Australian Journal of Crop Science AJCS
AJCS 7(4):469-483 (2013) ISSN:1835-2707

Biotechnological approaches for grain quality improvement in wheat: Present status and future possibilities

Umesh Goutam[1,2,3]*, Sarvjeet Kukreja[1], Ratan Tiwari[2], Ashok Chaudhury[3], R. K. Gupta[2], B.B. Dholakia[4] and Rakesh Yadav[3]

[1]**Lovely Professional University, Phagwara-144411, Punjab, India**
[2]**Directorate of Wheat Research, Karnal-132 001, Haryana, India**
[3]**Department of Bio & Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, India**
[4]**Division of Biochemical Sciences, National Chemical Laboratory, Pune- 411008, India**

***Corresponding author: umeshbiotech@gmail.com**

Abstract

Among cereals, wheat is a unique gift of nature to the mankind. Once wheat grain is converted into dough, it can be moulded into innumerable products of the choice, depending upon its suitability for making various end products. The criteria of assessing wheat grain quality traits are as varied as their different uses. Moreover, these quality traits and their parameters are very complex, often polygenic and still not well defined. However, the effects of some major genes for grain hardness, storage-protein encoding genes etc. have been well established. With the advent of molecular biology, it has become possible to develop DNA-based markers for traits of interest. These markers are now gaining the attention of the breeders, since PCR-based functional markers developed from gene sequences provide accurate and high throughput data for determination of allelic compositions in breeding materials. Thus, DNA markers complement conventional methods of breeding for developing novel cultivars with desirable attributes in less time. The present review describes the recent advancement in molecular markers and their utilization in breeding programs especially to improve traits relating to wheat grain.

Keywords: Grain quality, Molecular markers, Marker assisted selection, Wheat.

Introduction

Wheat is an important cereal crop which is cultivated worldwide and was one of the first crops to be domesticated some 10000 years ago. Unlike rice and maize, which prefer tropical environment, wheat is extensively grown in temperate regions occupying 17% of all crop acreage worldwide. It is the staple food for 40% of the world's population (Goyal et al., 2010; Peng et al., 2011). Wheat is the second only to rice which provides 21% of the total food calories and 20% of the protein for more than 4.5 billion people in 94 developing countries (Braun et al., 2010). Global wheat grain production must increase 2% annually to meet the requirement of consistently increasing world population (around 9 billion) till 2050 (Rosegrant and Agcaoili, 2010). World wheat production is based almost entirely on two modern species: common or hexaploid bread wheat (*Triticum aestivum*, 2n=6x=42, AABBDD) and durum or tetraploid wheat (*T. turgidum* subsp. durum, 2n=4x=28, AABB). These wheat cultivars are the result of extensive selection by breeders to meet both quality and agronomic requirements for the wide range of end products such as traditional pan, sponge and dough pan, flat or steamed breads, chapatti, frozen doughs, yellow alkaline or Udon style noodles, confectionary, biscuits, cakes and high-quality pastas (Gale, 2005). Today, the most challenging task for wheat breeders is not only to increase grain yield (Duveiller et al., 2007) but also to improve the grain quality for end products to meet the requirement of ever increasing population. Thus wheat quality becomes a major target in wheat breeding program for the world wheat trade (Gross et al., 2007). Quality refers to the desirability of the product and may include various physical and chemical aspects depending on the intended purpose. The factors that influence the wheat grain quality have been broadly classified in two groups- physical and chemical characteristics. The physical characters include grain appearance score, kernel or grain hardness, vitreousness of kernel, 1000-kernel weight, hectoliter weight (test weight), kernel size and shape whereas the chemical characters are protein content, protein quality and sedimentation test. Several other tests such as farinograph (Md Zaidul et al., 2004), extensograph (Abbasi et al., 2012), mixograph (Martinant et al., 1998) and alveograph (Codină et al., 2012) can estimate the dough mixing or visco-elastic properties.

Approaches for enhancing grain quality parameters

Breeding for quality

Conventional plant breeding usually involves production of variability by making sexual crosses among selected genotypes often having contrasting characters to be combined to develop population of plants with superior quality traits. Using conventional plant breeding methodologies, breeders have developed some of the varieties having good quality

characters in last few years and there has been a continued increase in productivity of wheat. However, many breeding constraints are involved in genetic improvement of wheat quality (Kadar et al., 1999). Schmidt et al. (1974) observed that environment influences are expressed in the loaf volume. It is a powerful constraint for improvement of wheat quality by breeding. Johnson et al. (1985) improved wheat protein in lines produced from cross between Atlas 66 and Naphal. Due to transgressive segregants, these lines produced grain with higher protein content than parent cultivars. Agricultural research station, Turda has released few high quality lines and three quality improved winter wheat varieties viz., Apullum, Turda 1995 and Turda 2000 under wheat breeding program (Kadar and Moldovan, 2003). *T. dicoccides* was used to improve grain protein content of durum wheat (Kovacs et al., 1998) and two hard spring wheats (Mesfin et al., 2000). Ciaffi et al. (1995) used this species to transfer an allelic variant of *Glu-A1* locus to durum wheat, which showed better quality than the donor and host wheats. Alvarez et al. (1999) developed durum wheat having high yellow pigment using *Hordeum chilinese* as a donor.

Role of glutenin and gliadin in wheat grain quality improvement

Among the cereals, wheat is unique because its flour alone has the ability to form dough that exhibits the rheological properties required for the wider diversity of foods. In dough, most of the proteins are converted into gluten complex. Wheat storage proteins namely, gliadin and glutenin are the main components of gluten, which is the major contributor to the rheological and end product making properties of wheat flour. Gel filtration (Huebner and Wall, 1976) and Flow fractionation studies (Stevenson and Preston, 1996; Wahlund et al., 1996) have revealed that glutenin proteins are among the largest protein molecules in nature (Wringley, 1996) having molecular weights over million daltons. Two major classes of glutenin subunits have been identified in wheat endosperm: the high molecular weight (HMW) and the low molecular weight (LMW) glutenin subunits (Huebner and Wall, 1976) based on their mobility on SDS-PAGE (Payne et al., 1980; Jackson et al., 1983; Singh et al., 1991). High molecular weight glutenin subunits (HMW-GS) are components of the glutenin polymer and therefore, play a major role in the determination of the unique visco-elastic properties of wheat dough (Payne et al.,1987; Nieto-Taladriz et al., 1994). The HMW-GS are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (1A, 1B, and 1D) designated as *Glu-1A*, *Glu-1B*, and *Glu-1D*, respectively (Payne et al., 1980; Butow et al., 2003). Each locus includes two genes linked together encoding two different types of HMW-GS, *x*-type (High molecular weight) and *y*-type (Low molecular weight) subunits after fractionation on SDS-PAGE (Payne et al., 1981; Shewry et al., 1992). To correlate the bread making potential with HMW subunit composition, two statistical approaches have been used. Based on the SDS sedimentation volume, each HMW-GS has been assigned a number, and the presence of certain combinations of these subunits is related to different quality aspects of end products (Payne, 1987; Payne et al., 1981). The quality scores assigned to the HMW-GS ranges from 1 (null allele) to 4 (Table1). In another approach, Ng and Bushuk (1988) developed an equation for predicting the unit loaf volume, based on HMW subunit composition of a bread wheat cultivar. Several HMW-GS are found to be associated with bread making quality. Subunit 5+10 had more significant effect on dough mixing time, SDS-sedimentation value and dough strength as compared to subunit 2+12 (Luo et al., 2001; Liang et al., 2010). Further it can be concluded that subunit 5+10 is more closely associated with better bread making quality whereas 2+12 with poor bread making properties (Kolster et al., 1991; Payne et al., 1981). Using NILs, Rogers et al. (1991) have shown that both types of subunits (5+10) are equally important for dough properties, SDS-sedimentation and loaf volume. Branlard and Dardevet (1985) have shown that the alveograph parameters W (gluten strength) and P (tenacity); and the Zeleny sedimentation value are correlated positively with subunits 7+9 and 5+10, and negatively with 2+12, whereas subunit 1 is correlated with W, and subunit 2* and 17+18 with G (swelling). Obukhova et al. (1997) have indicated that the high quality of dough is mainly determined by the *Glu-A1a* allele (subunit 1), whereas the low quality of dough is related to the *Glu-A1c* allele (null allele). Butow et al. (2003) found a significant association between dough strength and *1Bx7*. Radovanovic et al. (2002) analyzed 162 doubled haploid lines and found that 1Dx5 +1Dy10 had the greater effect on mixograph parameters, but the over expressed subunit *1Bx7* also had a significant effect. Allelic variations in composition of Low-molecular-weight glutenin subunits (LMW-GS) also contribute to bread making quality (Khelifi and Branlard, 1992; Manifesto et al., 1998). LMW-GS are encoded by the *Glu-3* loci on the short arms of homoeologous group 1 chromosomes (Singh and Shepherd, 1988). LMW-GS plays an important role in bread and noodle processing quality by influencing the viscoelasticity and extensibility of dough (Gupta and MacRitchie, 1994; Maucher et al., 2009). LMW-GS alleles have been found to be significantly correlated with dough properties in bread (Gupta et al., 1989, 1994) and durum wheat (Pogna et al., 1990; Ruiz and Carrillo, 1993). Payne et al. (1984) were the first to associate LMW-GS with the wheat quality characteristics of tetraploid wheat. Preliminary attempts to rank LMW-glutenin subunit alleles in order of quality have been reported subsequently (Gupta et al., 1989; Cornish, 1995; Cornish et al., 1999). In another study with double haploid lines, it has been observed that lines containing the *Glu-A3 d* allele have stronger gluten properties than lines with allele's f or g, while the *Glu-B3 b* is superior to its allelic counterpart (Cornish et al., 2001; Bekes et al., 2001; Eagles et al., 2002). However, it has been suggested that the effect of these alleles on quality can be more accurately assessed if considered in conjunction with the HMW-GS (Gupta et al., 1994; Eagles et al., 2002). Recently, 5 novel LMW-GS genes have been isolated and cloned from Tibetan wheat landraces. Conformation of identified LMW-GS was done by employing SDS-PAGE and MALDI-TOF-MS. They were further designated as LMW-m type genes and phylogenetic analysis revealed them to be gluten-like proteins. The five novel genes may be new candidate LMW-

Table 1. Quality scores assigned to HMW glutenin subunits encoded on the different chromosomes according to Payne et al. (1987).

Quality score of each subunit	HMW-GS at *Glu-1*		
	Glu-A1	*Glu-B1*	*Glu-D1*
1	Null	7	4+12
1	-	6+8	-
2	-	7+9	2+12
2	-	-	3+12
3	1	17+18	-
3	2*	7+8	-
4	-	-	5+10

GS genes with potential value for wheat quality improvement. (Lan et al., 2012). It is generally accepted that glutenins are mainly responsible for viscoelastic properties, but gliadins are important in conferring extensibility to dough. Gliadins are monomeric proteins, which are soluble in 70% aqueous alcohol. Based on their mobility in Acid Polyacrylamide gel electrophoresis (Acid-of A-PAGE, Bushuk and Zillman, 1978), Gliadins are divided into four groups: α–, β-, γ- and ω-gliadins. Gliadins of a single wheat grain can be separated into 20-25 components using one-dimensional electrophoresis (Bushuk and Zillman, 1978; Wrigley et al., 1982; Metakovsky et al., 1984) whereas two- dimensional electrophoresis and Reverse-phase high performance liquid chromatography (RP-HPLC) allows better separation with a resolution of up to 40-50 components (Payne et al., 1982; Pogna et al., 1990; Mamone et al., 2005). Three loci, *Gli-A1*, *Gli-B1* and *Gli-D1*, that are located on distal end of chromosome arms *1AS*, *1BS* and *1DS* respectively, encode γ-gliadins, β-gliadins and ω-gliadins. Separate complex loci on group-6 chromosome (*Gli-A2*, *Gli-B2* and *Gli-D2*) encode other gliadins (Skerritt, 1998). Dal Belin Peruffo et al. (1985) have identified individual gliadins encoded by *Gli-B1* as markers of dough strength in bread wheat. Moreover, significant correlations between gliadin components and gluten quality have been observed by several researchers. Metakovsky (1991) has shown significant correlations between gliadin alleles (*Gli-B1b, Gli-B2c and Gli-A2b*) and gluten strength in Italian bread wheat cultivars. Some gliadin alleles have also been shown to be positively associated to dough extensibility and dough strength (Branlard and Felix, 1994; Metakovsky et al., 1997). A consistent relationship has been found between the presence of γ-gliadin designated band 45 and gluten strength, and between the presence of γ-gliadin band 42 and gluten weakness (Damidaux et al., 1978). Boggini and Pogna (1989) have confirmed that γ-gliadin 45 has a strong and favourable influence on the bread making quality of durum wheat as well. Gliadins 42 and 45 have been found to be the genetic markers of quality (Pogna et al., 1990). Metakovsky (1991) has shown significant correlations between gliadin alleles (*Gli-B1b, Gli-B2c and Gli-A2b*) and gluten strength in Italian bread wheat cultivars.

Limitations of traditional approaches

Improvement of simple traits such as plant height and grain number through breeding is relatively simple and fast. However, in case of quality traits it requires assessment of an end product. Hence, only a limited number of lines can be analyzed. Direct estimation through milling and baking, is costly, time consuming and requires a large number of grain sample, which is usually not available in early-breeding generation (Gross et al., 2007). Therefore, to overcome this problem, indirect tests such as SDS sedimentation volume, alveograph and mixograph have been developed to determine the end product-making ability in earlier generations. However, these techniques are often time consuming and impose a major constraint on the breeder's resources (Howitt et al., 2007). As improving grain quality through breeding and indirect tests is a major challenge for wheat breeders, therefore, wheat storage Protein (glutenin and gliadin) markers give a good alternative for improvement of grain quality traits. The identification of allelic pairs present in a genotype is usually carried out at protein level by comparing the relative migration of glutenins (HMW and LMW glutenin subunits) and gliadins on Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Acid Polyacrylamide gel electrophoresis (ACID-PAGE), respectively. SDS-PAGE has been used extensively to identify HMW (Ram, 2003; Singh et al., 2007; Meng and CAI, 2008, Yang et al., 2010; Afshan and Naqvi, 2011) and LMW (Liu et al., 2010; Ribeiro et al., 2011) glutenin subunit alleles and to study their effects on bread quality (Payne et al., 1981; Singh and Shepherd, 1991; Gupta et al., 1989; Pogna et al., 1990; Figueroa et al., 2009). However, the drawback associated with the technique is that sometimes different glutenin subunits can appear to have the same relative mobility, resulting in the incorrect identification of some HMW-GS alleles that are functionally distinct (Butow et al., 2004). Moreover, the difficulty of resolving the large number of HMW and LMW glutenin subunits expressed in hexaploid wheat, results in the incorrect identification of functionally distinct alleles (Ma et al., 2003). Thus, it has hindered the selection of specific glutenin alleles in wheat breeding programs. Other techniques such as 2-D gel electrophoresis, MALDI-TOF-MS and RP-HPLC are also available for glutenin analysis (Skylas et al., 2000; Dong et al., 2009; Gao et al., 2010). But, these techniques are not in routine use due to large complexity, overlapping fractions and also time consuming procedure for high throughput analysis of large number of samples. Because of the above said restrictions it became absolutely necessary to search for more reliable and efficient alternative tools. Availability and use of various quality based markers served the purpose and aided the selection of desired trait with relative ease for wheat breeders.

Current status of DNA markers for quality traits

There are three major types of markers used by plant breeder's viz., morphological, biochemical and DNA markers (Collard et al., 2005). Morphological and biochemical markers have found wide applications in wheat breeding (Eagles et al., 2001; Gale, 2005). The major disadvantages of morphological and biochemical markers are that they are limited in number and are influenced by environmental factors (Winter and Kahl, 1995). DNA markers are the most widely used type of markers, predominantly due to their abundance and coverage of genome. They arise from different classes of DNA mutations such as substitution (point mutations), insertions, inversions, duplication, deletions, translocations or errors in replication of tandemly repeated DNA (Paterson, 1996). DNA markers are now gaining the attention of breeders for marker-assisted selection (MAS) in wheat breeding (William et al., 2007; Collard and Mackgill, 2008; Gupta et al., 2010). Till date, a large number of perfect and functional DNA markers are available for grain quality traits in wheat (Table 2).

Marker for High molecular weight glutenin subunits

The polymerase chain reaction (PCR) has been used as an efficient and reliable approach for the determination of HMW-GS allelic composition (Ahmad, 2000; De Bustos et al., 2000; D'Ovidio and Anderson, 1994; D'Ovidio et al., 1995; Lafiandra et al., 1997; Radovanovic and Cloutier, 2003; Xu et al., 2008) and is applicable to screening leaf material from the field prior to harvest of lines selecting the desired genotypes (Eagles et al., 2001; Gupta et al., 1999; Lei et al., 2006). PCR markers may be used to discriminate alleles based on very small differences in sequence identity between alleles, with as little as 1 bp polymorphism being sufficient for the development of allele specific PCR primers (Zhang et al., 2003). PCR-based functional markers

Table 2. An overview of DNA markers for wheat quality traits**.**

Phenotype	Gene/locus	Allele/s	Marker	References
Dough Strength	*Glu-A1*	*Ax ,Ax null, Ax1, Ax2*, Ax2*B, Ay*	Allele specific	D'Ovidio et al.(1995), Lafiandra et al.(1997)), De Bustos et al.(2000), Juhasz et al. (2001), Ma et al. (2003), Radovanovic and Cloutier (2003), Liu et al.(2008)
	Glu-B1	*Bx, Bx6,Bx7/Bx17, Bx7*,Bx7^{OE} , Bx13, Bx14,*	Allele specific	Ahmad (2000), Ma et al. (2003), Radovanovic and Cloutier (2003), Butow et al. (2003), Butow et al. (2004), Schwarz et al. (2004), Xu *et al*. (2008), Ragupathy et al. (2008), Espi et al. (2012)
		By8, By9, By16 & By null or (20)	Allele specific	Lei et al. (2006)
	Glu-D1	*Dx, Dy, Dx5, Dy10/Dy12*	Allele specific	D'Ovidio et al. (1994), Smith et al. (1994), D'Ovidio and Anderson (1994), Ahmad (2000), De Bustos et al. (2001), Ma et al. (2003), Radovanovic and Cloutier (2003), Liu et al.(2008)
	Glu-A3	*GluA3a,b,d,e,f, g,ac,*	SSR, STS	D'Ovidio (1993), Devos et al. (1995), Zhang et al. (2004), Wang et al. (2010)
	Glu-B3	*GluB3a,b,c,d,ef,g,h, I,e, f,g*	STS	Van Campenhout *et al. (*1995), De froidmont (1998), Zho et al. (2007b), Wang et al. (2009a)
	Glu-D3	*Glu-D3allelles*	STS, Allele specific	Zho et al. (2007a), Appelbee et al. (2009)
Dough properties	Rye chromatin ω-secalin	*Secalin-2*, *Glu-B3j*	STS	Francis et al. (1995), Koebner (1995), de Froidmont (1998), Zhang et al. (2003), Chai et al. (2006)
Grain hardness (Puroindolines)	pina, pinb	*Pina-D1a,b Pinb-D1a,b Pinb-D1c Pina-D1r*	CAPS/ STS	Gautier et al. (1994), Giroux and Morris (1997) Limello and Morris (2000), Morris (2002), Chen et al. (2012)
Starch properties	*Wx-A1*, *Wx-B1*, *Wx-D1*	*Wx-B1a, Wx-D1a Wx-D1e, Wx-B1 Null Wx-B1*	CAPS/ STS	Briney et al. (1998), Shariflou et al. (2001), McLauchlan et al. (2001), Nakamura et al. (2002), Yanagisawa et al. (2003), Saito et al. (2009)
Polyphenol oxidase activity	*Ppo-A1*	*Ppo-A1a/b*	STS	Sun et al. (2005), Wang et al. (2008)
	Ppo-D1	*Ppo-D1a, Ppo-D1a b*		He et al. (2007)
	Ppo-B1	*Ppo-B1a, Ppo-B1b*		Si et al. (2012a)
Lipoxygenase activity	*TaLox-B1*	*TaLox-B1a, TaLox-B1b*	STS	Geng et al. (2012)
Yellow pigment content	*Psy-A1*	*PsyA1a,b*	STS	He et al. (2008, 2009a)
	Psy-B1	*Psy B1a,bc,d,e*		He et al. (2009a)
	Psy1-D1	*Psy1-D1a,g*		Wang et al. (2009b)
	TaZds-A1	*TaZds-A1a,b*		Dong et al. (2010)
	TaZds-D1	*TaZds-D1a,b*		Zhang et al. (2011b)

developed from gene sequences provide accurate and high throughput data for determination of allelic compositions in breeding materials (Bagge et al., 2007; Liu et al., 2012). D'Ovidio and Anderson (1994) have designed locus specific primers to detect the presence or absence of *1Dx5* gene and to select for the entire locus *1Dx5-1Dy10*. Smith et al. (1994) designed primers that distinguished the *Dx2* and *Dx*5 genes in a co-dominant manner, facilitating the identification of heterozygous lines, although additional PCR products were also obtained from other homeologous genes using these primers specific for the *Dx5* gene with two internal polymorphic nucleotides within the forward primer and one polymorphic nucleotide within the reverse primer relative to the Dx2 gene. D'Ovidio et al. (1995) designed PCR primer pair to simultaneously amplify the y-type genes from all three genomes. These primers are also effective for the amplification of the corresponding genes from diploid and tetraploid progenitor species. Ahmad (2000) has reported the utility of specific PCR primers to identify wheat genotypes carrying glutenin allelic combinations, which are related to good or poor bread making quality (BMQ). De Bustos et al. (2000, 2001) reported the development and application of six dominant markers based on the amplification of the entire coding regions of seven HMW-GS genes (*Ax2*, Ax1/Axnull, Dx5, Dy10, Dx2 and Dy12*) using polymorphisms in the regions flanking the gene of interest. A number of markers targeting different glutenin alleles have been reported, including markers for *Glu-B1* alleles that are based on

sequence variations of *Bx* type genes (Ma et al., 2003). Schwarz et al. (2004) reported a co-dominant DNA marker to select against the *Bx6* subunit, which has a negative effect on the bread making quality of wheat. Lei et al. (2006) designed primers which target DNA sequence variation of the coding and /or promoter regions of wheat HMW glutenin y-type genes located at the *Glu-B1* loci and this allowed the development of a set of PCR-based markers for specific HMW glutenin genes encoding *By*-subunits. Ragupathy et al. (2008) reported DNA markers specific to the *Glu-B1al* allele encoding the over expressed *Bx7* subunit. Liu et al. (2008) developed and validated a set of three co-dominant markers suitable for high throughput MAS for HMW glutenin subunits encoded at the *Glu-A1* and *Glu-D1* loci. Espi et al. (2012) reported a novel DNA marker for discrimination between the subunits *Bx7* and *Bx7** at *Glu-B1* loci. Using AS-PCR markers, 224 Chinese wheat cultivars and advanced lines (Yang et al., 2010), 273 CIMMYT wheat lines and advanced lines (Liang et al., 2010), 718 wheat cultivars including advanced lines (Jin et al., 2011) from 20 different countries and 142 bread wheat varieties from different countries were characterized for the HMW-GS (Table 3).

Markers for Low molecular weight glutenin subunits

More than 100 sequence tags of genes, partial genes and pseudogenes of the LMW-GS family have been cloned and sequenced from several bread wheat cultivars (Cloutier et al., 2001; Ikeda et al., 2002; Zhang et al., 2004; Zhao et al., 2006). Hai et al. (2005) retrieved 69 known LMW-GS genes from GenBank and classified them into nine groups based on the deduced amino acid sequence of the highly conserved N-terminal domain, and nine corresponding primer sets proved to be LMW-GS group-specific were established. Ikeda et al. (2006) also constructed ten group-specific markers according to the published nucleotide sequences. Based on the sequence polymorphisms between LMW glutenin genes, LMW glutenin-specific PCR marker was developed to distinguish durum wheat cultivars and a microsatellite marker has been developed for mapping in hexaploid wheat (Devos et al., 1995). Allele-specific PCR markers based on single nucleotide polymorphism (SNP) of γ-gliadin genes (*Gli-1*) were subsequently used to detect specific alleles linked to the *Glu-A3*, *Glu-B3*, *Glu-D3* LMW glutenin loci, respectively (Zhang et al., 2003). Six LMW-GS genes were characterized at the *Glu-D3* locus on chromosome 1D and seven STS markers were established to amplify the corresponding gene sequences in wheat cultivars containing five *Glu-D3* alleles viz., a, b, c, d and e (Zhao et al., 2006, 2007a). These markers can be useful to identify the *Glu-D3* gene haplotypes in wheat breeding programs (Zhao et al., 2007a). Zhao et al. (2007b) have developed STS markers based on SNPs in *Glu-D3* and *Glu-B3* genes. Further, it has been reported that *Glu-A3* and *Glu-B3* LMW-GSs have significant influence on the processing quality of the end-user products of common wheat. To characterize LMW-GS genes at the *Glu-A3* (Wang et al., 2010) and *Glu-B3* locus (Wang et al., 2009a), seven and ten gene specific PCR primers were designed to amplify different *Glu-A3* (a, b, c, d, e, f and g) and *Glu-B3* (a, b, c, d, e, f, g, h and i) alleles, respectively. Zhang et al. (2011a) used a combined approach (PCR with conserved primers and high resolution capillary electrophoresis) for development of a new marker system for identification of LMW-GS genes in family in bread wheat. This marker system is very useful for high throughput analysis of LMW-GS genes to improve the bread making quality in wheat breeding program. Characterization of 273 CIMMYT wheat cultivars (Liang et al., 2010), 182 Indian wheat cultivars (Ram et al., 2011) and 718 wheat cultivars including advanced lines (Jin et al., 2011) has been done using gene/allele specific marker for LMW-GS (Table 3).

Markers for Starch characteristics

Waxy genes encodes waxy (*Wx*) proteins or Granule-bound starch synthase1 (*GBSS1*) which is responsible for the synthesis of amylose in stored starch in the endosperm and pollen (Mason-Gamer et al., 1998). The level of amylose (low amylose or amylose free) in starch is affected by the lack of *GBSS1* activity (due to mutation at waxy locus) in the developing wheat endosperm (Wickramasinghe and Miura, 2003; Saito et al., 2010). Null alleles of each of the homeologous *GBSS1* proteins including *Wx-A1*, *Wx-B1* and *Wx-D1* are located on chromosome arms 7AS, 4AL and 7DS, respectively (Chao et al.,1989, Saito et al., 2010). Null alleles of *Wx-B1* and *Wx-A1* are much more common in cultivars than null alleles of *Wx-D1*. Measurement of amylose content using colorimetric assays was not accurate so other methods such as two-dimensional polyacrylamide gel electrophoresis (Nakamura et al., 1993a) was used to separate all three waxy proteins (*Wx-A1*, *Wx-B1* and *Wx-D1*) which allowed the identification of waxy mutants lacking one or more waxy proteins (Nakamura et al., 1993b; Yamamori et al., 1994). Colorimetric assays or electrophoretic separation of *Wx* proteins are time consuming processes and interpretation of results is not easy. Further it is difficult to screen large number of wheat cultivars. Therefore, PCR-based DNA markers provide a good alternative for MAS for partial waxy wheat lines. Briney et al. (1998) reported the development of a *Wx-B1* specific PCR assay, with *Wx-B1* null lines showing a negative result. Shariflou et al. (2001) reported the development of a co-dominant marker for the specific detection of a deletion mutant of the *Wx-D1* gene derived from a Chinese land-race. Based on nucleotide sequences of the wild-type and null waxy alleles (Murai et al., 1999; Vrinten et al., 1999), a set of primer pairs was designed for the assay of each *GBSS1* homeoallele (Nakamura et al., 2002). These markers developed were suitable for the analysis of *GBSS1* alleles from diverse eco-geographical origins of world. Saito et al. (2004) used the primer set developed by Nakamura to characterize *waxy* mutations in 168 wheat lines from 20 countries. Yanagisawa et al. (2003) used a SNP in the *Wx-D1* gene to develop a co-dominant derived cleaved amplified polymorphic sequence (dCAPS) marker for the *Wx-D1e* allele, which appears to express an inactive form of the *GBSS1* enzyme due to presence of the SNP. Characterization of 103 Argentinean bread wheat cultivars for genetic variability of waxy loci was done using three sets of new primer (Vanzetti et al., 2009) and one primer pair developed by McLauchlan et al. (2001). Two alleles (*Wx-A1a*, *Wx-A1g*) at *Wx-A1* locus, three alleles (*Wx-B1a*, *Wx-B1b*, *Wx-B1e*) at *Wx-B1* and one allele (*Wx-D1a*) at *Wx-D1* locus was found frequently (Vanzetti et al., 2009).

Markers for Grain hardness

Grain hardness in wheat is the most important quality characteristic which affects milling, baking and end-use quality. The techniques used for grain hardness measurement are grinding, crushing and abrasion, Particle Size Index (PSI), Near Infrared Reflectance (NIR) hardness, Single Kernel Characterization system (SKCS), pearling index, SDS-PAGE and PCR markers (Pasha et al., 2010).

Table 3. Application of DNA markers in wheat quality breeding.

Gene/alleles	Genotypes	Country	Marker Type	Reference
HMW- and LMW-GS alleles	224 wheat cultivars and advanced lines	China	AS-PCR	Yang et al. (2010)
	718 wheat cultivars and advanced lines	20 countries	AS-PCR,STS	Jin et al. (2011)
HMW-GS alleles	60 germplasm lines	-	AS-PCR	Gao et al. (2010)
	26	Saudi Arabia	AS-PCR	Ghazy et al. (2012)
	142 bread wheat varieties	Different countries	AS-PCR	Espi et al. (2012)
LMW-GS alllels	182 bread wheat	India	AS-PCR	Ram et al. (2011)
Ppo	57 wheat cultivars	India	AS-PCR	Singh et al. (2009a)
	311 wheat cultivars and advanced lines	China		Xiao et al. (2008)
	273 wheat cultivars	CIMMYT, Mexico		Liang et al. (2010)
1B/1R	57 wheat cultivars and advanced lines	India	STS	Singh et al. (2009b)
Puroindoline alleles	127 wheat cultivars	-	STS	Chen et al. (2007)

The difference between hard and soft wheat is controlled by a major gene, *Ha* which is present on the short arm of chromosomes 5D (Symes, 1969; Law et al., 1978; Morris and Beecher, 2012). The genes for puroindoline a (*pin-a*), puroindoline b (*pin-b*) and grain softness protein (*Gsp-1*), closely linked to the *Ha* (soft) locus have been shown to be associated with the expression of grain softness (Turner et al., 1999). Giroux and Morris (1998) have found that presence of a single mutation in either protein, a null in *pin-a* or glycine to serine sequence change at position 46 in *pin-b*, is associated with hard grain texture. Limello and Morris (2000) have also reported a leucine to proline mutation at position 60 in *pin-b* in hard wheats from Northern Europe. The absence of these genes results in a really hard grain especially in case of durum wheat. Giroux and Morris (1997, 1998) have further designed PCR primers for *pin-a* and *pin-b* sequences using the single nucleotide change in *pin-b*. Preliminary results have indicated that bread wheat varieties with the null *pin-a* deletion had harder grains than varieties with the glycine to serine mutation (Giroux and Morris, 1998). Tranquilli et al. (1999) have further developed a more reliable, co-dominant CAPS (cleavage amplified polymorphic sequence) marker based on the same point mutation in *pin-b*, which can be used to determine kernel texture. Seven new allelic forms of puroindolines (Pin a and Pin b) and five new forms of GSP were detected using specific PCR primers (Chen et al., 2005). Later, Chang et al. (2006) detected *pin a* and *pin b* allelic variations in 102 common wheat cultivars and related species using denaturing PAGE. In total, seven variations (Two of *Pina-Pina-D1b* and *Pina-D1p*; Five of *Pinb*-null, *Pinb-D1b*, *Pinb-D1u*, *Pinb-D1v* and *Pinb-D1w*) were identified in common and spelt wheats. Further, Chen et al. (2007) investigated 127 cultivars (40 Yunnan endemic wheats, 21 historical cultivars and 66 current cultivars) and advanced lines for kernel hardness and puroindoline alleles using molecular and biochemical markers (Table 3). Four puroindoline alleles (*Pina-D1b*, *Pinb-D1b*, *Pinb-D1d* and *Pinb-D1e*) and one new allele (*pinb-D1u*) were found in the studied wheat cultivars. Further recently, Chen et al. (2012) physically mapped four puroindoline *b-2* variants using nulli-tetrasomic lines of bread wheat cultivar Chinese Spring and substitution lines of durum wheat (*Triticum turgidum* L.) cultivar Langdon.

Markers for Polyphenol oxidase activit

Polyphenol oxidase (PPO) activity causes darkening and discoloration of wheat (*Triticum aestivum* L.) based products, greatly reducing the appearance quality of end-products. DNA markers PPO16, PPO18, STS01and F-8 for the loci *Ppo-D1* on chromosome 2DL, *Ppo-A1* on chromosome 2AL and *Ppo-B1* on chromosome 2B have been developed, respectively (Sun et al., 2005; He et al., 2007, Wang et al., 2009b, Si et al., 2012a). Molecular markers associated with PPO activity have the potential to accelerate the selection efficiency for low PPO activity in wheat improvement programmes. Validation of STS markers on chromosomes 2A and 2D for PPO activity in 311 Chinese wheat cultivars (Xiao et al., 2008), 57 Indian wheat cultivars (Singh et al., 2009a), 273 CIMMYT wheat lines (Liang et al., 2010) have been done. Si et al. (2012b) used two STS markers (Ppo18 and STS01) of *Ppo* genes for detection of allelic variability in 300 F_4 plants from cross between Yangmai 158 and Huaimai 18 and 362 wheat cultivars. Sun et al. (2011) characterized the expression of *Ppo-A1* gene using DNA sequencing, semi-quantitative RT-PCR, PPO activity assays and whole-grain staining methods during grain development. Alternative splicing of pre mRNA in the coding region of *Ppo-A1* directly influenced the polyphenol oxidase activity in common wheat. The differences in expression of *Ppo-A1a* and *Ppo-A1b* were confirmed by PPO activity assays and whole grain staining, providing direct evidence for the influence of alternative splicing in the coding region of *Ppo-A1* on polyphenol oxidase activity in common wheat grains (Table 3).

Markers for Lipoxygenase activity and yellow pigment content

Flour color is an important trait in the assessment of flour quality and exerts significant influence on noodles and other related products (Parker et al., 1998). Lipoxygenase (LOX)

activity and Yellow pigment content (YPC) have major effect on color and processing quality of wheat-based products (Hessler et al., 2002; Carrera et al., 2007; Geng et al., 2011). A bright yellow color (Hessler et al., 2002; Carrera et al., 2007) and a low LOX activity are desirable in breeding durum wheat cultivars. STS markers LOX16 and LOX18 located on chromosome 4BS were developed to differentiate between cultivars with higher and lower LOX activities (Geng et al., 2012). Functional markers are also available for Phytoene synthase (*PSY*) genes on chromosomes 7A, 7B (He et al., 2008; 2009a,b) and 7D (Wang et al., 2009b), and for f (zeta)-carotene desaturase (ZDS) genes on chromosomes 2A (Dong et al., 2012) and 2D (Zhang et al., 2011b) to differentiate between different alleles in wheat. Additionally, molecular markers which are associated with other grain quality related traits such as grain protein content, pre-harvest sprouting tolerance, milling yield, BMQ and grain weight have been also reported in wheat (Gupta et al., 2008; Elangovan et al., 2008).

Markers for 1B/1R tranlocation

The 1B/1R translocation has been widely used in global wheat breeding, even though it has a significant and negative effect on dough qualities for both bread and Chinese noodle (Dhaliwal et al., 1988, He et al., 2005). Selection for or against the presence of rye chromatin may be accomplished using DNA markers to detect the presence of rye repetitive DNA sequences (Francis et al., 1995; Koebner, 1995). Using the gene specific primers for ω-secalin, a 1076-bp fragment was generated in genotypes with the 1B/1R translocation (Chai et al., 2006), whereas a 636-bp fragment for *Glu-B3* locus was amplified in genotypes without 1B/1R (de Froidmont, 1998). One hundred seven Turkish wheat cultivars including landraces (Yediay et al., 2010) using nine rye-specific markers and sixty seven Indian wheat cultivars including advanced breeding lines (Singh et al., 2009b) using four STS markers were tested for the presence/absence of 1B/1R translocation, respectively (Table 3).

Recent approaches to improve the wheat quality

Genomics: Genomics can be defined as the study of all of the genes in an organism. Differences in processing or end use quality characteristics can be studied by using sequence of the gene in different cultivars or differences in the level of expression of the gene. Gene expression or transcription analysis allows association with wheat quality traits to be explored. Transcriptome of the developing wheat seed provide a key resource for discovery of the molecular basis of grain quality traits (Drea et al., 2005; McIntosh et al., 2007). Wheat has been a difficult cereal crop for genomics research because it has complex polyploidy large genome, high proportion of repetitive sequences and lack of polymorphism however, the availability of cytogenetics stocks has been an asset for genomics research (Hussain et al., 2007; Gupta et al., 2008; William et al., 2008). Map-based cloning, comparative genomics and sequencing the wheat genome are available to discover genes responsible for trait variation, whereas, Serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), micro- and macroarrays techniques are now available for the estimation of a large number of expressed genes simultaneously (Francki, 2009). The microarrays have also been successfully used in wheat for understanding alterations in the transcriptome of hexaploid wheat during grain development and germination (Wilson et al., 2004, Gupta et al., 2008). Positional cloning was used to isolate a gene from wheat that improved protein content, zinc and iron (Uauy et al., 2006). The expression of specific glutenins can be used to alter the properties of wheat doughs (Masci et al., 2003; He et al., 2005). Molecular analysis of these loci provides a more detailed understanding of the basis of product quality. Low cost and high level of automation of assays of SNP is currently the preferred approach for high throughput analysis (Sobrino et al., 2005).

Transformation

Genetic engineering techniques (Anderson et al., 1996; Shewry and Tatham, 1997) have advanced to the stage that now it is possible to alter the genes in various ways to experiment systematically with the properties of the polypeptides, and progress towards elucidating how ideal glutenin subunits would look like? Through genetic transformation, a number of HMW-GS and LMW-GS genes have been introduced into wheat (Altpeter et al., 1996; Barro et al., 1997; Blechl et al., 1998, 2007; Tosi et al., 2005; Bregitzer et al., 2006). The first report of wheat transformation involved microprojectile acceleration into immature zygotic embryos to introduce the 1Ax1 subunit into cultivar 'Bobwhite', resulting in a 71 % increase in total high molecular weight glutenin proteins (Altpeter et al., 1996). Later, Blechl et al. (1998) demonstrated that not all transformation effects were necessarily additive, whereas, transgenic wheat for HMW glutenins exhibited decreased accumulation due to transgene mediated suppression. For the first time, durum wheat was transformed by addition of *1Ax1* and *1Dx5* subunits genes of *T. aestivum*, resulting in increased mixing time and peak resistance, which are indicators of good dough strength (He et al., 1999). Expression of HMW-GS *1Ax1* was found to improve the rheological properties of flours from transgenic lines, whereas lines expressing *1Dx5* had unsuitable bread-making characteristics (Barro et al., 2003; Blechl et al., 2007). These reports clearly demonstrated that bread-making quality can indeed be improved by introduction of HMW-GS genes that have been shown to be associated with good bread-making qualities.

Proteomics

The proteomics is a new research area, which can combine the total proteome and physiology. Proteomics covers the systematic analysis of proteins expressed by a genome, from the identification of their primary amino-acid sequence to the determination of their relative amounts, their state of modification and association with other proteins or molecules of different types (Barbier-Brygoo and Joyard, 2004). This is a promising way to identify tissue specific proteins, their diversity, regulation and post-translational modifications. Two-dimensional electro-phoresis (2-DE), nano-liquid chromatography and mass spectrometry (MS), have made possible the efficient separation and identification of cereal proteins, and high-throughput analysis of functions and functional networks of these proteins (Hirano, 2007). Tremendous progress has been made in this field in the past few years, especially in plant biology; however, this approach is still in its early stages in plants in general and wheat in particular (Thiellement, 1999, 2002). A proteomic analysis was carried out on wheat grain endosperm proteins and a substantial amount of information has been obtained on the complex

heterogeneity of proteins synthesized in the wheat endosperm (Skylas et al., 2000; Amiour et al., 2002, Skylas et al., 2005). The hardness of wheat controlled by the proteins encoded by the pin genes allows the selection of wheat cultivars with desirable hardness (Morris, 2002). Analysis of the sequences of these genes allows hardness to be predicted (Chen et al., 2005). Gobaa et al. (2007, 2008) reported the impact of rye chromatin introgression on the grain proteome of wheat. The comparison of the 2-D electrophoresis profiles of doubled haploid lines, with or without the 1BL.1RS translocation, revealed quantitative and qualitative proteic variations in prolamines and other endosperm proteins. To understand the impact of the 1BL.1RS translocation on dough strength and how 1BL.1RS genotypes may overcome the loss of *Glu-B3* and *Gli-B1*, proteomic profiles of 16 doubled haploid (DH) lines of similar glutenin composition but of different strength were compared (Gobaa et al. 2008). Two bread wheat cultivars, Chinese Spring and Recital, were used in proteomics analysis of the aleurone layer of the seed due to its nutritional and health benefits. The two 2D protein profiles shared more than 80% identity and approximately 700 spots in the aleurone layer (Laubin et al., 2008). Majoul et al. (2003) analyzed the effect of heat stress on the water-soluble fraction, composed essentially of albumins and globulins. These proteins were separated by two-dimensional electrophoresis (2-DE and analyzed by Melanie-3 software). Similarly, Vensel et al. (2005) detected over 250 proteins that participate in 13 biochemical processes during wheat endosperm development. Prandi et al. (2012) analyzed peptide mixture by LC/MS which is obtained from flour samples for common wheat determination in durum wheat. Two marker peptides viz;, One peptide was present only in common wheat and the second was present in all wheat samples (both common and durum).

Challenges and Future prospects

Various biotechnological approaches like in vitro tissue culture, gene transfer and use of DNA markers have emerged as powerful tools to complement conventional methods of breeding by generating genetic variability necessary for desirable traits and reducing the time taken to produce cultivars with improved characteristics. Among various methods of wheat transformation, particle bombardment has been used widely to develop highly efficient transformation systems for wheat (Vasil et al., 1993; Altpeter et al., 1996). Gene dosage studies have indicated that bread-making quality of wheat could be improved by integration and expression of specific HMW-GS genes (Flavell et al., 1989; Shewry et al., 1995). Since, bread making quality is the major thrust of wheat quality improvement studies, efforts have been made to manipulate the proportion of HMW-GS that are known to be associated with good bread making quality. The DNA markers for quality traits are of extremely useful in breeding programs. For simple traits, PCR that require small amount of DNA is becoming very useful for screening large population of segregating progenies. Unfavorable alleles can be eliminated or greatly reduced during the early stages of plant development through MAS (Howitt et al., 2007; Landjeva et al., 2007). Current DNA marker technologies have relatively high cost associated with their development and analysis, low-throughtput and need of sequence information. Hence, novel marker systems of high-throughput analysis are required to identify and screen large numbers of markers, rapidly and at low cost for modern wheat breeding for the rapid and precise analysis of germplasm, mapping and MAS. In conclusion, development of various DNA-based markers during last two decades has revolutionized biological and agricultural science. DNA markers are highly efficient for screening genotypes in wheat breeding programs targeting quality improvement, especially for early generation screening/testing. However, various challenges for MAS in crop breeding still exist, and practical benefits are taking longer time than expected to achieve. The main reasons for this delay are the inadequate quality of markers and high costs of DNA assays (Koebner and Summers, 2002; Bonnett et al., 2005). The stability and robustness of the PCR used markers allows more than one marker to be scored in a single reaction. The multiplexing has a potential to combine dominant and co-dominant marker in a single reaction (Ma et al., 2003). The identification of three alleles in one reaction is still not powerful as SDS-PAGE, which can resolve all alleles in a lane. However, due to the multigenic nature of many quality traits and the requirements to assess the effect of environment on the trait, it will not replace the use of traditional wheat-quality testing procedures for later generation screening and cultivars evaluation. Instead, they will become extremely valuable tools that allow the breeder to identify lines of interest for more in-depth analysis at an earlier stage in the breeding program.

References

Abbasi H, Ardabili SMS, Emam-Djomeh Z, Mohammadifar MA, Zekri M, Aghagholizadeh R (2012) Prediction of Extensograph properties of wheat-flour dough: Artificial neural networks and a genetic algorithm approach. J Texture Studies. DOI: 10.1111/j.1745-4603.2011.00342.x

Afshan S, Naqvi FN (2011) Allelic variation in high molecular weight glutenin subunits in Pakistani bread wheat genotypes. Cereal Res Commu. 39:109-119

Ahmad M (2000) Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor App Genet. 101:892–896

Altpeter F, Vasil V, Srivastava V, Vasil IK (1996) Integration and expression of the high-molecular-weight glutenin subunit 1Ax1 into wheat. Nat Biotechnol. 14:1155–1159

Alvarez JB, Martin LM, Martin A (1999) Genetic variation for the carotenoid pigments content in the amphiploid *Hordeum chilense* × *Triticum trugidum* conv. durum. Plant Breed. 118:187-189

Amiour N, Merlino M, Leroy P, Branlard G (2002) Proteomic analysis of amphiphilic proteins of hexaploid wheat kernels. Proteomics. 2:632-641

Anderson OD, Kuhl JC, Tam A (1996) Construction and expression of a synthetic storage protein gene. Gene. 174:51-58

Appelbee MJ, Mekuria GT, Nagasandra V, Bonneau JP, Eagles HA, Eastwood RF, Mather DE (2009) Novel allelic variants encoded at the Glu-d3 locus in bread wheat. J Cereal Sci. 49: 254-261

Barbier-Brygoo H, Joyard J (2004) Focus on Plant Proteomics. Plant Physio Biochem. 42: 913–917

Bagge M, Xia XC, Lubberstedt T (2007) Functional markers in wheat. Curr Opin Plant Biol. 10:211–216

Barro F, Barcelo P, Lazzeri PA, Shewry PR, Ballesteros J, Martin A (2003) Functional properties of flours from field grown transgenic wheat lines expressing the HMW glutenin subunit 1Ax1 and 1Dx5 genes. Mol Breed.12:223–229

Barro F, Rooke L, Bekes F, Gras P, Tatham AS, Fido R, Lazzeri P, Shewry PR, Barcelo P (1997) Transformation of wheat with HMW subunit genes results in improved functional properties. Nat Biotechnol. 15:1295–1299

Bekes F, Gras PW, Anderssen RS, Apples R (2001) Quality traits of wheat determined by small-scale dough testing methods. Aust J Aric Res. 52:1325-1338

Blechl A, Lin J, Nguyen S, Chan R, Anderson OD, Dupont FM (2007) Transgenic wheats with elevated levels of Dx5 and/or Dy10 high molecular weight glutenin subunits yield doughs with increased mixing strength and tolerance. J Cereal Sci. 45:172–183

Blechl AE, Le HQ, Anderson OD (1998) Engineering changes in wheat flour by genetic transformation. J Plant Physiol. 152:703–707

Boggini G and Pogna NE (1989) The breadmaking quality and storage protein composition of Italian durum wheat. J Cereal Sci. 9:131-138

Bonnett DG, Rebetzke GJ and Spielmeyer W (2005) Strategies for efficient implementation of molecular markers in wheat breeding. Mol Breed. 15:75–85

Branlard G and Dardevet M (1985) Diversity of grain proteins and bread wheat quality. I Correlation between gliadin bands and flour quality characteristics. J Cereal Sci. 3: 29-343.

Branlard G, Felix I (1994) Wheat Kernel Proteins: molecular and functional aspects In*:* Martinal CS (ed) Proceedings of the Int. Meeting, Viterbo, Italy, 1999

Bregitzer P, Blechl AE, Fiedler D, Lin J, Sebesta P, De Soto JF, Chicaiza O, Dubcovsky J (2006) Changes in high molecular weight glutenin subunit composition can be genetically engineered without affecting wheat agronomic performance. Crop Sci. 46: 1553–1563

Briney A, Wilson R, Potter RH, Barclay I, Crosbie G, Appels R, Jones MGK (1998) A PCR-based marker for selection of starch and potential noodle quality in wheat. Mol Breed. 4:427–433

Braun, HJ, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds, CRP (ed) Climate change and crop production, CABI, London, UK.

Bushuk W, Zillman RR (1978) Wheat cultivar identification by gliadin electrophoregrams, Appratus, method and nomenclature. Can J Plant Sci. 58:505-515

Butow BJ, Gale KR, Ikea J, Juhasz A, Bedo Z, Tamas L, Gianibelli MC (2004) Diversity and dissemination of Bx7 glutenin subunits revealed by novel PCR markers and RP-HPLC. Theor Appl Genet. 109:1525-1535

Butow BJ, Ma W, Gale KR, Cornish GB, Rampling L, Larroque O, Morell MK, Bekes F (2003) Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high molecular weight glutenin allele has a major impact on wheat flour dough strength. Theor Appl Genet. 107:1524-1532

Carrera, A, Echenique V, Zhang W, Helguera M, Manthey F, Schrager A, Picca A, Cervigni G, Dubcovsky J (2007) A deletion at the Lpx-B1 locus is associated with low lipoxygenase activity and improved pasta color in durum wheat. J Cereal Sci 45:67–77

Chai JF, Zhou RH, Jia JZ and Liu X (2006) Development and application of a new co-dominant PCR marker for detecting 1BL.1RS wheat-rye chromosome translocations. Plant Breed. 125:302-304

Chang C, Zhang H, Xu J, Li W, Liu G, You M, Li B (2006) Identification of allelic variations of puroindoline genes controlling grain hardness in wheat using a modified denaturing PAGE. Euphytica. 152: 225-234

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homeologous group 7 chromosomes. Theor Appl Genet. 78:495–504

Chen F, He ZH, Xia XC, Limello M, Morris C (2005) A new puroindoline b mutation in Chinese winter wheat cultivar jingdong 11. J Cereal Sci. 42:267-269

Chen F, Shang X, Morris CF, Zhang F, Dong Z, Cui D (2012) Molecular characterization and diversity of puroindoline b-2 variants in cultivated and wild diploid wheat. Genet Resour Crop Evol DOI: 10.1007/s10722-012-9813-y

Chen F, Yu Y, Xia X, He Z (2007) Prevalence of a novel puroindoline b allele in Yunnan endemic wheats (*Triticum aestivum* ssp. yunnanense King) Euphytica 156: 39-46

Ciaffi M, Lafiandra D, Turchetta T, Ravaglia S, Bariana H, Gupta R, MacRitchie F (1995) Bread making potential of durum wheat lines expressing both x- and y- type subunits at the *Glu-A1* locus. Cereal Chem. 72:465-469

Cloutier S, Rampitsch C, Penner GA, Lukow OM (2001) Cloning and expression of a LMW-i glutenin gene. J Cereal Sci. 33:143–154

Codina GG, Mironeasa S, Mironeasa C, Popa CN, Tamba-Berehoiu R (2012) Wheat flour dough Alveograph characteristics predicted by Mixolab regression models. J Sci Food and Agric. 92: 638–644

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica. 142:169–196

Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Phil Trans R Soc B. 363:557-572

Cornish GB (1995) Wheat proteins and end-product quality In: William YA, Wringley CW (eds) Proceedings of 45th Aust. Cereal Chem. Conf. Royal Australian Chemical Institute, Melbourne, Australia.

Cornish GB, Bekes F, Allen HM, Martin DJ (2001) Flour proteins linked to quality traits in an Australian doubled haploid wheat population. Aust J Agric Res. 52:1339-1348

Cornish GB, Panozzo JF, Wringley CW (1999) Victorian wheat protein families. In: L. O'Brien, Blakeney AB**,** Ross AS, Wringley CW (eds*) Proceedings of* 48th Aust. Cereal Chem. Conf. Royal Australian Chemical Institute, Melbourne , Australia.

Damidaux RE, Autran JC, Griganac P, Feillet P (1978) Evidence of relationships useful for breeding between the electrophoretic patterns of gliadins and the viscoelastic properties of the gluten in *Triticum durum* (in French). C.R.Hebd Seances Acad Sci Ser D Sci Nat. 287:701-704

Dal Belin Peruffo A, Pogna NE, Tealdo E, Tutta C, Albuzio A (1985) Isolation and partial characterization of gamma-gliadins 40 and 43.5 associated with quality in common wheat. J Cereal Sci. 3:355-362

De Bustos A, Rubio P, Jouve N (2000) Molecular Characterization of the inactive allele of the gene Glu-A1 and the development of a set of ASPCR markers for HMW glutenins of wheat. Theor Appl Genet. 100:1085–1094

De Bustos A, Rubio P, Soler C, Garcia P, Jouve N (2001) Marker assisted selection to improve HMW-glutenins in wheat. Euphytica. 119:69-73

de Froidmont D (1998) A co-dominant marker for the 1BL/1RS wheat-rye translocation via multiplex PCR. J Cereal Sci. 27:229-232

Devos KM, Bryan GJ, Collins AJ, Stephenson P (1995) Application of two microsatellite sequences in wheat

storage proteins as molecular markers. Theor Appl Genet.90: 247–252.
Dhaliwal AS, Mares DJ, Marshall DR, Skerritt JH (1988) Protein composition and pentosan content in relation to dough stickiness of 1B/1R translocation wheats. Cereal Chem. 65:143-149
Dong K, Hao CY, Wang AL, CAI MH, Yan YM (2009) Characterization of HMW Glutenin Subunits in Bread and Tetraploid Wheats by Reversed-Phase High-Performance Liquid Chromatography. Cereal Res Commun 37: 65–72
Dong CH, Ma ZY, Xia XC, Zhang LP, He ZH (2012) Allelic variation at the TaZds-A1 locus on wheat chromosome 2A and development of a functional marker in common wheat. Agric Sci China. (in press)
D'Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with bread making quality. Theor Appl Genet. 88:759- 763
D'Ovidio R, Masci S, Porceddu E (1995) Development of a set of oligonucleotide primers specific for genes at the Glu-1 complex loci of wheat. Theo Appl Genet. 91:189-194
Drea S, Leader D, Arnold B, Shaw P, Dolan L, Doona, J (2005) Systematic spatial analysis of gene expression during wheat caryopsis development. Plant Cell.17:2173-2183
Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica. 157:417–430
Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke P H, Carter M (2001) Implementation of markers in Australian wheat breeding. Aus J Agric Res.52:1349–1356
Eagles HA, Hollamby GJ, Bariana HS, Gorroro NN, Eastwood RF (2002) Estimation and utilization of glutenin gene effects from the analysis of unbalanced data from wheat breeding programs. Aus J Agric Res. 53:367-377
Elangovan M, Rai R, Dholakia BB, Lagu MD, Tiwari R, Gupta RK, Rao VS, Roder M, Gupta VS (2008) Molecular genetic mapping of quantitative trait loci associated with loaf volume in hexaploid wheat (*T. aestivum*) J Cereal Sci. 47:587-598
Espi A, Giraldo P, Rodriguez-Quijano M, Carrilo, JM (2012) A PCR-based method for discriminating between high molecular weight glutenin subunits Bx7 and Bx7* in *Triticum aestivum* L. Plant Breed. DOI:10.1111/j.1439-0523.2012.01961.x
Figueroa JDC, Maucher T, Reule W, Pena RJ (2009) Influence of high molecular weight glutenins on viscoelastic properties of intact wheat kernel and relation to functional properties of wheat dough. Cereal Chem. 86:139–144
Francki M G (2009) Genomics for Wheat Improvement .In: Jain M S and Brar D S (eds) Molecular Techniques in crop improvement, 2nd edn. Springer, New York
Flavell RB, Goldsbrough AP, Robert LS, Schnick D, Thompson RD (1989) Genetic variation in wheat HMW glutenin subunits and the molecular basis of bread-making quality. Biotechnology. 7:1281-1285
Francis HA, Leitch AR, Koebner RMD (1995) Conversion of a RAPD-generated PCR product, containing a novel dispersed repetitive element, into a fast and robust assay for the presence of rye chromatin in wheat. Theo Appl Genet. 90:636–642
Gale KR (2005) Diagnostic DNA markers for quality traits in wheat. J Cereal Sci. 41:181-192
Gao LY, Ma WJ, Chen J, Wang K, Li J, Wang SL, Bekes F, Appels R, Yan YM (2010) Characterization and comparative analysis of wheat high molecular weight glutenin subunits by SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS. J Agric Food Chem. 58:2777–2786
Geng HW, Zhang Y, He ZH, Zhang LP, Appels R, Qu YY, Xia XC (2011) Molecular markers for tracking variation in lipoxygenase activity in wheat breeding. Mol Breed. 28:117-126
Geng HW, He ZH, Zhang LP, Qu YY, Xia XC (2012) Cloning the lipoxygenase gene on chromosome 4BS and development of functional markers in common wheat. Crop Sci. 52. doi: 10.2135/cropsci2011.07.0365
Ghazi AI, Zanouny AI, Moustafa KA, AI-Doss AA (2012) Molecular screening of high molecular weight glutenin genes in spring bread wheat genotypes in Saudi Arabia. J Food Agri Environ. 10: 157-161
Giroux MJ and Morris CF (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
Giroux MJ and Morris CF (1998) Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. Proc Natl Acad Sci. 95: 6262-6266
Gobaa S, Bancel E, Branlard G, Kleijer G, Stamp P (2008) Proteomic analysis of wheat recombinant inbred line Variations in prolamin and dough rheology. J Cereal Sci. 47: 610–619
Gobaa S, Bancel E, Kleijer G, Stamp P, Branlard G (2007) Effect of the 1BL.1RS translocation on the wheat grain proteome as revealed by proteomics analysis. Proteomics doi:10.1002/pmic.200700488
Goyal A, Prasad R (2010) Some Important Fungal Diseases and their Impact on wheat Production. In: Arya A, Perelló AEV (eds) Management of fungal plant pathogens. CABI (H ISBN 9781845936037), pp.362
Gross C, Bervas E, Chanliaud G, Charmet G (2007) Genetic analysis of bread making quality scores in bread wheat using a recombinant inbred line population Theor Appl Genet.115:313-323
Gupta PK, Landridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. Molecular Breed. 26 :145-161
Gupta PK, Mir RR, Mohan A, Kumar J (2008) Wheat Genomics: Present Status and Future Prospects. International J Plant Genom. doi:10.1155/2008/896451
Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed. 118:369–390
Gupta RB, MacRitchie F (1991) A rapid one-step one-dimensional SDSPAGE procedure for analysis of subunit composition of glutenin in wheat. J Cereal Sci. 14: 105–109
Gupta RB, MacRitchie F (1994) Allelic variation at glutenin subunit and gliadin loci, *Glu-1, Glu-3* and *Gli-1* of bread wheats: biochemical basis of the allelic effects on dough properties. J Cereal Sci. 19:19-28
Gupta RB, Paul JG, Cornish GB, Palmer GA, Bekes F, Rathjen AJ (1994) Allelic variation at glutenin subunit and gliadin loci, Glu-1-Glu-3 and Gli-1 of common wheats. I. Its additive and interaction effects on dough properties. J Cereal Sci. 19: 9–17
Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theo Appl Genet. 77:57–64
Hai L, Yu MW, Ze HY, Bernard B, Eviatar N, You LZ (2005) Classification of wheat low-molecular-weight glutenin

subunit genes and its chromosome assignment by developing LMW-GS group-specific primers. Theor Appl Genet. 111:1251–1259

He XY, He ZH, Ma W, Appels R, Xia XC (2009a) Allelic variants of phytoene synthase 1 (Psy1) genes in Chinese and CIMMYT wheat cultivars and development of functional markers for flour colour. Mol Breed. 23:553–563

He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Xia XC (2007) Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. Theor Appl Genet. 115:47-58

He GY, Rooke L, Steele S, Bekes F, Gras P, Tatham AS, Fido R, Barcelo P, Shewry PR, Lazzeri P A (1999) Transformation of pasta wheat (*Triticum durum* L. var. durum) with high-molecular-weight glutenin subunit genes and modification of dough functionality. Mol Breed. 5:377–396

He ZH, Liu L, Xia XC, Liu JJ, Pena RJ (2005) Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread wheats. Cereal Chem. 82:345-350

He XY, Wang JW, Ammar K, Pena RJ, Xia XC, He ZH (2009b) Allelic variants at the Psy-A1 and Psy-B1 loci in durum wheat and their associations with grain yellowness. Crop Sci. 49:2058–2064

He XY, Zhang YL, He ZH, Wu YP, Xiao YG, Ma CX, Xia XC (2008) Characterization of phytoene synthase 1 gene (Psy1) located on common wheat chromosome 7A and development of a functional marker. Theor Appl Genet. 116:213–221

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

Hirano H, Islam N, Kawasaki H (2004) Technical aspects of functional proteomics in plants. Pytochemistry 65:1487-1498

Howitt CA, Gale KR Juhasz A (2007) Diagnostic markers for quality. In: Wringley C, Bekes F, Bushuk W (eds) Gliadin and Glutenin: The unique balance of wheat quality, AACC International, U.S.A

Huebner FR, Bietz JA 1985 Detection of quality differences among wheats by high-performance liquid chromatography. J Chromat. 327:333-342

Huebner FR, Wall JS (1976) Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258-269

Hussain ss, qamar r (2007) wheat genomics: challenges and alternative strategies. Proc. Pakistan Acad Sci 44:305-327

Ikeda TM, Araki E, Fujita Y, Yano H (2006) Characterization of low-molecular-weight glutenin subunit genes and their protein products in common wheats. Theor Appl Genet. 112:327–334

Ikeda TM, Nagamine T, Fukuoka H, Yano H (2002) Characterization of new low molecular weight glutenin subunit genes in wheat. Theor Appl Genet. 104:680–687

Jackson EA, Holt LM, Payne PI (1983) Characterization of high molecular weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal location of their controlling genes. Theo Appl Genet. 66:29-37

Jackson EA, Morel MH, Sontag-Strohm T, Branlard G, Metakovsky EV, Radaelli R (1996) Proposal for combining the classification systems of alleles of Gli-1 and Glu-3 loci in bread wheat (*Triticum aestivum* L.). J Genet Breed. 50:321–336

Jin H, Yan J, Pena RJ, Xia XC, Morgounov A, Han LM, Zhang Y, He ZH (2011) Molecular detection of high- and low-molecular 4weight glutenin subunit genes in common wheat cultivars from 20 countries using allele-specific markers. Crop Pasture Sci. 62:746-754

Johnson VA, Mattern PJ, Patterson CJ, Kuhr SL (1985) Improvement of Wheat protein by traditional breeding and genetic techniques. Cereal Chem. 62: 350-355

Kadar R, Moldovan V (2003) Achievement by breeding of winter wheat varieties with improved bread-making quality Cereal Res Commun 31: 89-95.

Kadar R, Moldovan V, Tianu M, Marca V (1999) Cercetaqri privind calitatea de panificatie a graului de toamma-In:Contributii ale Cercetaqrii stiintifice la dezvoltarea agriculturii, Tipografia Boema Turda, Vol. VI, 25-34

Khelifi D, Branlard G (1992) The effects of HMW and LMW subunits of glutenin and of gliadins on the technological quality of the progeny from four crosses between poor breadmaking quality and strong wheat cultivars. J Cereal Sci. 16:195-209

Koebner RMD (1995) Generation of PCR-based markers for the detection of rye chromatin in a wheat background. Theor Appl Genet. 90:740-745

Koebner RMD, Summers RW (2002) The impact of molecular markers on the wheat-breeding paradigm. Cell Mol Biol Lett. 7:695-702

Kolster P, Van Eeuwijk FA, Van Gelder WMJ (1991) Additive and epistatic effects of allelic variations at the high molecular weight glutenin subunit loci determining the bread making quality of breeding lines of wheat. Euphytica. 55:277-285

Kovacs MP, Howes NK, Clarke JM, Leisle D (1998) Quality characteristics of durum wheat lines deriving high protein from a *Triticum dicoccoides* (6B) substitution. J Cereal Sci. 27:47-51

Lafiandra D, Tucci GF, Pavoni A, Turchetta T, Margiotta B (1997) PCR analysis of x- and y-type genes present at the complex Glu-A1 locus in durum and bread wheat. Theor Appl Genet. 94:235–240

Lan Q, Feng B, Xu Z, Wang T (2012) Molecular cloning and characterization of five novel low molecular weight glutenin subunit genes from Tibetan wheat landraces (Triticum aestivum L.). Genet Resour Crop Evol. DOI: 10.1007/s10722-012-9877-8

Landjeva S, Korzun V, Börner A (2007) Molecular markers: actual and potential contributions to wheat genome characterization and breeding. Euphytica.156:271-296

Laubin B, Lullien-Pellerin V, Nadaud I, Gaillard-Martinie B, Chambon C, Branlard G (2008) Isolation of the wheat aleurone layer for 2D electrophoresis and proteomics analysis. J Cereal Sci. 48:709-714

Law CN, Young CF, Brown JWS, Snape JW, Worland JW (1978) The study of grain protein control in wheat using whole chromosome substitution lines. In: Seed Protein Improvement by Nuclear Techniques. International Atomic Energy Agency, Vienna, Austria

Liang D, Tang JW, Pena RJ, Singh R, He XY, Shen XY, Yao DN, Xia XC, He ZH (2010) Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. Euphytica. 172:235–250

Lei ZS, Gale KR, He ZH, Gianibelli C, Larroque O, Xia XC, Butow BJ, Ma W (2006) Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the Glu-B1 locus in hexaploid wheat. J Cereal Sci. 43:94-101

Limello M, Morris CF (2000) A leucine to proline mutation in puroindoline b is frequently present in hard wheats from Northern Europe. Theor Appl Genet. 100:1100–1107

Liu S, Chao S, Anderson JA (2008) New DNA markers for high molecular weight glutenin subunits in wheat Theor Appl Genet. DOI 10.1007/s00122-008-0886-0

Liu Y, He Z, Apples R, Xia X (2012) Functional markers in wheat: current stauts and future prospects. Theor Appl Genet. 125: 1-10

Liu L, Ikeda TM, Branlard G, Pena RJ, Rogers WJ, Lerner SE, Kolman MA (2010) Comparison of low molecular weight glutenin subunits identified by SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR in common wheat. BMC Plant Biol. 10:124

Luo C, Griffin WB, Branlard G, McNeil DL (2001) Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. Theor Appl Genet. 2:1088-1098.

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica. 134:51–60

Martinant JP, Nicolasa Y, Bouguennec A, Popineaub Y, Saulnierc L, Branlard G (1998) Relationships between mixograph parameters and indices of wheat grain quality. J Cereal Sci. 27: 179-189

Maucher T, Figueroa JDC, Reule W, Pena RJ (2009) Influence of low molecular weight glutenins on viscoelastic properties of intact wheat kernels and their relation to functional properties of wheat dough. Cereal Chem. 86:372–375

Majoul T, Bancel E, Triboi E, Ben Hamida, J, Branlard G (2003) Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from total endo-sperm. Proteomics. 3:175–183

Mason-Gamer RJ, Weil CF, Kellog EA (1998) Granule bound starch synthase: structure, function, and phylogenetic utility. Mol Biol Evol. 15:1658–1673

Masci S, D'Ovidio R, Scossa F, Patacchini C, Lafiandra D, Anderson OD, Blechl AE (2003) Production and characterization of a transgenic bread wheat line over-expressing a low-molecular-weight glutenin subunit gene. Mol Breed. 12:633-647

McIntosh SR, Watson L, Bundock P, Crawford AC, White J, Cordeiro GM, Barbary D, Rooke L, Henry RJ (2007) SAGE of the most abundant transcripts in the developing wheat caryopsis. Plant Biotechnol J. 5:69-83

Mamone G, Addeo F, Chianese L, Di Luccia A, De Martino A, Nappo A, Formisano A, De Vivo P, Ferranti P (2005) Characterization of wheat gliadin proteins by combined two-dimensional gel electrophoresis and tandem mass spectrometry. Proteomics 5: 2859-2865

Manifesto MM, Feingold S, Hopp HE, Schlatter AR, Dubcovsky J (1998) Molecular markers associated with differences in breadmaking quality in a cross between bread wheat cultivars with the same HMW glutenins. J Cereal Sci. 27:217-227

Md Zaidul IS, Abd Karim A, Manan DMA, Ariffin A, Nik Norulaini, NA, Mohd Omar AK (2004) farinograph study on the viscoelastic properties of sago/wheat flour dough systems. J Sci Food and Agric. 84:616-622

Meng XG, Cai SX (2008) Association between glutenin alleles and Lanzhou alkaline stretched noodle quality of northwest China spring wheats. II. Relationship with the variations at the Glu-1 loci. Cereal Res Commun. 36:107–115

Mesfin A, Frohberg RC, Khan K, Olson TC (2000) Increased grain protein content and its association with agronomic and end use quality in two hard red spring wheat populations derived from *Triticum turgidum* L. var. *dicoccoides*. Euphytica. 116: 237-242

Metakovsky EV (1991) Gliadin allele identification in common wheat II Catalogue of gliadin alleles in common wheat. J Genet Breed. 45:325-344

Metakovsky EV, Felix I, Branlard G (1997) Association between dough quality (W values) and certain gliadin alleles in French common wheat cultivars. J Cereal Sci. 26:371-373

Metakovsky EV, Novoselskaya AY, Kopus MM, Sobko T A, Sozinov AA (1984) Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis. Theor Appl Genet. 67:559-568

Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol. 48:633–647

Morris CF, Beecher BS (2012) The distal portion of the short arm of wheat (Triticum aestivum L.) chromosome 5D controls endosperm vitreosity and grain hardness. Theor Appl Genet 125: 247-254

Murai J, Taira T, Oht D (1999) Isolation and characterisation of the three Waxy genes encoding the granule-bound starch synthase in hexaploid wheat. Gene 234: 71–79.

Nakamura T, Vrinten P, Saito M, Konda M (2002) Rapid classification of partial waxy wheats using PCR-based markers. Genome. 45:1150-1156

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993a) Identification of three Wx proteins in wheat (*Triticum aestivum* L.). Biochem. Genet. 31:75–86

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993b) Decrease of waxy (Wx) protein in 2 common wheat cultivars with low amylose content. Plant Breed. 111:99–105

Ng PKW, Bushuk W (1988) Statistical relationships between high molecular weight subunits of glutenin and bread making quality of Canadian-grown wheats. Cereal Chem. 65: 408-413

Nieto-Taladriz MT, Perretant MR, Rousset M (1994) Effect of gliadins and HMW and LMW subunits of glutenin on dough properties in the F6 recombinant inbred lines from a bread wheat cross. Theo Appl Genet. 88:81–88

Obukhova LV, Maystrenko OI, Generalova GV, Ermakova MF, Popova RK (1997) Composition of HMW Glutenin subunits in common wheat substitution lines from cultivars with contrasting bread making qualities. Russian J Plant Genet. 33:1005-1009

Parker GD, Chalmers KJ, Rathgen AJ, Langridge P (1998) Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.). Theor Appl Genet. 97:238–245

Pasha I, Anjum FM, Morris CF (2010) Grain Hardness: A Major Determinant of Wheat Quality. Food Sci Tech Int. 16:512-522

Paterson AH (1996) Making genetic maps. In: Paterson A H (ed) R. G. Landes Company, San Diego, California; Academic Press, Austin, Texas

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol. 38:141–153

Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theor Appl Genet. 60:229–236

Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Theor Appl Genet. 63:129-138

Payne PI, Jackson EA, Holt LM (1984) The association between γ-gliadin 45 and gluten strength in durum wheat

varieties: a direct causal effect or the result of genetic linkage. J Cereal Sci. 2:73–81

Payne PI, Law C N, Mudd E E (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor Appl Genet 58:113-120.

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric. 40:51–65

Peng J, Sun D, Nevo E (2011) Wild emmer wheat, *Triticum dicoccoides*, occupies a pivotal position in wheat domestication. AJCS 5:1127-1143

Pogna NE, Autran JC, Mellini F, Lafiandra D, Feillet P (1990) Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to glutenin strength. J Cereal Sci.11:15–34

Prandi B, Bencivenni M, Tedeschi T, Marchelli R, Dossena A, Galaverna G, Sforza S (2012) Common wheat determination in durum wheat samples through LC/MS analysis of gluten peptides. Analytical Bioanalytical Chem 403: 2909-2914

Radovanovic N, Cloutier S (2003) Gene-assisted selection for high molecular weight glutenin subunits in wheat doubled haploid breeding programs. Mol Breed. 12:51–59

Radovanovic N, Cloutier S, Brown D, Humphreys DG, Lukow OM (2002) Genetic variance for gluten strength contributed by high molecular weight glutenin proteins. Cereal Chem. 79:843–849

Ragupathy R, Naeem HA, Reimer E, Lukow OM, Sapirstein HD, Cloutier S (2008) Evolutionary origin of the segmental duplication encompassing the wheat GLU-B1 locus encoding the overexpressed Bx7 (Bx7OE) high molecular weight glutenin subunit. Theor Appl Genet. 116:283–296

Ram S (2003) High Molecular Weight Glutenin Subunit Composition of Indian Wheats and Their Relationships with Dough Strength. J Plant Biochemistry Biotechnology. 12: 151-155

Ram S, Sharma S, Verma A, Tyagi BS, Pena RJ (2011) Comparative analyses of LMW glutenin alleles in bread wheat using allele-specific PCR and SDS-PAGE. J Cereal Sci. 54:488–493

Rogers WJ, Payne PI, Seekings JA, Sayers EJ (1991) Effect on bread-making quality of x-type and y-type high molecular weight subunits of glutenin. J Cereal Sci. 14:209- 221

Ribeiro M, Carvalho C, Carnide V, Guedes-Pinto H, Igrejas G (2011) Towards allelic diversity in the storage proteins of old and currently growing tetraploid and hexaploid wheats in Portugal. Genet Resour Crop Evol. 58:1051-1073

Rosegrant, MW, Agcoili M (2010) Global food demand, supply and food prospects. International food policy research Institute, Washington, D.C., USA.

Ruiz M, Carrillo JM (1993) Linkage relationships between prolamin genes on chromosomes 1A and 1B of durum wheat. Theor Appl Genet. 87:353-360

Saito M, Konda M, Vrinten\\ P, Nakamura K, Nakamura T (2004) Molecular comparison of waxy null alleles in common wheat and identification of a unique null allele. Theo Appl Genet. 108:1205–1211

Saito M, Vrinten P, Ishikawa G, Graybosch R, Nakamura T (2009) A novel codominant marker for selection of the null Wx-B1 allele in wheat breeding programs. Mol Breed. 23:209–217

Saito M, Vrinten P, Narkamura T (2010) DNA markers for identifying waxy mutations and improving noodle quality in wheat. Jpn Agri Res Quart. 44:109–115

Schwarz G, Felsenstein FG, Wenzel G (2004) Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele Glu-B1-1d (Bx-6) in wheat. Theor Appl Genet. 109:1064–1069

Shariflou MR, Hassani M, Sharp PJ (2001) A PCR-based DNA marker for detection of mutant and normal alleles of the Wx-D1 gene of wheat. Plant Breed. 120:121–124

Shewry PR, Halford NG and Tatham AS (1992) High molecular-weight subunits of wheat glutenin. J Cereal Sci. 15:105–120

Shewry PR and Tatham AS (1997) Disulphide bonds in wheat gluten proteins. J Cereal Sci. 25:207-227

Shewry PR, Tatham AS, Barro F, Barcelo P and Lazzeri P (1995) Biotechnology of breadmaking: unrevealing and manipulating the multi-protein gluten complex. Biotechnology. 13:1185-1190

Schmidt JW, Mattern PJ, Johnson VA, Morris R (1974) Investigations of the genetics of bread wheat baking quality. Genetics Lectures, Vol. III: 83-101

Si H, Zhou Z, Wang X, Ma C (2012a) A novel molecular marker for the polyphenol oxidase gene located on chromosome 2B in common wheat. Mol Breed. DOI 10.1007/s11032-012-9723-x.

Si H, Ma C, Wang X, He X (2012b) Variability of polyphenol oxidase (PPO) alleles located on chromosomes 2A and 2D can change the wheat kernel PPO activity. Aus J Crop Sci. 6:444-449

Singh AM, Deveshwar JJ, Ahlawat AK, Singh BB (2007) Identification of novel variants of High Molecular Weight Glutenin Subunits in Indian bread wheat landraces. Cereal Res Commu. 35:99-108

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

Singh R, Goutam U, Gupta RK, Pandey GC, Shoran J, Tiwari R (2009a) Allelic variations of functional markers for polyphenol oxidase (PPO) genes in Indian bread wheat (Triticum aestivum L.) cultivars. J Genet 88 : 325–329

Singh R, Tiwari R, Priyamvada, Gupta RK, Shoran J, Mishra B (2009b) 1RS.1BL translocation and grain yield as well as bread loaf volume in Indian wheats. Cereal Res Commu 37:441-448

Skerritt JH (1998) Gluten proteins: genetics, structure and dough quality- a review. AgBiotech News Information. 10(8):247-270

Skylas DJ, Mackintosh JA, Cordwell SJ, Walsh BJ, Harry J, Blumenthal C, Copeland L, Wrigley CW, Rathmell WG (2000) Proteome approach to the characterisation of protein composition in the developing and mature wheat-grain endosperm. J Cereal Sci. 52:169-188

Skylas DJ, Van Dyk DV, Wrigley CW (2005) Proteomics of wheat grain. J Cereal Sci. 41:165-179

Smith RL, Schweder ME, Barnett RD (1994) Identification of glutenin alleles in wheat and triticale using PCR-generated DNA markers. Crop Sci. 34:1373–1378

Sobrino B, Brion M, Carracedo A (2005) SNPs in forensic genetics: A review on SNP typing methodologies. Forensic Sci Int. 154:181-194

Stevenson SG, Preston KR (1996) Flow field-flow fractionation of wheat proteins. J Cereal Sci. 23:121-131

Stuber CW, Polacco M, Senior ML (1999) Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci. 39:1571–1583

Sun YW, He ZH, Ma WJ, Xia XC (2011) Alternative splicing in the coding region of Ppo-A1 directly influences the

polyphenol oxidase activity in common wheat (*Triticum aestivum* L.). Funct Integr Genomics. 11:85–93

Sun DJ, He ZH, Xia XC, Zhang LP, Morris CF, Appels R, Ma WJ, Wang H (2005) A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breed. 16:209-218

Symes KJ (1969) Influence of a gene causing hardness on the milling and baking quality of two wheats. Aust J Agric Res. 20:971–979

Thiellement H, Bahrman N, Damerval C, Plomion C, Rossignol M, Santon PY, de Vienne D, Zivy M (1999) Review: Proteomics for genetic and physiological studies in plants. Electrophoresis. 20:2013-2026

Thiellement H, Zivy M, Plomion C (2002) Review: Combining proteomic and genetic studies in plants. J Chromat. 782:137-149

Tosi P, Masci S, Giovangrossi A, D'Ovidio R, Bekes F, Larroque O, Napier J, Shewry P (2005) Modification of the low molecular weight (LMW) glutenin composition of transgenic durum wheat: effects on glutenin polymer size and glulten functionality. Mol Breed. 16:113-126

Tranquilli G, Lijavetzky D, Muzzi G, Dubcovsky J (1999) Genetic and physical characterization of grain texture-related loci in diploid wheat. Mol Gen Genet. 262: 846-850

Turner M, Mukai Y, Leroy P, Charef B, Appels R, Rahman S (1999) The *Ha* locus of wheat: Identification of a polymorphic region for tracing grain hardness in crosses. Genome. 42:1242-1250

Uauy C, Distelfeld A, Fahima T, Blechi A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein zinc and iron content in wheat. Science. 314:1298-1301

Vanzetti LS, Pfluger LA, Rodríguez-Quijano M, Carrillo JM, Helguera M (2009) Genetic variability for *waxy* genes in Argentinean bread wheat germplasm. Electron J Biotechnol. DOI: 10.2225/vol12-issue1-fulltext-2

Vasil V, Srivastava V, Castillo AM, Fromm ME, Vasil IK (1993) Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. Biotechnology. 11:1553–1558

Vensel WH, Tanaka CK, Cai N, Wong JH, Buchanan BB, Hurkman WJ (2005) Developmental changes in the metabolic protein profiles of wheat endosperm. Proteomics 5:1594-1611

Vrinten P, Nakamura T, Yamamori M (1999) Molecular characterization of waxy mutations in wheat. Mol Gen Genet. 261:463–471

Wahlund KG, Gustavsson M, MacRitchie F, Nylander T, Wannerberger L (1996) Size characterization of wheat proteins, particularly glutenin, by asymmetrical flow field-flow fractionation. J Cereal Sci. 23:113-119

Wang XB, Ma CX, Si HQ, Qiao YQ, Chang C, He XF, Xia YX (2009a) Gene markers for grain polyphenol oxidase activity in common wheat. Mol Breed. DOI 10.1007/s11032-008-9223-1

Wang LH, Li GY, Pena RJ, Xia XC, He ZH (2010) Development of STS markers and establishment of multiplex PCR for Glu-A3 alleles in common wheat (*Triticum aestivum* L.). J Cereal Sci. 51:305–312

Wang LH, Zhao XL, He ZH, Ma W, Appels R, Pena RJ, Xia XC (2009b) Characterization of low-molecular-weight glutenin subunit Glu-B3 genes and development of STS markers in common wheat (Triticum aestivum L.). Theor Appl Genet. 118:525–539

Weegels PL, Hamer RJ, Schofield JD (1996) Functional properties of wheat glutenin. J Cereal Sci. 23:1-18

Wickramasinghe HAM, Miura H (2003) Gene dosage effects of the wheat *Wx* alleles and their interaction on amylose synthesis in the endosperm. Euphytica. 132:303–310

Williams HM, Trethowan R, Crosby-Galvan EM (2007) Wheat breeding assisted by markers: CIMMYT's experience. Euphytica. 157:307–319

William M, Landrige P, Trethowan R, Dreisigacker S, Crouch J (2008) Genomics of Wheat: the basis of our daily Bread. In: Moore P H, Ming R (eds) Genomics of Tropical Crop Plants, Springer

Wilson ID, Barker GLA, Beswick RW (2004) A transcriptomics resource for wheat functional genomics. Plant Biotechnology J. 6:495–506

Winter P, Kahl G (1995) Molecular marker technologies for plant improvement. World J Microbio Biotechnol.11:438–448

Wrigley CW, Lawrence GJ, Shepherd D K W (1982) Association of glutenin subunits with gliadin composition and grain quality in wheat. Aus J Plant Physiol. 9: 15.

Wrigley CW (1996) Giant proteins with flour power. Nature. 381:738-739

Xiao YG, He XY, Liu JJ, Sun DJ, Xia XC, He ZH (2008) Molecular identification and distribution of the polyphenol oxidase genes in Chinese winter wheat cultivars. Sci Agric Sin. 41:954–960

Xu Q, Xu J, Liu CL, Chang C, Wang CP, You MS, Li BY, Liu GT (2008) PCR-based markers for identification of HMW-GS at Glu-B1x loci in common wheat Journal of Cereal Sci. 47:394–398

Yamamori M, Nakamura T, Endo T, Nagamine T (1994) Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor Appl Genet. 89:179–184

Yanagisawa T, Kiribuchi-Otobe C, Hirano H, Suzuki Y, Fujita M (2003) Detection of single nucleotide polymorphism (SNP) controlling the waxy character in wheat by using a derived cleaved amplified polymorphic sequence (dCAPS) marker. Theor Appl Genet. 107:84–88

Yang FP, Wang LH, Wang JW, He XY, Zhang XK, Shang XW , Yang WX, Xia XC, He ZH (2010) Characterisation of high- and low-molecular-weight glutenin subunit genes in Chinese winter wheat cultivars and advanced lines using allele-specific markers and SDS-PAGE. Crop Pasture Sci. 61:84–91

Yediay FM, Baloch FS, Kilian B, Hakan H (2010) Testing of rye-specific markers located on 1RS chromosome and distribution of 1AL.RS and 1BL.RS translocations in Turkish wheat (*Triticum aestivum* L., *T. durum* Desf.) varieties and landraces. Genet Resour Crop Evol. 57:119–129

Zhang CY, Dong CH, He XY, Zhang LP, Xia XC, He ZH (2011b) Allelic variation at the TaZds-D1 locus on wheat chromosome 2DL and their association with yellow pigment content. Crop Sci. 51:1580–1590

Zhang W, Gianibelli MC, Ma W, Rampling L, Gale KR (2003) Identification of SNPs and development of allele-specific PCR markers for γ-gliadin alleles in *Triticum aestivum*. Theor Appl Genet. 107:130–138

Zhang W, Gianibelli MC, Rampling L, Gale KR (2004) Characterisation and marker development for low molecular weight glutenin genes from Glu-A3 alleles of bread wheat (*Triticum aestivum* L.). Theor Appl Genet. 108:1409–1419

Zhang X, Liu D, Yang W, Liu K, Sun J, Guo X, Li Y, Wang D, Ling H, Zhang A (2011a) Development of a new marker system for identifying the complex members of the low-molecular-weight glutenin subunit gene family in bread

wheat (Triticum aestivum L.) Theor Appl Genet. 122: 1503–1516.

Zhao XL, Ma W, Gale KR, Lei ZS, He ZH, Sun QCX, Xia XC (2007b) Identification of SNPs and development of functional markers for LMW-GS genes at Glu-D3 and Glu-B3 loci in bread wheat (*Triticum aestivum* L.). Mol Breed. 223-231

Zhao XL, Xia XC, He ZH, Gale KR, Lei ZS, Appels R, Ma W (2006) Characterization of three low-molecular-weight *Glu-D3* subunit genes in common wheat. Theor Appl Genet. 113:1247-1259

Zhao XL, Xia XC, He ZH, Lei ZS, Appels R, Yang Y, Sun QX, Ma W (2007a) Novel DNA variations to characterize low molecular weight glutenin *Glu-D3* genes and develop STS markers in common wheat. Theor Appl Genet.114:451–460