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

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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 Agcaolli, 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 (Codinà et al., 2012) can estimate the dough mixing or viscoelastic 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. dicoccoides 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 chilense 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 (Table 1). 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, Rodgers 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 strength and P tenacity); and the Zeleny sedimentation value are correlated positively with subunits 7b9 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 IBs7. Radovanovic et al. (2002) analyzed 162 doubled haploid lines and found that 1D5+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).**

<table>
<thead>
<tr>
<th>Quality score of each subunit</th>
<th>HMW-GS at Glu-1</th>
</tr>
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<tbody>
<tr>
<td>Glu-A1</td>
<td>Glu-B1</td>
</tr>
<tr>
<td>1</td>
<td>Null</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2*</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
</tbody>
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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; Wrilegy 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 Perulfo 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., 2003). 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 gliadens (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 gliadin 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; Lafuanda 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.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene/locus</th>
<th>Allele/s</th>
<th>Marker</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Dough Strength</td>
<td>Glu-A1</td>
<td>Ax, Ax null, Ax1, Ax2*, Ax2*B, Ay</td>
<td>Allele specific</td>
<td>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)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bx, Bx6, Bx7/Bx17, Bx7*, Bx7*DE, Bx13, Bx14, By8, By9, By16 &amp; By null or (20) Ds, Ds, Ds5, Ds10/Ds12</td>
<td>Allele specific</td>
<td>Ahmad (2000), Ma et al. (2003), Radovanovic and Cloutier (2003), Butow et al. (2003), Butow et al. (2004), Schwartz et al. (2004), Xu et al. (2008), Ragupathy et al. (2008), Espi et al. (2012), Lei et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Glu-D3</td>
<td>Glu-D3alleles Secalin-2, Glu-B3j</td>
<td>STS, Allele specific</td>
<td>Zhao et al. (2007a), Appelbee et al. (2009)</td>
</tr>
<tr>
<td>Grain hardness</td>
<td>pina, pinb</td>
<td>Pina-D1a,b, Pinb-D1a,b, Pinb-D1c, Pina-D1r, Wx-B1a, Wx-D1a</td>
<td>CAPS/STS</td>
<td>Gautier et al. (1994), Giroux and Morris (1997), Limello and Morris (2000), Morris (2002), Chen et al. (2012)</td>
</tr>
<tr>
<td>(Puroindolines)</td>
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<td></td>
<td>Ppo-D1</td>
<td>Ppo-D1a, Ppo-D1a b</td>
<td></td>
<td>He et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Ppo-B1</td>
<td>Ppo-B1a, Ppo-B1b</td>
<td></td>
<td>Si et al. (2012a)</td>
</tr>
<tr>
<td>Lipoygenase activity</td>
<td>TaLox-B1</td>
<td>TaLox-B1a, TaLox-B1b</td>
<td>STS</td>
<td>Geng et al. (2012)</td>
</tr>
<tr>
<td>Yellow pigment content</td>
<td>Psy-A1</td>
<td>Psy-A1a,b</td>
<td>STS</td>
<td>He et al. (2008, 2009a)</td>
</tr>
<tr>
<td></td>
<td>Psy-B1</td>
<td>Psy-B1a,b,Psy-B1a,b, Psy-B1a,c,d,e</td>
<td></td>
<td>He et al. (2009a)</td>
</tr>
<tr>
<td></td>
<td>Psyl-D1</td>
<td>Psyl-D1a,g</td>
<td></td>
<td>Wang et al. (2009b)</td>
</tr>
<tr>
<td></td>
<td>TaZds-A1</td>
<td>TaZds-A1a,b</td>
<td></td>
<td>Dong et al. (2010)</td>
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<td></td>
<td>TaZds-D1</td>
<td>TaZds-D1a,b</td>
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<td>Zhang et al. (2011b)</td>
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</table>

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 Ds2 and Ds5 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 Ds5 gene with two internal polymorphic nucleotides within the forward primer and one polymorphic nucleotide within the reverse primer relative to the Ds2 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, Ds5, Dy10, Ds2 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 γ-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, Rapaghaty 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 (GI-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 synthase (GBSSI) 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 GBSSI 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 GBSSI 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 (Murali et al., 1999; Vrinten et al., 1999), a set of primer pairs was designed for the assay of each GBSSI homeoallele (Nakamura et al., 2002). These markers developed were suitable for the analysis of GBSSI 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 GBSSI enzyme due to presence of the SNP. Characterization of 103 Argentinian 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-A1u, 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).
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 (1997, 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. Linello 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 breed 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 (Pinb-D1b, Pinb-D1h, Pinb-D1l 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 null-tetrasomic lines of bread wheat cultivar Chinese Spring and substitution lines of durum wheat (Triticum turgidum L.) cultivar Langdon.

**Markers for Polyphenol oxidase activity**

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, STS01 and 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 (Li 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 F4 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-A1u 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 (Hesseler et al., 2002; Carrera et al., 2007; Geng et al., 2011). A bright yellow color (Hesseler 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 Phytorene 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 translocation

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 (Dhalwal 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 u-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 (Yediyi 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 gluten 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 electrophoresis (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 (ThillEremeev, 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 prolamin 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-throughput 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.

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