Indian J. Genet., 72(2): 208-216 (2012)

Distribution of alleles of grain quality genes in Indian bread wheat varieties

Anju M. Singh*, Santosh K. Singh, Arvind K. Ahlawat, Neelu Jain, G. P. Singh, Indu Ravi[1], Mamata Yadav[2] and P. C. Misra[3]

Grain Quality Laboratory, Division of Genetics, IARI, New Delhi 110 012
[1]IGNOU Regional Centre, Jaipur 304 022; [2]NBPGR, New Delhi 110 012
[3]Zonal Agricultural Research Centre, Powarkheda, Hoshangabad 461 110

Abstract

The prevalence of puroindoline hardness mutations (alleles) among Indian wheat varieties, germplasm lines, *Triticum spelta* and synthetic derivative accessions was surveyed in the present study. A total of 356 wheat genotypes were examined. While most *T. spelta* accessions had soft grains, very few among the rest of the lines were soft and possessed wild- type alleles of the two puroindoline genes. The majority were, however, uniformly hard. The null mutation of *Pina* and the wild type *Pinb* allele (*Pina-D1b/Pinb-D1a*) was the most frequent combination in the Indian wheat germplasm. Out of the 356 lines examined, only 42 possessed the *Pinb-D1b* allele indicating gly46serine mutation in *Pinb.* One landrace MPG33 possessed the *Pinb-D1e* allele as found by sequencing the 447 bp product obtained with *Pinb-D1* primer in this line. Distribution of the alleles of the serpin (*Srp5B*) gene was also studied in 90 Indian wheat cultivars released during 1985-2010. Only 11 of the 90 genotypes possessed the *b-type* allele indicating the prevalence of the desirable *a-type* allele in the Indian wheat varieties. The role of the alleles of puroindoline and serpins genes in end-use quality of wheat is discussed.

Key words: PINA, PINB, kernel texture, *PinaD1*, *PinbD1*, serpins, *Srp5B,* flour yield

Introduction

Cultivated hexaploid or 'bread' wheat (*Triticum aestivum* L., genome *AABBDD*, 2n=6x= 42) can be classified as soft or hard wheat depending on the force required to crush its grains. Soft wheat has soft endosperm texture and requires less energy for milling. Hard wheats produce coarser textured flours and require more grinding energy so that fracture planes produce broken starch granules and hence higher levels of starch damage. Thus, kernel texture is a primary determinant of technological and processing properties of wheat and the assessment of the grain endosperm texture is necessary in characterizing the end-use quality of wheat [1].

Kernel texture has long been associated with a group of ~13 kDa proteins termed 'friabilin' due to their abundant association with water washed starch from soft friable grains, scarce in hard wheat and complete absence in durum [2]. The electrophoretic separation and amino acid sequencing of friabilin resolved two major polypeptides, Puroindoline a (*PINA*) and Puroindoline b (*PINB*), and a third minor but related polypeptide, Grain Softness Protein (GSP-1) [3]. Kernel texture in wheat is a highly heritable trait, with the two major texture classes controlled by the Hardness (*Ha*) locus on the short arm of chromosome 5D [4]. This locus contains the genes *Puroindoline a* and *Puroindoline b* (*Pina-D1* and *Pinb-D1*) that encode the main components of friabilin, *PINA* and *PINB*, mentioned above, and the gene *Grain Softness Protein-1* (*Gsp-1*), encoding GSP-1 [3]. In hexaploid wheat, the tight linkage among these three genes has not been broken, such that the *Ha* locus haplotype (*Pina-D1, Pinb-D1* and *Gsp-D1*) is associated with soft kernel texture phenotype [5]. Both *Pin* genes have been deleted from chromosomes 5A and 5B during the evolution of tetraploid wheat (the contributor of A and B genomes of common wheat) that resulted in the loss of the softness-conferring PIN proteins in durum wheat; consequently durum has very hard kernel texture. In hexaploid wheat, both the *Pina* and *Pinb* genes are required to be in their wild state for grain softness. Mutation in either of the genes results

*Corresponding author's e-mail: anju_mahendru@yahoo.co.in
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

into a hard phenotype. A number of puroindoline alleles have been documented variation in these alleles has been associated with differences in wheat grain quality. Recently, more variants of puroindoline b at other loci have been reported and their association with grain traits and yield components has been investigated [6]. It is, therefore, pertinent for wheat breeding programmes to identify alleles of these genes present in elite germplasm since different hard genotypes may confer differences in functional quality and yield [6-8].

Another group of proteins called as serpins (serine proteinase inhibitors) have assumed importance due to reports of their influence on wheat grain quality. Serpins are potent irreversible inhibitors of serine proteinase of the chymotrypsin family, they control and regulate proteolysis which is important for plant growth, development, stress response and defense against insects and pathogens. Serpins have been found in diverse species of the plant kingdom [9], in cereals, serpins are collectively called Z proteins, are salt soluble [10] and constitute ~5% of the total albumin in grains [11]. Wheat serpins are unable to inhibit endogenous grain proteinases but may function to protect the prolamines from the digestive proteinases of insects [12, 13]. Serpins are suggested to influence the grain quality traits because they are concentrated in the endosperm protein of aqueous phase of bread dough [14]. In addition, they have two other properties that may influence grain quality traits: amino acid sequence motifs in their structure which resemble the Gln-rich repeat sequences found in the prolamin storage proteins gene in the endosperm, and the ability to form complexes by intermolecular disulfide bridges between serpins and β-amylase protein molecules found in cereal endosperm [9]. Thus, serpins may have pleiotropic functions in wheat. Gene coding for the serpin protein are present on the long arm of the chromosome 5B and termed as *Srp5B*. Cane *et al*. [15] identified two alleles of the serpin gene, the wild type *a* allele (*Srp5Ba*), and a null type *b* (*Srp5B*) produced by the early termination of the product due to a single nucleotide polymorphism (SNP) at position 340. The null allele of serpin gene was found o be frequently present in Australain wheats and affects the milling yield adversely by reducing ~0.4 g of flour per 100 g of grain [15]. The reduction appears small but the margins in milling industry are narrow. Considering the large quantities (~15 MT) of wheat processed in our own country, any reduction in flour recovery assumes huge importance for millers. Therefore, it is important to find out the distribution of alleles of *Srp5B* gene in Indian wheats and determine the adverse effects, if any of the alleles of this gene in our germplasm. In the present study, we report the distribution of puroindoline alleles in 356 wheat germplasm lines and the distribution of serpin alleles in ninety Indian bread wheat cultivarss.

Materials and methods

Germplasm and grain hardness

The material consisted of 356 wheat lines including 166 varieties released in India between 1985-2010, 117 indigenous germplasm (including high protein advanced lines, elite lines, germplasm lines from Madhya Pradesh and IC numbers from National Bureau of Plant Genetic Resources, New Delhi) and 41 exotic germplasm (including Australian cultivarss, three durum lines, elite lines from CIMMYT and EC numbers obtained from NBPGR). In addition, 25 *T. spelta* accessions and 8 synthetic derivatives obtained from CIMMYT, Mexico were also included in the study.

Ninety cultivars released during 1965-2010 were screened for serpin alleles. Australian cultivars EGA Jitaring and Gladius, with published *Srp5B* profile [15] were used as positive controls for serpin alleles *a* and *b* respectively along with molecular weight standard DNA ladder.

Materials were grown in *rabi* season in 2009-10 and 2010-11 at IARI, New Delhi farm and *T. spelta* accessions in nethouse with recommended additional photoperiod.

Measurement of kernel hardness

Grain hardness was determined in each genotype on an approximately 300-kernel sample of each season harvest by the Perten Single Kernel Characterisation System (SKCS) 4100, following the manufacture's operational procedure. Damaged kernels were removed prior to hardness measurement. Average, standard deviation, and distribution of SKCS hardness data were automatically obtained from the measurements. The instrument gives Hardness Index (HI) as a numerical value. The higher the value, the greater is the hardness. According to manufacturer, score below 35 denotes a soft grain while a score above 75 is considered to denote a hard grain. However, classification as per [16] consisting of four classes given below was followed.

Classification of kernel hardness index obtained through SKCS

Type	*As per* Morris *et al.* [16]
Hard	≥ 60
Med Hard	34-46
Med Soft	47-59
Soft	≤ 33

DNA extraction

Total genomic DNA was extracted from 12-days young leaves of wheat germplasm, by the cetyl trimethyl ammonium bromide (CTAB) method [17]. The resulting DNA was spooled out, washed twice with 70% ethanol, dissolved in TE (10mM Tris, 0.1mM EDTA, pH 8.0) containing 25 mg/ml RNase-A, incubated at 37^{o}C for 30 min, and extracted with chloroform: iso-amyl alcohol (24: 1 v/v). DNA was re-precipitated, dissolved in TE buffer, and checked for its quality and quantity by 1% agarose gel electrophoresis using a standard containing 100 ng/mL genomic λDNA.

Detection of puroindoline alleles

Pina was amplified with the forward primer 5'ATGAAGGCCCTCTTCCTCA3' and the reverse primer 5'TCACCAGTAATAGCCAATAGTG3' [18] yielding an expected PCR product of 441bp. *Pinb* was amplified with the forward primer 5'ATGAAGACCTT ATTCCTCCTA3' and the reverse primer 5'TCACCAGTAATAGCCACTAGGGAA3' [18] that amplified a PCR product of 447bp. The lack of amplification by *Pina* specific primer from a sample and a simultaneous amplification of *Pinb* was taken as an indication of the *Pina*-null mutation (*Pina-D1b*). Where, the grain phenotype was found hard *i.e.* with a HI above 60 and both the specific primers (for *Pina* and *Pinb* genes) gave amplification product, allele- specific primers for *PinbD1a* with glycine specific forward primer 5'ATGAAGGCCCTCTTCCTCA3' and reverse primer 5'CTCATGCTCACAGCCGCC3' [19] and for the detection of *Pinb-D1b* with serine specific forward primer 5'ATGAAGGCCCTCTTCCTCA-3'and reverse primer 5' CTCATGCTCACAGCCGCT3' [19] was used. Where these primers failed to give amplification, information on other alleles was obtained by DNA sequencing of 447bp products from such lines.

For PCR amplifications, MyCyclerTM Thermal Cycler (Bio-RAD) was used. For kernel hardness alleles, PCR reaction was performed in 25 µl volumes containing 10 pmols of each primer, 250 µM of each of dNTPs,1X PCR buffer, 1.5mM of $MgCl_2$, 0.5 unit of Taq DNA polymerase (MBI Fermentas, Germany), and 100ng of genomic DNA. The samples, denatured at 95°C, 1 min annealing at 60°C, and 1 min elongation at 72°C, with a final extension of 8 min at 72°C at the end. Amplification products were resolved on 2% Agarose gel using 1xTBE buffer, stained with Ethidium bromide and visualized on Gel Documentation System (G-Box, SYNGENE Synoptics, USA) under UV trans illumination. Amplification products were scored in relation to molecular weight standard 100bp DNA ladder Plus (MBI Fermentas) and to positive controls.

Detection of serpin gene alleles

The gene specific primers for the analysis of serpin alleles were synthesized by Sigma-Aldrich based on sequences obtained from Dr Karen Cane, Australia. CAP-PCR amplifications were carried out following protocol [15] with minor modifications. PCR reactions were performed on MyCyclerTM Thermal Cycler (Bio-RAD) using forward and reverse primers in a single 15 µl reaction containing 40 ng of each primer with a PCR profile of 94^{o}C for 2 min followed by 35 cycles of 94^{o}C for 30 s, 60^{o}C for 30 s, 72^{o}C for 1min.

Amplification products were resolved on 2% Agarose gel using 1xTBE buffer, stained with Ethidium bromide and visualized on Gel Documentation System (G-Box, SYNGENE Synoptics, USA) under UV trans illumination. Amplification products were scored in relation to molecular weight standard 100 bp DNA ladder Plus (MBI Fermentas) for presence of 730 bp bands. Further 5 µl amplified product was restricted using enzyme *Hind III* (MBI Fermentas) for 45 minutes at 37^{o}C. After restriction, amplified products were separated on 2% of Agarose gel which produced two different alleles: the wild type *a*-allele identified by presence of a pair of bands of 430 bp and 300 bp and the null type *b*- allele identified by presence of a pair of bands of 510 bp and 220bp.

Results and discussion

The range of kernel hardness score (HI) in different categories of germplasm is depicted in Table 1. Majority of the Indian wheat cultivars had the hardness index ≥ 60, one (Safed Lerma) had HI of 27 and three cultivars (Lerma rojo, HDK10 and HB208) scored HI between 34-46. Only one variety (Choti Lerma) fell in medium hard category with HI of 48. Among the indigenous germplasm, only 4% of the genotypes represented the

Table 1. Distribution of kernel texture among the varied germplasm screened

Hardness index	Indian cultivars	Indigenous collections	Exotics	Australian cultivars	*T. spelta* and synthetic derivatives
≤33	2	5	1	2	10
34-46	3	4	0	0	13
47-64	1	2	1	0	5
≥65	160	106	29 (3 durums)	8	5
Total	166	117	31	10	33

first three categories and the rest fell in Hard texture class. Among the exotics, only one (Ist SAMNYT 406) was in medium soft class while there were two (Longreach Orion and Drysdale) in soft class among the ten Australian cultivars. The hard textured phenotype was observed in rest of the lines. In contrast, one of the *T. spelta* accessions had HI= 33 (PI 348597) while majority (20) of *T spelta* and synthetic derivatieves had HI between 34-46. Six synthetic derivatives had HI between 47-59 while six fell in the hard calss. The amplicons of *Pinb*-D1with allele-specific primers as visualized on agarose gel are shown in Fig. 1 and 2 respectively. Screening of the 356 wheat lines for puroindoline genes using gene specific primers showed (Table 2) that 26 of the 33 Speltas and synthetic derivatives had amplicons of expected sizes with both the primers and had HI below 60 (*i.e.* soft grains and *Pina-D1a/Pinb-D1a*). This combination was observed only in two Indian cultivars, 5 indigenous lines, one exotic and two Australian cultivars. In rest of the genotypes (except Australian cultivars), null allele (*Pina-D1b*) of the puroindoline gene *Pina* and the wild type allele of the other gene *Pinb* (i.e.*Pinb-D1a*) was the most predominant. This combination (*Pina-D1b/Pinb-D1a*) was observed in 285 out of 305 cases and they were phenotypically hard textured (HI above 60). The Australian cultivars, however, showed a reverse trend. Out of the ten cultivars studied, only one hard textured line had this combination. Six genotypes among the Indian cultivars, 27 of the indigenous germplasm, four of the exotic lines and six Australian cultivars produced amplicons of the sizes 441bp and 447 bp with the *Pina* and *Pinb* primers respectively alongwith HI = 60. These 43 lines were further screened with glycine and the serine specific primers (that could detect *Pinb-D1b* allele). Except one line (MPG 33), all 42 failed to show amplification with glycine specific primer and produced ~ 250 bp product with serine specific primer, thus, a *Pina-D1a/Pinb-D1b* combination was present in these lines except in MPG 33.

In MPG 33 (germplasm collection from Madhya Pradesh, India), with HI of 94±9.56, the 447bp product was sequenced and compared with the wild type sequence [18]. A single base substitution from G to A giving the codon TGA (stop codon) instead of the normal TGG (tryptophan) at the 39th position (shown in bold letters in the sequence shown below) was found representing the allele *Pinb-D1e.* This mutation was first discovered in 2004 in *Aegilops tauschii* Coss [21]. Thus, 42 of the 356 lines studied had the *Pinb-D1b* allele while only one line had the *Pinb-D1e* allele.

GAGCACAACCTTCGCGCAATACTCAGAAGT
TGGCGGCTGGTACAATGAAGTTGGCGGAGG
AGGTGGTTCTCAACAATGTCCGCAGGAGCGGCCG
AAGCTAAGCTCTTGCAAGGATTACGTGA
TGGAGCGA TGTTTCACAATGAAGGATTTTCCAG
TCACC/**TGA/**CCC/ACA/AAA/TGG/TGG/AAG/
GGCGGCTGTGAGCATGAGG TTCGGGAGAAGTGC
TGCAAGCAGCTGAGCCAGATAGCACCACAATGT
CGCTGTGATTCTATCCGGCGAGTGATCCA
AGGCAGGCTCGGTGGCTTCTTGGGCA
TTTGGCGAGGTGAGGTATTCAAACAACTTCAG
AGGGCCCAGAGCCTCCCCTCAAAGTGCA
ACATGGGCGCCGACTGCAAGTTCCCTAGTGGCTA
TTACTGGTGAA

Table 2. Distribution of the alleles of puroindoline genes among the germplasm lines

Germplasm group	HI range	*Pina-D1b/ Pinb-D1a*	*Pina-D1a/ Pinb-D1a*	*Pina-D1a/ Pinb-D1b*	*Pina-D1b/ Pinb-D1b*	Total
Indian	27-108	158	2	6	0	166
Indigenous	28-108	85	5	26	0	116
Exotics	23-116	23	1	4	3(durums)	31
Australian	23-86	2	2	6	0	10
T. spelta & Synthetic derivatives	33-105	7	26	0	0	33

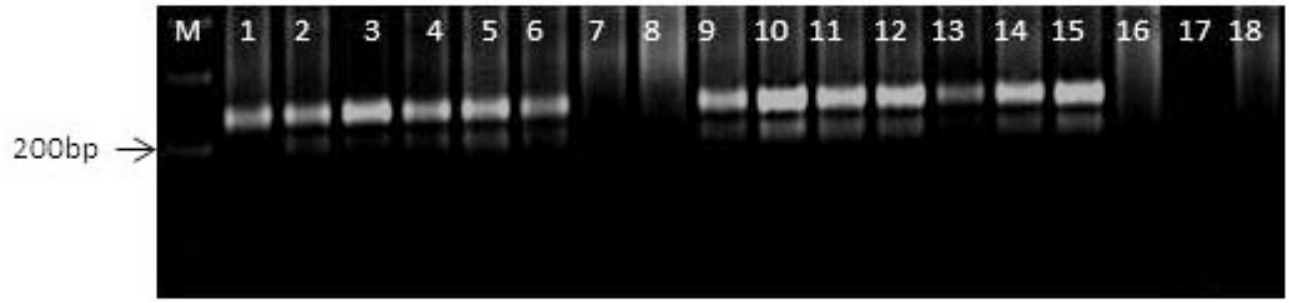

Fig. 1. Amplification with glycine specific primer to detect *Pinb-D1a* allele. Wheat lines in the lanes 7-8 and 16-18 failed to show amplification indicating *P1Nb* mutation in these lines.

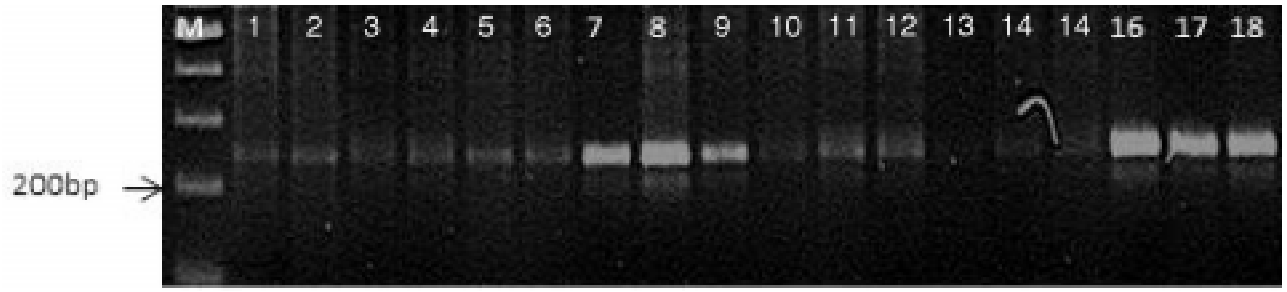

Fig. 2. Amplification with cerine specific primer to detect *Pinb-D1b* allele. Same wheat lines as in Fig. 1. in the lanes 7-8 and 16-18 show amplification here indicating the presence of *Pinb-D1b* in them. Lanes : M: 100bp DNA ladder, 1: Drysdale, 2: HD 2285, 3: PBW343, 4: HI1077, 5: PBW175, 6: Raj3077; 7: Sunnlin, 8: Yitpi, 9: Raj3765, 10: WH542, 11: UP2525, 12: PBW502, 13: WR544, 14: HD2851, 15: HD2643, 16: Baxter, 17: Wyalkatchem and 18: Janz

Thus, majority of Indian wheat genotypes in the present study were shown to have the null allele of *Pina* (*Pina-D1b*) and the wild type allele of *Pinb (Pinb-D1a)*. These two mutations are the most predominant mutations occurring in the global hexaloid wheat germplsm [5]. *Pina-D1b* was the first mutation to be reported in cultivars 'Falcon'. This allele has been reported widely in landraces and cultivars of common wheat of diverse origins. SNPs and INDELs in this gene are infrequently encountered. The reverse is true of *Pinb* gene. A much higher number of SNPs, INDELS, duplications etc. than the null mutation in global germplasm are noted in *Pinb*. The SNP within *Pinb-D1* leading to Gly46Ser change (P*inb-D1b* allele) was the first mutation associated with hard texture in common wheat [19]. This mutation has now been reported in several studies worldwide [5]. Majority of hard wheats of Australia carry the Gly46Ser mutation in the *Pinb* gene with a wild type *Pina* gene (Howard Eagles per. commun.) and also observed in the present study. The *Pinb-D1b* allele confers slightly softer kernel texture than *Pina-D1b* but is reported to give higher flour yield compared with the *Pina-D1b* allele [7, 16]. Since majority of Indian cultivars have the *Pina-D1b* allele in them, there is scope to improve the flour yields further in Indian wheat cultivars by breeding for *Pinb-D1b* types.

Flour yield is a complex trait and in addition to the grain hardness, it is affected by a number of other factors such as the grain and test weight, environmental conditions at the time of grain filling and maturity as well as the genotypic effects. Recently, a group of proteins called serpins controlled by the *Srp5B* locus have been reported to exert significant influence on flour yields of Australian wheat [15]. The *Srp5B*-b is a null allele and reduced the flour yields significantly in Australian wheats [14]. In our study, ninety Indian wheat cultivars were screened for *Srp5B* alleles. The distribution of the wild type (*a*) and the null type allele (*b*) in Indian wheat cultivars in different zones is depicted in Tables 3 and 4. The CAP-PCR based amplified products as visualised on the agarose gel are shown in

Table 3. Distribution of the alleles of the serpin gene (*Srp5B*) in the Indian wheat *cvs* screened in the present study

Zone	*Srp5B*		Total no. of cultivars
	a-type	*b-type*	
North Western Plains Zone (NWPZ)	21	6	27
North Eastern Plains Zone (NEPZ)	23	1	24
Northern Hills Zone (NHZ)	11	3	14
Central Zone (CZ)	12	0	12
Penninsular Zone (PZ)	9	1	10
Southern Hills Zone (SHZ)	3	0	3
Total cultivars	79	11	90

Fig. 3. About 90% of the Indian wheat cultivars studied showed the desirable (*a*) allele of the serpin gene. Thus, only a few varieties possess the null type allele (Table 4). Also, the expression levels of serpin gene may differ in genotypes and thus exert their influence by differences in amounts of serpins in the grain. In a differential expressed unigene analysis, higher levels of expression of serpins and b-purothionin precursor were observed in hard wheats than in soft wheat lines [22]. Flour recovery is also known to be higher in hard wheats than in soft wheats.

Considerable variation in the flour yields in different varieties within and between zones, irrigation conditions and over the years has been observed in India. Mean values of the flour yields and grain yields recorded at Directorate of Wheat Research [23, 24] in a few prominent varieties during the years 2005-2008 is shown in Table 5 and in two or more sowing conditions, in Table 6. There were significant interactions between irrigation condition and year and variety and year for flour yield in addition to genotypic effects on flour yield. Thus, in Indian wheat varieties where type-*a* allele is frequently present, differences in the level of expression of the allele, varietal grain character and irrigation conditions may play a more significant role in determination of the flour yield than the mere presence of the serpin allele. Therefore, serpins need to be investigated thoroughly to understand their role in quality and in defence mechanisms in wheat and other cereals.

Table 4. Allelic composition of Indian wheat varieties for serpin gene (*Srp5B*). All these varieties have the *Pina-D1b/Pinb-D1a* combination of puroindoline genes

Cultivar	*Srp5B*	Cultivar	*Srp5B*	Cultivar	*Srp5B*
NWPZ					
HD-2285	*a*	PBW-65	***b***	UP-2425	***b***
DL-153-2	*a*	PBW-226	*a*	PBW-502	*a*
HD-2270	*a*	PBW-175	***b***	WR-544	*a*
HD-2687	*a*	RAJ-3077	*a*	HD-2851	*a*
RAJ-1972	***b***	RAJ-3765	*a*	KRL-1-4	*a*
WH-291	***b***	PBW-343	*a*	WH-157	*a*
RAJ-2184	***b***	UP-2338	*a*	KRL-19	*a*
PBW-154	*a*	WH-542	*a*	DBW-16	*a*
WH-416	*a*	PBW-396	*a*	PBW- 550	*a*
NEPZ					
HDR-77	***b***	DL-788-2	*a*	K-9107	*a*
HP-1633	*a*	HD-2733	*a*	NW-1012	*a*
DL-784-3	*a*	HW-2045	*a*	K-9465	*a*
CPAN-3004	*a*	HUW-234	*a*	HUW-468	*a*
HP-1731	*a*	HUW-213	*a*	PBW- 443	*a*
HD-2643	*a*	K-9006	*a*	NW-2036	*a*
HP-1744	*a*	K-8962	*a*	DBW-14	*a*
HP-1761	*a*	K-8804	*a*	JOB-666	*a*
NHZ					
CPAN-1796	*a*	HS-365	***b***	VL-738	*a*
HS-207	*a*	HS-420	*a*	VL-804	***b***
HS-240	*a*	UP-1109	*a*	VL - 832	*a*
HS-295	*a*	VL-616	***b***	VL - 829	*a*
HS-277	*a*	HPW-42	*a*		
CZ					
HI-1077	*a*	J-405	*a*	MP-4010	*a*
DL-803-3	*a*	GW-190	*a*	GW-273	*a*
HW-2004	*a*	GW-173	*a*	GW-366	*a*
HI-1500	*a*	GW-322	*a*	HD-2987	*a*
PZ					
HD-2501	*a*	DWR-162	*a*	K-9644	*a*
HD-2380	***b***	DWR-195	*a*	RAJ-4037	*a*
MACS-2496	*a*	NIAW-34	*a*		
HD-2781	*a*	HUW-510	*a*		
SHZ					
HW-1085	*a*	HW-2044	*a*	HUW-318	*a*

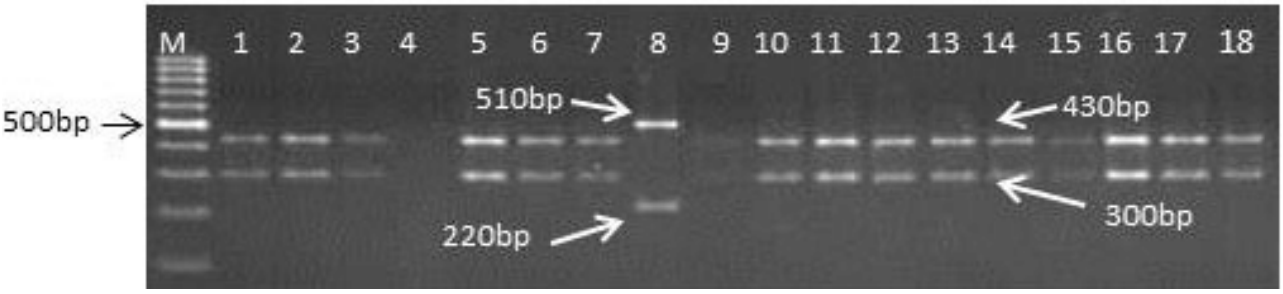

Fig. 3. Amplification of *Srp5B* alleles in Indian wheat varieties : a-type with 430bp and 300bp bands and b-type with 510bp and 220bp bands respectively. Lines : M: 100 bp DNA ladder, 1: HD2285, 2: HD2270, 3: HD2687, 4: PBW343, 5: WH542, 6: HD2643, 7: HUW234, 8: PBW175, 9: K9107, 10: HUW468, 11: VL738, 12: HW2004, 13: HI1500, 14: GW322, 15: DWR195, 16: HD2781, 17: HW1085, 18: HW2044

Table 5. Flour recovery of popular wheat varieties during the years 2005-06, 2006-07 and 2007-08

Cultivar	Zone	Year of release	*Srp5B*	TGW (g)	HI	Average yield(t/ha)	Flour recovery %
HD-2687	NWPZ	1999	*a*	35.21	85.9	4.94	66.56
PBW 175	NWPZ	1989	***b***	45.48	78.8	2.80	68.95
RAJ 3765	NWPZ	1996	*a*	38.35	80.7	3.65	69.36
WH 542	NWPZ	1992	*a*	34.99	102.4	4.8	66
PBW 396	NWPZ	2000	*a*	38.12	82.4	3.70	66.96
UP 2425	NWPZ	1999	***b***	45.32	62.7	3.88	68.2
DL-788-2	NEPZ	1997	*a*	42.11	70.7	3.93	70.46
HD 2733	NEPZ	2001	*a*	42.47	79.5	4.81	70.53
HW 2045	NEPZ	2002	*a*	38.97	78.4	3.75	69.3
HUW 234	NEPZ	1985	*a*	38.69	71.9	3.34	70.43
K 9107	NEPZ	1996	*a*	42.45	74.0	4.02	70.83
HUW 468	NEPZ	1999	*a*	37.7	63.9	4.28	71.95
NW 2036	NEPZ	2002	*a*	37.38	77.0	3.87	69.26
DBW 14	NEPZ	2003	*a*	40.45	64.8	3.68	70.16
HS 295	NHZ	1992	*a*	38.05	72.8	2.56	64.9
HS 277	NHZ	1993	*a*	33.72	81.4	2.88	66.03
HS 420	NHZ	2003	*a*	33.87	77.3	2.5	65.23
VL 616	NHZ	1986	***b***	39.32	81.3	2.71	66.13
VL 829	NHZ	2003	*a*	39.75	81.6	2.90	64.73
HI 1500	CZ	2003	*a*	45	81.9	1.81	66.5
GW 173	CZ	1994	*a*	37.97	77.6	4.1	70.23
GW 322	CZ	2002	*a*	38.63	73.5	4.47	68.8
MP 4010	CZ	2003	*a*	41.0	78.0	3.66	70.63
HD 2781	PZ	2002	*a*	44.1	81.8	1.58	70.03
NIAW 34	PZ	1997	*a*	38.67	71.3	3.06	69.93
RAJ 4037	PZ	2003	*a*	38.68	79.4	4.09	70.66

Source: DWR Progress Report. Vol 4.: Wheat Quality, AICW & BIP

Table 6. Flour recovery of some Indian wheat varieties grown in two or more sowing conditionsduring three years

Variety	Zone and sowing conditions	Year of release	*Srp5B* allele	Flour recovery(%)			
				2005-06	2006-07	2007-08	Mean
HS 240	NHZ (I/TS,HF)	1988	*a*	64.1	63.7	63.9	63.9
	NHZ (RF/TS,LF			64.9	64.6	64.6	64.7
HW 2004	CZ, Res Irig, TS	1995	*a*	65.8	66.2	70.3	67.43
	CZ, RF, TS			66.7	67.1	70.6	68.13
	PZ, Res Irig / TS			68.6	68.3	71.3	69.4
PBW343	NWPZ, I/TS	1996	*a*	66.2	66.9	68.5	67.2
	NEPZ, I/TS			68.8	69.4	68.5	68.9
VL 738	NHZ, I/TS, HF	1997	*a*	66	65.1	65.2	65.43
	RF, TS, LF			65.1	65.2	65.4	65.23
VL 804	NHZ, I/TS	2002	*b*	63.2	65.6	65.1	64.63
	RF,TS, LF			64.8	63.6	65.9	64.76

I= Irrigated, Res Irig= Restricted irrigation, RF= Rainfed; TS= Timely sown, LS= Late sown; HF= High fertility, LF= Low fertility

Acknowledgements

The authors acknowledge the grant-in-aid from ICAR for the project CIM/2010/14 under which this work was carried out. The indigenous and exotic collections (IC and EC numbers) provided by National Bureau of Plant Genetic Resources is also greatfully acknowledged. We thank Karen Cane, DPI, Horsham, Australia and Howard Eagles, University of Adelaide, Australia for providing the primer sequence, protocol for amplification and detection of serpin alleles, and vibrant discussions.

References

1. **Morris C. F. and Rose S. P.** 1996. Chapter 1. Wheat. p. 3-54. *In* R.J. Henry and P.S. Kettlewell (Eds.). Cereal Grain Quality. Chapman Hall, London.
2. **Jolly C. J., Rahman S., Kortt A. A.** 1993. Characterisation of the wheat Mr 15,000 'grain-softness protein' and analysis of the relationship between its accumulation and the whole seed and grain softness. Theor. Appl. Genet., **86**: 589-597.
3. **Morris C. F.** 2002. Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol. Biol., **48**: 633-647.
4. **Mattern P. J., Morris R., Schmidt J. W. and Johnson V. A.** 1973. Location of genes for kernel properties in wheat variety 'Cheyenne' using chromosome substitution lines. *In*: Sear, E.R., Sears, L.M.S. (Eds.), Proceedings of Fourth International Wheat Genetics Symposium. University of Missouri, Columbia, pp.703-707.
5. **Bhave M. and Morris C. F.** 2008. Molecular genetics of puroindolines and related genes: allelic diversity in wheat and other grasses. Plant Mol. Biol., **66**: 205-219.
6. **Chen F., Zhang F., Cheng X., Morris C., Xu X., Dong Z., Zhan J. and Cui D.** 2010. Association of Puroindoline b-B2 variants with grain traits, yield components and flag leaf size in bread wheat (*Triticum aestivum* L.) varieties of the Yellow and Huai Valleys of China. J. Cereal Sci., **52**: 247-253.
7. **Martin J. M., Frohberg R. C., Morris C. F.** 2001. Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. Crop Sci., **41**: 228-234.
8. **Eagles H. A., Cane K. and Eastwood R. F.** 2006. Contributions of glutenin and puroindoline genes to grain quality traits in southern Australian wheat breeding programs. Aus. J. Agric. Res., **57**: 179-186.
9. **Roberts T. H. and Hejgaard J.** 2008. Serpins in plants and green algae. Funct. Integr. Genomics, **8**: 1-2.
10. **Rasmussen S. K.** 1993. A gene coding for a new plant serpin. Biochim. Biophys. Acta, **172**: 151-154.
11. **Hejgaard J.** 1982. Purification and properties of protein Z- a major albumin of barley endosperm. Physiol. Plant., **54**: 174-182.
12. **Rasmussen S. K., Klausen J., Hejgaard J., Svensson B. and Svendsen I.** 1996. Primary structure of the plant serpin BSZ7 having the capacity of chymotrypsin inhibition. Biochim. Biophys. Acta, **1297**: 127-130.
13. **Oéstergaard H., Rasmussen S. K., Roberts T. H. and Hejgaard J.** 2000. Inhibitory serpins from wheat grain with reactive centers resembling glutamine-rich repeats of prolamin storage proteins. Cloning and

characterization of five molecular forms. J. Biol. Chem., **275**: 33272-33279.

14. **Salt L. J., Robertson J. A., Jenkins J. A., Mulholland F. and Mills E. N. C.** 2005. The identification of foam-forming soluble proteins from wheat (*Triticum aestivum*) dough. Proteomics, **5**: 1612-1623.
15. **Cane K., Sharp P. J., Eagles H. A., Eastwood R. F., Hollamby G. J., Kuchel H., Lu M. and Martin P. J.** 2008.The effect on grain quality traits of a grain serpin protein and the VPM1 segment in southern Australian wheat breeding. Aust. J. Agric. Res., **59**: 883-890.
16. **Morris C. F., Lillemo M., Simeone M. C., Giroux M. J., Babb S. L. and Kidwell K. K.** 2001. Prevalence of puroindolinegrain hardness genotypes among historically significant North American spring and winter wheats. Crop Sci., **41**: 218-228.
17. **Murray M. G. and Thompson W. F.** 1980. The isolation of high molecular weight plant DNA. Nucleic Acids Res., **8**: 4321-4325.
18. **Gautier M. F., Aleman M. E., Guirao A., Marion D. and Joudrier P.** 1994. *Triticum aestivum* puroindolines, two basic cystine-rich seedproteins: cDNA sequence analysis and developmental gene expression. Plant Mol. Biol., **25**: 43-57.
19. **Giroux M. J. and Morris C. F.** 1997. A glycine to serine change in puroindoline-b is associated with wheat grain hardness and low levels of starch-surface friabilin. Theor. Appl. Genet., **95**: 857-864.
20. **Giroux M. J., Talbert L. and Habernicht D. K.** 2000.Association of puroindoline sequence type and grain hardness in hard red spring wheat. Crop Sci., **30**: 370-374.
21. **Massa A. N., Morris C. F. and Gill B. S.** 2004. Sequence diversity of puoindoline-a, puoindoline-b and the grain softness protein genes in *Aegilops tauschii* Coss. Crop Sci., **44**: 1808-1816.
22. **Eujayl I. and Morris C.** 2009. Identification of differentially expressed Unigenes in developing wheat seed using Digital Differential Display. J Cereal Sci., **49**: 316-318.
23. **Gupta R. K., Mohan D., Ram S. and Mishra B.** 2006, 2007 and 2008. (Eds). DWR Progress Report. Vol **4**. Wheat Quality, AICW & BIP.
24. **Kundu S., Shoran J., Mishra B. and Gupta R. K.** 2006. Indian wheat varieties at a glance. Directorate of wheat research, Karnal-132001, India. Research Bulletin No. **21**: p. 447.