0.225683	0.35966	0.573058	0.6418	8.640153	11.11636	11.77667	11.86675

SD- Standard deviation Cv- Coefficient of Variation

ated from the slurry by centrifuging at 5000 rpm for 10 min (Singh et al., 1991). The gliadin samples were filtered with 0.4 µm filters before injecting into Nucleocil, C18 columns of RPHPLC (WATERS system, USA) and the gliadin fractions were detected at 220 nm (SHIMADZU UV detector). The samples were injected after 1 h equilibration with water containing 0.05% Tri Fluoro Acetic acid (TFA) and 60% acetonitrile with 0.01% TFA. The data in the form of chromatogram were integrated for different peaks, designated as ω, α, β and γ gliadin, as prescribed by Daniel and Triboi, (2000). The area under the peaks was calculated by statistical integration (ORIGIN ver. 6.1, USA) and plotted as graphs using MS-EXCEL software. The areas under different fractions were separated for RILs and parents. For parents, the fraction values were analyzed for variance (ANOVA) using MS-EXCEL software and ANOVA with two factors (data analysis package). The fractions of RILs were subjected to paired t-Test using MS-EXCEL software. For loaf volume (Lv) estimation, the tempered grains from both the locations (Pune and Kota) were ground and Lv was estimated at DWR karnal wheat quality laboratory as described in our earlier report (Elangovan et al., 2008).

Results

Analyis of variance (ANOVA)

The ANOVA for various gliadin fractions extracted from HI977 and HD2329 grown at Kota and Pune location are presented in the Table 1. The three gliadin fractions showed significant variation among the genotypes (HI977 and HD2329), due to location. Further in Table 1, the difference among the genotypes HI977 and HD2329 for ω gliadin was insignificant, while it was significant for α+β and γ gliadin. For α+β gliadin the genotypes showed significant differential accumulation by itself,

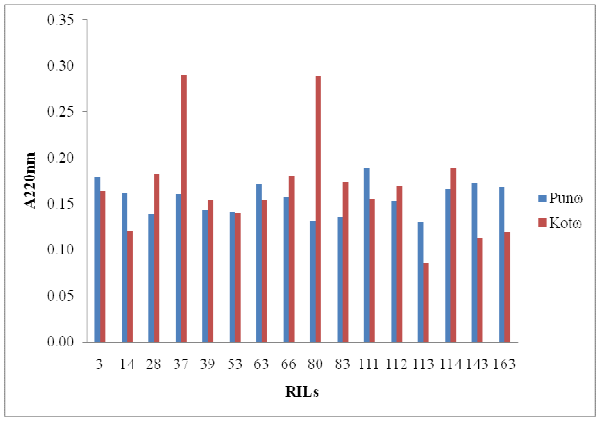

Fig 1. Comparison of ω gliadin fraction accumulated in the RILs grown at Pune and Kota. The gliadin fractions were estimated by absorption at 220nm and plotted against RILs. The ω gliadin of Pune and Kota location are denoted by Punω and Kotω, respectively.

while the interaction with the location was insignificant.

The average of gliadin fractions for the parents HI977 and HD2329 are tabulated in the Table 2. Overall, the composition of the γ gliadin was highest among other fractions followed by α +β and ω gliadin. The standard deviation and coefficient of variation was higher for γ gliadin compared to other gliadin fractions. For γ gliadin, all these factors such as location, genotype and location x genotype interaction were significant. The differential accumulation of γ gliadin was higher compared to α and β gliadin.

The correlation among the gliadin fractions and Lv of RILs grown at two locations are shown in the Table 3. All fractions showed a positive relationship with respect-tive Lv i.e. Pune gliadin fractions with Pune Lv and similarly, Kota gliadin fractions with Kota Lv. Initially the data was plotted as scatter plot to identify the

Table 3. Correlation among gliadin fractions and Lv

	PunLv	KotLv	Punω	Kotω	Punα+β	Kotα+β	Punγ
KotLv	0.638						
Punω	0.368	-0.033					
Kotω	0.105	0.154	-0.159				
Punα+β	0.072	-0.147	0.660	-0.359			
Kotα+β	0.268	0.498	-0.272	0.835	-0.362		
Punγ	0.388	0.092	0.676	-0.356	0.498	-0.189	
Kotγ	0.267	0.210	-0.108	0.821	-0.227	0.666	-0.324

PunLv- Pune Loaf volume, KotLv- Kota Loaf volume, Pun- Pune, Kot-Kota

relationship, the gliadin fractions with Lv, did not show a linear relationship, but in turn scattered along the axis (data not shown). So, the correlation of the gliadin with Lv is not a valid relationship, instead mere positive and negative relationship can be interpreted with the Table 3. The gliadin fractions of Pune grown RIL showed negative relationship with gliadins of Kota grown RILs. Further, Figs 1, 2 and 3 depict the graphical presentation of ω, α+β and γ gliadin accumulation estimated in the RILs, grown at both Pune and Kota respectively.

The α+β gliadin accumulation was higher in RILs grown at Kota than Pune as evident from the Fig 2, while the γ was higher in RILs grown at Pune than Kota, location as shown in the Fig 3.

Paired t-Test analysis of gliadin fractions

A paired t-Test was done between Kota and Pune gliadin fractions along with Lv, using 16 RILs. The results were tabulated in Table 4. It revealed significant difference in accumulation of α +β and γ gliadin of RILs grown at Pune and Kota location, while it was insignificant for α gliadin. The Lv also showed insignificant difference in RILs grown at both locations.

Discussion

Wheat protein content and bread making quality depend on genotype and environmental factors and it is considered that variation in protein content and composition significantly alter the end use (Triboi et al., 2000). The gliadins being major component of wheat protein were often used to study their impact using dough mixing behavior. Our experiment with four replicates two genotypes (HI977 and HD2329) along with 16 non replicating genotypes forms statistically viable experimental material as compared to two samples, used by Triboi et al., (2000) to study the differential accumulation of gliadin proteins. Also the 16 genotypes (RILs) of the population were selected based on their loaf volume ranging from 500 ml to 600 ml to avoid bias.

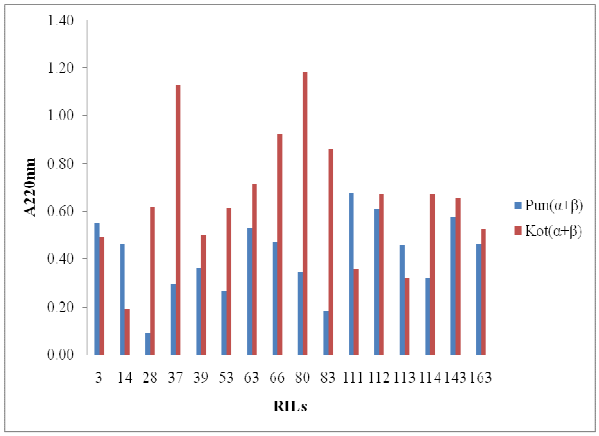

Fig 2. Comparison of α+β gliadin fraction accumulated in the RILs grown at Pune and Kota. The gliadin fractions were estimated at 220 nm and plotted against RILs. The α+β gliadin of Pune and Kota location are denoted by Punα+β and Kotα+β, respectively.

Analysis of variance for different components of gliadin revealed a statistically significant difference in accumulation due to location or environment. This shows that there is a significant effect of environment in controlling the expression of gliadin protein. The differential accumulation of gliadin due to environment was well documented by Triboi et al., (2000). The HI977 and HD2329 were compared for gliadin accumulation and identified that both accumulated similar amount of ω gliadin, but significantly different amount of α+β and γ gliadin. The High Performance Capillary Electrophoresis (HPCE) studied on popular wheat varieties Chinese Spring, Eagle and Cheyenne revealed that the quantity and quality of gliadin fractions were unique and genotype dependent (Werner et al., 1994).

The different gliadin fractions showed different interactions due to location. The ω gliadin showed significant interaction due to environment, while it was insignificant for α+β gliadin. But, the genotypes (HI977 and HD2329), showed statistically significant difference in α+β gliadin accumulation, while it was insignificant for ω gliadin. This revealed that similar amount of ω

Table 4. Paired t-Test analysis for various traits of RILs grown at Pune and Kota

	ω		α+β		γ		Lv	
RILs	Pune	Kota	Pune	Kota	Pune	Kota	Pune	Kota
P≤ 0.05	0.44ns		0.01*		0.00***		0.07ns	
t-Test	no significant		significant		significant		not significant	

gliadin accumulated in both HI977 and HD 2329, which varies due to environment in which it is grown. But for α+β gliadin, both the parents show statistically significant difference, but the response due to environment was dissimilar for these genotypes, hence the interaction due to environment was not significant (Table 1). This fact is supportive to the α+β gliadin accumulated in the RILs, shown in Fig 2. The accumulation of α+β gliadin was higher in most of the RILs grown at Kota compared to RILs grown at Pune location. Such genotype dependent differential accumulation were reported by Werner et al., (1994).

The analysis of variance for γ gliadin shown in Table 1, revealed great difference between HI977 and HD2329, coupled with significant interaction in γ gliadin accumulation due to location. Among the gliadin fractions, the quantity of γ gliadin was high compared to other two gliadin fractions (ω and α+β), which is the reason behind high standard deviation and coefficient of variation. This shows that γ gliadin expression and accumulation are very different from other two gliadin fraction (ω and α+β). The HI977 with an average of 8.64 units of γ gliadin in Pune, recorded 11.77 units in Kota, while there was a less variation for HD2329 between these two locations. This showed that there was a significant difference in γ gliadin accumulation due to environment and was genotype dependent. The analysis of γ gliadin accumulation in RILs reveal that RILs grown at Pune location had higher accumulation compared to RILs grown at Kota location, as shown in the Fig 3. Studies on mixing behavior of dough suggest that gliadin components impart significant effect on dough. According to Uthayakumaran et al., (2000) γ gliadin positively correlated with Lv, while ω gliadin had negative effect and α+β gliadin had least effect on Lv. The γ gliadin decreased mixing time and increased the maximum resistance to dough extension to a great extent. Table 4 displays paired t-Test results of gliadin fractions and loaf volume recorded at Kota and Pune locations. Our study revealed a significant differential accumulation of γ gliadin among the parents (HI977 and HD2329). This differential accumulation should also be realized in RILs. Significant difference was observed in γ gliadin and α+β accumulation in the 16 RILs, while it was insignificant for ω gliadin and Lv. This is evident through ω, α+β and γ gliadin fractions measured as shown in Fig 1, 2 and 3, respectively. The RILs 37 and 80 grown at Kota recorded higher ω, α+β and γ gliadin, compared to Pune location. This may be due to specific

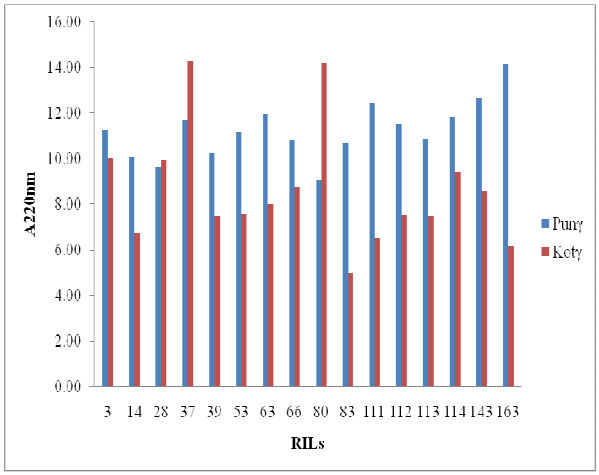

Fig 3. Comparison of γ gliadin fraction accumulated in the RILs grown at Pune and Kota. The gliadin fractions were estimated at 220 nm and plotted against RILs. The γ gliadin of Pune and Kota location are denoted by Punγ and Kotγ, respectively.

interaction of the genotype with environment. It has been postulated that α+β gliadins and γ gliadin associate with other proteins through a disulphide interchange mechanism, through hydrophobic and hydrogen bonding (Bietz and Wall, 1980). The addition of various gliadin fractions on dough mixing behavior suggested that γ gliadin had highest positive effect, followed by α and β gliadin (Khatkar et al., 2002). However, the above study was performed with 20 mg (1% w/ w, flour basis) of various gliadins added into 10 gm flour. In our study only 100 mg of flour was taken for analysis, so this suggested that the natural variation due to γ gliadin accumulation, though significant, was too less to create a profound variation in Lv. Hence, the Lv, recorded at both Kota and Pune location did not show significant difference, when subjected to paired t-Test.

Acknowledgement

Elangovan M acknowledges Council of Scientific and Industrial Research (CSIR) for financial support through Senior Research Fellowship. The financial assistance for this project from Department of Biotechnology (DBT), New Delhi is gratefully acknowledged. We thank Dr. Ratan Tiwari, Senior Scientist, DWR and Dr. R.K. Gupta, Senior Scientist, DWR, Karnal for sharing the plant material and Lv data used in this study.

References

Bietz JA, Wall JS (1980) Identity of high molecular weight gliadin and ethanol soluble glutenin subunits of wheat: relationship to gluten structure. Cereal Chemistry 57**:**415–421.

Daniel C, E Triboi (2000) Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition. Journal of cereal sciences 32:45-56

Elangovan M, Rai R, Dholakia BB, Lagu MD, Tiwari R, Gupta RK, Rao VS, Röder MS, Gupta VS (2008) Molecular genetic mapping of quantitative trait loci associated with loaf volume in hexaploid Wheat (*Triticum aestivum* L.) Journal of Cereal Science 47:587-598

Hessler TG, Thomson MJ, Benscher D, Nachit MM, Sorrells ME (2002) Association of a lipoxygenase locus, Lpx-B1, with variation in lipoxygenase activity in durum wheat seeds. Crop Science 42:1695-1700

Khatkar BS, Fido RJ, Tatham AS and Schofield JD (2002) Functional properties of wheat gliadins I. effects on mixing characteristics and bread making quality. Journal of Cereal Science 35:299-306

Payne PI, Holt LM, Lawrence GJ, Law CN (1982) The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Qual. Plant. Foods. Hum. 31:229-241

Pogna PE, Redaelli R, Dachkevitch T, Curoni A, Dal Belin Peruffo A (1994) Genetics of wheat quality and its improvement by conventional and biotechnological breeding. In Bushuk W and Rasper VF (eds) Wheat: production, properties and quality, by Chapman and Hall pp 205-224.

Singh NK, Shepherd KW, Cornish GB (1991) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. Journal of Cereal Science 14:203-208

Triboi E, Abad A, Michelena A, Lloveras J, Ollier JL, Daniel C (2000) Environmental effects on the quality of two wheat genotypes: 1. Quantitative and qualitative variation of storage proteins, European Journal of Agronomy 13:47–64

Uthayakumaran S, Tomoskozi S, Tatham AS, Sewage AWJ, Gianibelli MC, Stoddard FL, Bekes F (2000). Effects of gliadin fractions on properties of wheat dough depending on molecular size and hydro-phobicity. Cereal Chemistry 78:138–141.

Werner WE, Wiktorowicz JE, Kasarda DD, (1994) Wheat varietal identification by capillary electrophoresis of gliadins and high molecular weight glutenin subunits. Wheat Cereal Chemistry 71:397-402.