Chapter 1

Marker-Assisted Breeding in Wheat

Nana Vagndorf, Peter Skov Kristensen, Jeppe Reitan Andersen, Ahmed Jahoor and Jihad Orabi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74724

Abstract

Selection is an integral component in plant breeding, which ensures the progressive values of the breeding material, in terms of yield and quality. However, selection is influenced by the environment in any given growing season. The observed phenotype is a product of the genotype (G), the environment (E), and/or genotype × environment (G×E). Therefore, phenotypic selection is not always the best predirector of the genotype. Therefore, an environment-independent method is preferred by the breeder. The development of molecular markers in plants has facilitated marker-assisted selection (MAS). MAS requires the establishment of correlation between a desired trait such as disease resistance and molecular marker(s). This can be obtained, e.g., by phenotyping a genetic mapping population followed by QTL analysis. Initially, this process was slow due to the laborious nature of the first DNA molecular marker system, such as restriction fragment length polymorphism (RFLP). Later, with the discovery of various marker systems amenable to automation and the development of genotyping techniques and instruments, MAS has become a standard procedure in plant breeding. In wheat breeding, MAS helped to accelerate the introgression of many genes that contribute to improve quality and resistance.

Keywords: wheat, marker-assisted breeding, molecular markers, wheat diseases, wheat quality

1. Introduction

Wheat is one of the most important sources of food worldwide. Data from FAOSTAT indicate that the need is still growing, indicated by the steadily increasing yield since 1961 (**Figure 1**). The need for an enhanced wheat production combined with stagnation in the area cultivated (**Figure 1**) leads to a demand for a more effective and higher yielding wheat production.

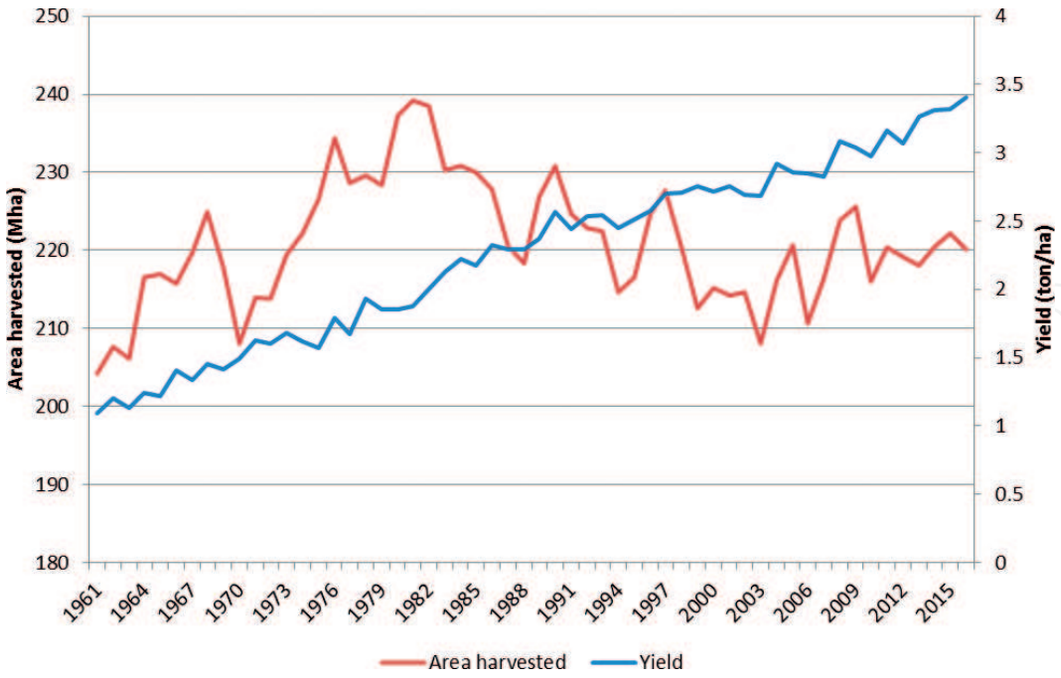

Figure 1. Development in wheat cultivation in the years 1961–2016. The primary y-axis displays the area harvested in Mha (red line) and the secondary y-axis displays the yield in tones pr. ha (blue line). Data from FAOSTAT.

Marker-assisted selection (MAS) or molecular breeding offers an opportunity to accelerate the traditional breeding. Traditional breeding is based on phenotypic selection of genotypes obtained from crosses. Genotype × environment (G×E) interaction is a common problem including time-consuming and costly procedures of phenotyping. By employing molecular markers, desirable genes can be fixated in early generations of the breeding program. In addition, molecular markers are unaffected by environmental conditions and are detectable in all stages of the plant growth. Scientists and breeders across the world implement MAS in breeding programs [1].

2. Linkage and molecular markers

MAS is based on the concept of genetic linkage between loci. This describes the tendency of loci located closely together on the same chromosome being more likely to be inherited together in a recombination event during meiosis. Thus, two alleles located very close on the chromosome will almost always be inherited together.

Molecular markers are used in MAS to highlight a place on the chromosome close to or in a specific gene of interest. The technique is based on detecting different alleles (polymorphisms) between several individuals. Due to genetic linkage, the molecular marker will reveal if the linked allele is present or not in a line. Several types of molecular markers exist, depending on the type of polymorphism. In today's MAS, markers detecting single nucleotide differences are usually employed.

3. Application of MAS in breeding for disease resistance in wheat diseases

Plant diseases are a major constraint in wheat production and significant resources are allocated to control various diseases. The relatively long growing season of winter wheat renders it vulnerable to a range of diseases and breeding for disease resistance is generally thought to be the first line of defence [2]. Disease resistance is generally separated into quantitative and qualitative resistance. Qualitative resistance is most often controlled by a single gene and follows the gene-for-gene hypothesis. Thus, an R gene in the host can specifically interact with an *Avr* gene in the pathogen to induce a defence reaction in the host. Qualitative resistance often mediates a complete resistance response, whereas quantitative resistance is regarded as an incomplete or partial resistance. This type of resistance is usually mediated by several minor genes, which are designated as a quantitative trait locus (QTL) [3]. Following the development of MAS, targeted pyramiding of several resistance genes in single lines is now possible. In the following sections, three severe diseases of wheat and correlated resistance genes are described. Common to these diseases is that fungicides are becoming less efficient. Hence, more effective approaches are desired.

3.1. Septoria tritici blotch

One of the most important foliar diseases in wheat is Septoria tritici blotch (STB), caused by the fungus *Zymoseptoria tritici* formally known as *Septoria tritici* (anamorph) and *Mycosphaerella graminicola* L (teleomorph) [4]. STB is a devastating disease causing massive yield losses worldwide every year in wheat. Severe epidemics can reduce wheat yield by 35–50% [5]. Symptoms of the disease are chlorotic lesions on the leaf with black fruiting bodies containing fungal spores. Breeding strategies over the years have primarily focused on breeding for higher yield, in turn increasing the susceptibility towards STB [6]. Disease control can be performed by delaying sowing time, probably due to less time in the autumn for *Z. tritici* to infect seedlings and produce inoculum. Furthermore, the application of fungicides and implementation of resistant cultivars in breeding programs are widely used [5]. In previous years, several studies have identified STB resistance genes using molecular techniques.

Stb1, *Stb2* and *Stb3* were the first qualitative genes for STB resistance to be named [7]. Prior to that, STB resistance was thought of as a quantitative, polygenic trait. *Stb1* was mapped to the long arm of chromosome 5B in the cultivar Bulgaria88 [8]. *Stb2* was mapped to the short arm of chromosome 1B in the cultivar Veranopolis [9]. Additionally, *Stb11* was mapped to the short arm of 1B [10]. However, no studies have included an allelism test of *Stb2* and *Stb11*. *Stb3* was mapped to the short arm of 7A in Israel493 [11]. *Stb6* was mapped to the short arm of chromosome 3A in the cultivar Flame [12]. This gene is the only STB-resistant gene found to possess a gene-for-gene relationship, in which a specific R gene in the host interacts with an *Avr* gene in the pathogen. This was demonstrated in a study where Flame was found to confer specific resistance towards the *Z. tritici* isolate IPO323 [12]. This study conforms to the original gene-for-gene model proposed by Flor [13]. *Stb6* was subsequently found to be one of the most abundant STB-resistant genes in European wheat [14]. In total, 18 *Stb* genes have been

identified and mapped using various molecular markers. Additionally, several QTL have been identified conferring STB resistance [7, 15]. **Table 1** summarises major STB-resistance genes together with linked markers suitable for MAS. Additionally, several QTL have been identified conferring STB resistance [7, 15].

One of the more promising resistance genes identified in recent years is *Stb16*. This gene was identified in synthetic hexaploid wheat lines, which represent a rich source of variation [23]. *Stb16* explained a high proportion of STB disease resistance and conferred resistance at the seedling stage to all tested *Z. tritici* isolates. Moreover, 20 tested isolates were all avirulent to this gene, indicating that *Stb16* confers broad-spectrum resistance. If this is the case, *Stb16* holds promise for future breeding of efficient and durable STB resistance.

In order to obtain the most resistant wheat variety, breeders should take a number of things into account. Since qualitative resistance genes often conform to the gene-for-gene hypothesis, they are readily overcome by the pathogen. Due to the high frequency of genetic recombination of *Z. tritici,* the specific recognition of R proteins by the host is lost [26]. Furthermore, the strong

Resistance gene	Marker type	Marker name	Location	Reference
Stb1	SSR	Xbarc74, Xgwm335	5BL	[8]
Stb2	SSR	Xwmc406, Xbarc008	1BS	[9]
Stb3	SSR	Xwmc83	7AS	[11]
Stb4	SSR	Xgwm111, Xgwm44	7DS	[16]
Stb5	SSR	Xgwm44	7DS	[17]
Stb6	SSR	Xgwm369	3AS	[12]
Stb7	SSR	Xgwm160, Xwmc219, Xwmc319	4AL	[18]
Stb8	SSR	Xgwm146, Xgwm577	7BL	[19]
Stb9	SSR	Xfbb226, XksuF1b	2BL	[20]
Stb10	SSR	Xgwm848	1D	[21]
Stb11	SSR	Xbarc008	1BS	[22]
Stb12	SSR	Xwmc219, Xgw313	4AL	[21]
Stb13	SSR	Xwmc396	7BL	Wheat gene catalogue
Stb14	SSR	Xwmc500, Xwmc632	3BS	Wheat gene catalogue
Stb16	SSR	Xgwm494	3DL	[23]
Stb17	SSR	Xhbg247	5AL	[23]
Stb18	SSR	Xgpw5176, Xgpw3087	6DS	[24]
StbWW	SSR	Xbarc119b	1BS	[25]

The name of the resistance gene, marker type, marker name, the location on the genome and the reference are indicated.

Table 1. An overview of the named and mapped genes for STB resistance.

selection pressure placed on the pathogens by one major resistance gene promotes the rise of new adapted races in the pathogen population [27]. An earlier study has proved that commercial cultivation of a highly resistant cultivar can result in loss of resistance towards STB. The intensive cultivation of the variety Gene in the 1990s in Oregon, US, resulted in resistance breakdown. Gene was found to be resistant to two specific isolates, which were avirulent to *Stb6* and *Stb10* [28]. Gradually, an adaptation of *Z. tritici* to one of the resistance genes occurred and the resistance was lost [26]. In general, it appears that most *Z. tritici* isolates used in earlier studies are virulent to almost all *Stb* genes [7]. This may indicate that *Z. tritici* easily can overcome single qualitative resistance genes. In contrast, the quantitative resistance is regarded as more durable. This is due to a lower selection pressure on the pathogen as a result of smaller resistance effects of individual QTL [3]. Furthermore, since quantitative resistance is often polygenic, the mutation of one gene does not necessarily break down disease resistance completely.

3.2. Fusarium head blight

Fusarium head blight (FHB) is an important disease in all wheat growing countries. Epidemics occur frequently, especially under seasons with regular rainfall [29]. The United States Department of Agriculture (USDA) has stated that FHB is the most devastating plant disease since the rust epidemics in the 1950s. FHB contaminates the grain with mycotoxins, in turn restricting its use for both animal and human consumption [30]. The disease is caused by several species of *Fusarium*; however, the predominant causal agent is the fungus *Fusarium graminearum* (teleomorph *Gibberella zeae*). The first symptoms of FHB on wheat plants occur shortly after flowering as diseased spikelets display premature bleaching. The bleaching usually spreads to the whole spike as the pathogen grows. When conditions are optimal for the pathogen, i.e., in a warm and moist environment, light pink coloured spores, called sporodochia, appear on individual spikelets. Later during the season, black fruiting bodies will appear. These are the sexual structures of the fungus, called perithecia. Disease progression results in shrinking and wrinkling of the grain inside the spike. As with the pathogen causing STB, *F. graminearum* produces both sexual and asexual spores: ascospores and macroconidia, respectively [30]. The major toxin produced by FHB in wheat is deoxynivalenol (DON). DON is a protein synthesis inhibitor also known as vomitoxin due to its negative impact on the digestive system of pigs. Several recommendations and restrictions have been made in order to keep DON levels sufficiently low in wheat for both animal and human consumption [31].

Chemical control and crop management are not sufficient to control FHB; thus, breeding resistant varieties plays a key role. Conventional breeding involves repeated testing of breeding lines under natural or artificial inoculations. This process is time-consuming, costly, and prone to influence by environment. Thus, it is relevant to supplement phenotypic selection with MAS for FHB resistance. [32]. FHB resistance is generally divided into three types: resistance to initial infection (type I), resistance to spreading of the pathogen in infected tissue (type II) and resistance to DON accumulation (type III) [33]. Several studies have demonstrated that FHB resistance is of quantitative nature [29]. Furthermore, the expression of resistance is highly dependent on the pathogen, the environment and the host [34], in turn complicating phenotypic selection. Several QTL for FHB resistance have been identified and

located during recent years [29]. The first QTL for type II resistance was identified in the spring wheat 'Sumai 3' on chromosome 3BS. This QTL was named *Fhb1* and characterised by molecular markers [35–37]. Recently, *Fhb1* was cloned from Sumai 3 and a pore-forming toxin-like (PFT) gene was found to confer FHB resistance [38]. *Fhb1* has been found to reduce FHB disease severity tremendously and MAS is employed to incorporate the resistance in breeding programs [29]. A QTL, named *Fhb2,* on chromosome 6BS was found to confer type II FHB resistance [39, 40]. Additionally, *Fhb4* was identified and located on chromosome 4B [41]. **Table 2** lists all FHB-resistant genes identified by molecular markers. Currently, breeders are pyramiding *Fhb1, Fhb2* and *Fhb4* in single breeding lines to obtain optimal FHB resistance [34]. Several additional QTL have been identified and located in numerous studies [29].

Resistance gene	Marker type	Marker name	Location	Reference
Fhb1	SSR	Xgwm493, Xgwm533	3BS	[42]
Fhb2	SSR	Xgwm133, Xgwm644	6BS	[40]
Fhb4	SSR	Xhbg226, Xgwm149	4B	[41]
Fhb5	SSR	Xgwm304, Xgwm415	5A	[43]
Fhb6	KASP	Wg1s_snp1	1AS	[44]
Fhb7	SSR	XsdauK66, Xcfa2240	7DS	[45]

Table 2. Overview of the FHB-resistant genes identified in wheat using molecular markers.

3.3. Wheat stripe rust (yellow rust)

Wheat stripe rust, mostly designated as 'yellow rust' (YR), causes major yield losses every year. The disease is caused by *Puccinia striiformis,* which belongs to the family *Pucciniaceae* of rust fungi. The most devastating epidemics occur in temperate areas with cool and humid summers or in warmer areas with cool nights. The fungus is heteroecious, i.e., it requires at least two hosts in order to proliferate. *P. striiformis* uses cereals as a primary host and *Berberis* spp. as a secondary host for sexual recombination. Typical, yellow stripes develop on the leaf in lesions. Spores continue to be produced as stripes spread longitudinally on the leaf. After the onset of senescence, *P. striiformis* will produce teliospores. Teliospores can infect the secondary host, *Berberis* spp., and initiate onset of pycnia infection of the *Berberis* leaf [46].

Breeding for YR resistance was initiated in 1905 by Biffen [47]. To date, more than 70 genes (*Yr* genes) conferring YR resistance have been identified [48]. Most of the catalogued genes confer seedling resistance, while relatively few confer adult plant resistance. In general, studies have shown that seedling resistance is conferred by single genes and the resistance is therefore easily overcome by the pathogen by mutations in virulence genes. Adult plant resistance is generally thought to be more durable [49]. High-temperature adult plant (HTAP) genes are expressed as the plants grow older and the weather becomes warmer [50]. HTAP genes confer a non-specific, quantitative resistance. Studies have proven that varieties with HTAP genes display resistance

Resistance gene	Marker type	Marker name	Location	Reference
Yr5	SSR	Xgwm501	2BL	[55]
Yr7	SSR	Xgwm526	2BL	[56]
Yr15	SSR	Xbarc8, Xgwm493	1BS	[57]
Yr18	CAPS	Cssfr6	7D	[58]
Yr36	SSR	Xgwm508, Xbarc136	6BS	[54]
Yr60	SSR	Xwmc776	4AL	[59]
Yr76	SSR	Xwmc11, Xwmc532	[60]	
Yr78	SNP	IWA7257	6BS	[61]

Table 3. A selection of the genes conferring YR resistance identified by molecular markers.

to YR even after having been cultivated for 60 years [51]. Additionally, several studies have mapped QTL to all wheat chromosomes except chromosome 1D and 3A [49]. Commonly used resistance genes employed in wheat breeding programs include *Yr*18, *Yr*29 and *Yr*36 [52–54]. *Yr36* is tightly linked to *Gpc-B*1, a high-protein gene, rendering varieties with *Yr36* and *Gpc-B*1 useful in breeding for YR resistance and improved quality. **Table 3** lists a selection of *Yr* genes that have been characterised and mapped with molecular markers suitable for MAS.

Several incidences have been reported where *Yr* genes have been classified as ineffective. Some of the most widely used resistance genes including *Yr17* [62], *Yr27* [63] and *Yr31* [64] have recently lost resistance towards YR.

4. Marker-assisted wheat breeding for improving quality traits

Wheat is grown in large parts of the world and is used for animal feed or for a wide range of products such as pasta, biscuits, cakes and bread. The end-use quality differs greatly between wheat cultivars and is influenced by several traits, e.g., grain hardness, grain protein content, gluten content and composition and starch properties. Quality should therefore be an important focus in wheat breeding programs. However, wheat quality cannot be easily determined phenotypically, and different methods are preferred in different countries and industries. Methods for testing quality are typically time-consuming and costly and require relatively large amounts of grain, which is typically not available until late stages of breeding programs. Thus, markers for wheat quality traits can be very useful, as they enable screening of a high number of lines and can be used early in breeding programs [65, 66].

4.1. Grain hardness

Grain hardness influences milling, flour and end-use properties of wheat. Flour from grain with hard endosperm texture has higher water absorption than flour from soft grain and is therefore preferred for bread-making. A soft endosperm texture leads to less starch granule damage

during the milling and consequently to lower water absorption, which is preferred in the production of biscuits and cakes. Grain hardness is primarily controlled by the *Hardness* locus on chromosome 5DS. This locus consists of three small genes: *Pina-D1*, *Pinb-D1* (*Puroindoline a/b*) and *grain softness protein-1 (Gsp-1*). Wheat varieties with the wild-type alleles *Pina-D1a* and *Pinb-D1a* normally have soft grain, while deletions or other loss-of-function mutations in one or both *Pin* genes cause harder grain (**Table 4**) [67, 68]. *Pinb-D1* mutations are positively associated with many quality traits, but the alleles are not equally useful in breeding for improved quality. *Pinb-D1d* has been reported to have a lower effect on gluten quality and loaf volume than the *b*- or *c*-allele [69]. Alleles of *Pinb-D1* can be detected using PCR primers that target a specific mutation (*Pinb-D1b*), using a restriction enzyme on the amplified *Pinb-D1* gene (*Pinb-D1c*), or by sequencing the amplified gene (*Pinb-D1d-g*) [67, 70, 71].

Allele	Change in protein	Primer sequences, 5′–3′	PCR product	References
Pina-D1a	Wild-type	F: ATGAAGGCCCTCTTCCTCA R: TCACCAGTAATAGCCAATAGTG	448 bp	[73, 74]
Pina-D1b	Large deletion	F: ATGAAGGCCCTCTTCCTCA R: TCACCAGTAATAGCCAATAGTG	Null (0 bp)	[73, 74]
Pinb-D1a	Wild-type	F: ATGAAGACCTTATTCCTCCTA R: CTCATGCTCACAGCCGC**C**	240 bp	[70, 73]
Pinb-D1b	Gly to Ser pos. 46	F: ATGAAGACCTTATTCCTCCTA R: CTCATGCTCACAGCCGC**T**	240 bp	[70, 73]
Pinb-D1c	Leu to Pro pos. 60	F: ATGAAGACCTTATTCCTCCTA R: TCACCAGTAATAGCCACTAGGGAA	448 bp*	[67, 73]
Pinb-D1d	Trp to Arg pos. 44	F: TGCAAGGATTACGTGATGGA R: TCACCAGTAATAGCCACTAGGGAA	300 bp for pyrosequencing	[67, 71]
Pinb-D1e	Trp to stop codon pos. 39	F: TGCAAGGATTACGTGATGGA R: TCACCAGTAATAGCCACTAGGGAA	300 bp for pyrosequencing	[71, 75]
Pinb-D1f	Trp to stop codon pos. 44	F: TGCAAGGATTACGTGATGGA R: TCACCAGTAATAGCCACTAGGGAA	300 bp for pyrosequencing	[71, 75]
Pinb-D1g	Cys to stop codon pos. 56	F: TGCAAGGATTACGTGATGGA R: TCACCAGTAATAGCCACTAGGGAA	300 bp for pyrosequencing	[71, 75]

Wild type alleles confer soft endosperm; mutations confer hard endosperm. For additional alleles, see reviews [68, 72].*Digest with restriction enzyme *Pvu*II to cut other alleles into 264 bp and 184 bp. *Pinb-D1c* is not cut.

Table 4. Alleles of *Pina-D1* and *Pinb-D1* and the change in amino acid sequence of the encoded protein.

4.2. Gluten

The characteristic viscoelastic properties of wheat dough are due to a network of gluten proteins that is formed when flour is mixed with water. Thus, gluten is a major factor contributing to wheat quality. High grain protein content is typically associated with high quality, since roughly 80% of the grain protein is gluten [76]. However, both the amount and the composition of gluten affect wheat quality. Gluten consists of two types of proteins: polymeric glutenins and monomeric gliadins. Glutenins can be classified as low or high molecular weight (LMW or HMW) subunits, while gliadins can be classified as α, β, γ or ω types [77, 78]. The most important HMW glutenins, LMW glutenins, and gliadins are encoded by the *Glu-1*, *Glu-3* and *Gli-1* loci, respectively (**Table 5**). HMW glutenins generally have the largest impact on wheat quality. Each of the three *Glu-1* loci comprises two genes that can encode an x- or a y-type HMW subunit. In hexaploid wheat, only three to five of the HMW subunits are expressed (zero to two from *Glu-A1*, one to two from *Glu-B1*, and two from *Glu-D1*) [79]. The *Glu-1* alleles with the largest positive effect on baking quality are *Glu-D1d*, *Glu-A1a* or *Glu-A1b* and *Glu-B1al* [80, 81]. SDS-PAGE electrophoresis can be used to screen varieties for their HMW glutenin proteins. DNA markers have also been developed to discriminate between different alleles of *Glu-1*, *Glu-3* and *Gli-1* loci [82, 83]. For *Glu-A1* and *Glu-D1*, KASP markers are available that can be used to select varieties with the optimal alleles [84]. Each of the *Glu-3* loci (*Glu-A3*, *Glu-B3* and *Glu-D3*) contains several linked genes, and many alleles have been found for all three loci [85–89]. Markers are available for individual alleles of *Glu-A3* and *Glu-B3*, and multiplex PCR can be used to screen for certain combinations of alleles simultaneously [87]. However, the alleles of *Glu-3* loci with the largest effects are not consistent across studies [90–92]. The exact effects of the individual alleles on wheat quality traits are challenging to determine, since they can be influenced by genetic background, environment and G×E interactions [91, 93]. Furthermore, the alleles can have both additive effects and epistatic interactions [94, 95]. Ref. [93] showed that the *d*-allele of *Glu-B3* might increase the positive effects of the HMW loci *Glu-B1i* and *Glu-D1d*. The *Glu-A3b* or *d*-allele and *Glu-B3b*, *d-* or *g*-allele can possibly be used for improving dough strength and extensibility [90–92]. *Glu-B3i* has been reported to be positively associated with wheat quality in some lines and negatively associated in other lines. This discrepancy is possibly due to linkage with different *Gli-B1* alleles [90]. The *Gli-1* loci encode γ and ω gliadins and are linked to the *Glu-3* loci [96], while *Gli-2* loci encode α and β gliadins and are located on chromosome 6AS, 6BS and 6DS [78]. Overview of markers (including primer sequences) for more alleles of *Glu* loci and other quality genes can be found in [82].

4.3. Wheat-rye translocation and falling number

The wheat-rye translocation 1BL.1RS has been employed in many breeding programs as it carries resistance genes against powdery mildew and rusts. Markers for the resistance genes can be used to test for the absence or presence of the translocation in wheat varieties [100]. Alternatively, markers for *Glu-B3* or *Gli-B1* might be used (**Table 6**), since many wheat varieties with the 1BL.1RS translocation do not have these two loci, but instead can have the rye secalin locus *Sec-1* [96]. Therefore, wheat quality can be negatively affected by the translocation [101]. Additionally, the 1BL.1RS translocation can have a negative effect on falling number. Falling number is an indirect measure of α-amylase enzyme activity. The α-amylases are encoded by

Locus	Chr. arm	Primer sequences, 5′–3′	PCR product	References
Glu-A1	1AL	FAM: AAGTGTAACTTCTCCGCAACA VIC: AAGTGTAACTTCTCCGCAACG Common: GGCCTGGATAGTATGAAACC	FAM: ***Glu-A1a*** or ***Glu-A1b*** VIC: *Glu-A1c*	[84, 97]
Glu-B1	1BL	F: ACGTGTCCAAGCTTTGGTTC R: GATTGGTGGGTGGATACAGG	***Glu-B1al***: 447 bp Others: 0 bp	[98]
Glu-D1	1DL	FAM: ATAGTATGAAACCTGCTGCGGAG VIC: ATAGTATGAAACCTGCTGCGGAC Common: TACTAAAAAGGTATTACCCAAGTGTAACTT	FAM: *Glu-D1a* or others VIC: ***Glu-D1d***	[84, 99]
Glu-A3	1AS	F: TTCAGATGCAGCCAAACAA R: GCTGTGCTTGGATGATACTCTA	***Glu-A3b***: 894 bp Others: 0 bp	[86, 87]
		F: TTCAGATGCAGCCAAACAA R: TGGGGTTGGGAGACACATA	***Glu-A3d:*** 967 bp Others: 0 bp	
Glu-B3	1BS	F: ATCAGGTGTAAAAGTGATAG R: TGCTACATCGACATATCCA	**Glu-B3b***:* 1549 bp Others: 0 bp	[88]
		F: CACCATGAAGACCTTCCTCA R: CACCATGAAGACCTTCCTCA	***Glu-B3d***: 662 bp Others: 0 bp	
		F: CCAAGAAATACTAGTTAACACTAGTC R: GTTGGGGTTGGGAAACA	***Glu-B3g***: 853 bp Others: 0 bp	

Favourable alleles are marked in bold.

Table 5. Important HWM and LMW glutenin loci, their chromosomal location and primer sequences for detection of alleles with positive effects on wheat quality.

Locus	Primer sequences, 5′–3′	PCR product	References
Gli-B1	F: TGATCTGGCCACAAAGGGA	***Gli-B1.1*****:** 369 bp	[96]
(1BL.1RS)	R: CATTGGCCACCAATTCCTGT	*Gli-B1.2* or 1BL.1RS: 0 bp	
	F: TGATCTGGCCACAAAGGGC	***Gli-B1.2*****:** 397 bp	
	R: CATTGGCCACCAATTCCTGT	*Gli-B1.1* or 1BL.1RS: 0 bp	
Rht-D1	F: GGTAGGGAGGCGAGAGGCGAG	**Rht-D1b***:* 237 bp	[106]
	R: CATCCCCATGGCCATCTCGAGCTA	*Rht-D1a:* 0 bp	
wbm	F: CCGTCACAAGATTTACAGGGTTG R: TTATGGATCTCTTTATGTCTGTGT	**High *wbm* expression**: 961 bp Others: 0 bp	[103]
Gpc-B1	F: TCTCCAAGAGGGGAGAGACA	***Gpc-B1***: 122 bp	[105]
	R: TTCCTCTACCCATGAATCTAGCA	No *Gpc-B1*: 126 bp	

Favourable alleles are marked in bold.

Table 6. Additional loci influencing wheat quality traits.

the loci *α-Amy-1*, *α-Amy-2* and *α-Amy-3* located on the homoeologous chromosome groups 6, 7 and 5, respectively. High falling number reduces the risk of pre-harvest sprouting, which has a considerable negative impact on quality. Environmental conditions around the time of harvest influence falling number, but it is also influenced genetically. The *b*-allele of the *Rht-D1* (*reduced height*) gene on chromosome 4D is correlated with increased falling number [102].

4.4. Other genes for improving quality

Ref. [103] identified a gene, *wheat bread making* (*wbm*), that was highly expressed in developing seeds of wheat varieties with good bread-making quality. Polymorphisms in the promoter region sequence were identified between good- and poor-quality varieties. The allele identified in the good quality varieties was positively associated with gluten and bread-making quality in CIMMYT (The International Maize and Wheat Improvement Center) germplasm [104].

Genes from wild wheat relatives might also be used for improving quality in modern cultivars. Backcrossing can be used to transfer the genes into breeding material. In this case, MAS is useful since offspring containing the desired genes easily can be detected, and linkage drag can be reduced. One example of such a gene is *Gpc-B1* (*grain protein content*), which was found in wild emmer (*Triticum turgidum* L. ssp. *dicoccoides*). This gene has been used for increasing grain protein content in both durum and common wheat [105]. Markers tightly linked to *Gpc-B1* were identified, but require digestion with restriction enzymes. Therefore, [105] recommends the use of the marker shown in **Table 6** for MAS, although it is not completely linked to *Gpc-B1*.

5. Conclusion and perspectives

Trait-linked DNA markers have been identified for numerous traits in wheat, including disease resistance and grain quality. Employing such markers in MAS offers several advantages to wheat breeding compared to conventional phenotypic selection and laborious analysis of grain quality. These advantages include the fixation of desirable traits at an early stage of the breeding program and marker-assisted backcrossing in order to transfer agronomically important genes from wild relatives to cultivated wheat.

In addition, DNA markers are neutral to both environment and tissue type. Thus, they can be employed at any plant developmental stage and independent on environmental conditions during selection. This is particularly relevant for selection for disease resistance. DNA markers further offer the possibility for targeted pyramiding of several resistance genes, a task impossible by phenotypic selection due to complex host-pathogen interactions. To secure durable resistance, it is important to combine qualitative and quantitative resistance in a given line. Here, molecular markers can be used to combine both resistances.

As DNA markers have been correlated to numerous traits, they can be employed to combine, e.g., resistance and grain quality in the early generations. Consequently, DNA markers are being employed in early generations to select for several traits, in turn reducing the number of lines entering replicated, multi-location trials. Similarly, the number of samples for laboratory analysis of grain quality can be reduced. In effect, the application of MAS can lead to an

optimisation of resources demanded by any given breeding program, allowing the breeder to focus phenotypic selection on highly multi-genic traits, difficult to handle with MAS, e.g., yield.

Following developments in technologies and statistical genetics, the application of DNA markers in breeding is rapidly changing. While MAS has been employed to select for traits controlled by one/few genes, genomic selection will allow accurate selection for traits affected by numerous genes.

Once genomic selection has been validated in breeding programs, it can be implemented in combination with MAS. This will further improve selection efficiency and accuracy for disease resistance and quality parameters as well as for multi-genic traits such as yield.

Author details

Nana Vagndorf[1,2], Peter Skov Kristensen[1,3], Jeppe Reitan Andersen[1], Ahmed Jahoor[1,4] and Jihad Orabi[1]*

*Address all correspondence to: jior@nordicseed.com

1 Nordic Seed A/S, Odder, Denmark

2 Department of Agroecology, Flakkebjerg Research Centre, Aarhus University, Slagelse, Denmark

3 Department of Molecular Biology and Genetics, Center for Quantitative Genetics and Genomics, Aarhus University, Tjele, Denmark

4 Department of Plant Breeding, The Swedish University of Agricultural Sciences, Alnarp, Sweden

References

[1] Dubcovsky J. Marker Assisted Selection in Wheat. http://maswheat.ucdavis.edu/

[2] Oerke EC. Crop losses to pests. The Journal of Agricultural Science. 2006;**144**(1):31

[3] Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ. Shades of gray: The world of quantitative disease resistance. Trends in Plant Science. 2009;**14**:21-29

[4] Eyal Z, Scharen AL, Prescott JM, Van Ginkel M. The Septoria Diseases of Wheat: Concepts and Methods of Disease Management. Mexico: CIMMYT; 1987. 54 p

[5] Ponomarenko A, Goodwin SB, Kema GHJ. Septoria tritici blotch (STB) of wheat. Plant Health Instructor. 2011. http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Septoria.aspx

[6] Torriani SFF, Melichar JPE, Mills C, Pain N, Sierotzki H, Courbot M. Zymoseptoria tritici: A major threat to wheat production, integrated approaches to control. Fungal Genetics and Biology. 2015;**79**:8-12

[7] Brown J, Chartrain L, Lasserre-Zuber P, Saintenac C. Genetics of resistance to Zymoseptoria tritici and applications to wheat breeding. Fungal Genetics and Biology. 2015;**79**: 33-41

[8] Adhikari TB, Yang X, Cavaletto JR, Hu X, Buechley G, Ohm HW, et al. Molecular mapping of Stb1, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theoretical and Applied Genetics. 2004;**109**(5):944-953. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15490099

[9] Liu Y, Zhang L, Thompson IA, Goodwin SB, Ohm HW. Molecular mapping re-locates the Stb2 gene for resistance to Septoria tritici blotch derived from cultivar Veranopolis on wheat chromosome 1BS. Euphytica [Internet]. 2012;**190**(1):145-156. Available from: http://link.springer.com/10.1007/s10681-012-0796-8

[10] Arraiano LS, Chartrain L, Bossolini E, Slatter HN, Keller B, JKM B. A gene in European wheat cultivars for resistance to an African isolate of Mycosphaerella graminicola. Plant Pathology [Internet]. 2007;**56**(1):73-78. Available from:. DOI: 10.1111/j.1365-3059.2006.01499.x

[11] Goodwin SB, Cavaletto JR, Hale IL, Thompson I, Xu SS, Adhikari TB, et al. A new map location of gene *Stb3* for resistance to Septoria tritici blotch in wheat. Crop Science [Internet]. 2015;**55**(1):35-43. Available from: https://www.crops.org/publications/cs/abstracts/55/1/35

[12] Brading P, Verstappen E, Kema G, Brown J. A gene-for-gene relationship between wheat and mycosphaerella graminicola, the septoria tritici blotch pathogen. Phytopathology [Internet]. 2002;**92**(4):439-445. Available from http://www.ncbi.nlm.nih.gov/pubmed/18942957

[13] Flor HH. Current status of the gene-for-gene concept. Annual Review of Phytopathology. 1971;**9**:275-296

[14] Arraiano LS, Brown J. Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. Plant Pathology. 2006;**55**(6):726-738

[15] Vagndorf N, Nielsen NH, Edriss V, Andersen JR, Orabi J, Jørgensen LN, et al. Genomewide association study reveals novel quantitative trait loci associated with resistance towards Septoria tritici blotch in North European winter wheat. Plant Breeding. 2017;**136**:474-482

[16] Adhikari TB, Cavaletto JR, Dubcovsky J, Gieco JO, Schlatter AR, Goodwin SB. Molecular mapping of the Stb4 gene for resistance to septoria tritici blotch in wheat. Phytopathology [Internet]. 2004;**94**(11):1198-1206. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18944455

[17] Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM. Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat " Synthetic 6x". Theoretical and Applied Genetics. 2001;**103**:758-764

[18] McCartney CA, Brûlé-Babel AL, Lamari L, Somers DJ. Chromosomal location of a race-specific resistance gene to Mycosphaerella graminicola in the spring wheat ST6. Theoretical and Applied Genetics [Internet]. 2003;**107**(7):1181-1186. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12898022

[19] Adhikari TB, Anderson JM, Goodwin SB. Identification and Molecular Mapping of a Gene in Wheat Conferring Resistance to *Mycosphaerella graminicola*. Phytopathology [Internet]. 2003;**93**(9):1158-1164. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18944101

[20] Chartrain L, Sourdille P, Bernard M, Brown JKM. Identification and location of Stb9, a gene for resistance to septoria tritici blotch in wheat cultivars Courtot and Tonic. Plant Pathology [Internet]. 2009;**58**(3):547-555. Available from: http://doi.wiley.com/10.1111/j.1365-3059.2008.02013.x

[21] Chartrain L, Berry S, Brown J. Resistance of wheat line kavkaz-k4500 L.6.a.4 to septoria tritici blotch controlled by isolate-specific resistance genes. Phytopathology [Internet]. 2005;**95**(6):664-671. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18943783

[22] Chartrain L, Joaquim P, Berry S, Arraiano L, Azanza F, Brown J. Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. Theoretical and Applied Genetics [Internet]. 2005;**110**(6):1138-1144. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15759105

[23] Ghaffary SMT, Faris JD, Friesen TL, Visser RGF, van der Lee TJ, Robert O, et al. New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat. Theoretical and Applied Genetics [Internet]. 2012;**124**(1):125-142. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3249545&tool=pmcentrez&rendertype=abstract

[24] Ghaffary SMT, Robert O, Laurent V, Lonnet P, Margalé E, van der Lee TJ, et al. Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache. Theoretical and Applied Genetics [Internet]. 2011;**123**(5):741-754. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3155673&tool=pmcentrez&rendertype=abstract

[25] Raman R, Milgate AW, Imtiaz M, Tan MK, Raman H, Lisle C, et al. Molecular mapping and physical location of major gene conferring seedling resistance to Septoria Tritici blotch in wheat. Molecular Breeding. 2009;**24**(2):153-164

[26] Cowger C, Hoffer ME, Mundt CC. Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. Plant Pathology. 2000;**49**(4):445-451

[27] Orton ES, Deller S, Brown JKM. *Mycosphaerella graminicola*: From genomics to disease control. Molecular Plant Pathology. 2011;**12**(5):413-424

[28] Chartrain L, Brading P, Makepeace J, Brown J. Sources of resistance to septoria tritici blotch and implications for wheat breeding. Plant Pathology. 2004;**53**:454-460

[29] Buerstmayr H, Ban T, Anderson JA. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breeding. 2009;**128**:1-26

[30] Schmale DG, Bergstrom GC. Fusarium Head Blight (FHB) or Scab. APSNET. The Plant Health Instructor; 2010. https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx

[31] Anonymous. Commision Regulation (EC) No 856/2005 of 6 June 2005 amending regulation (EC) no 466/2001as regards Fusarium toxins. 2005

[32] Anderson JA. Marker-assisted selection for Fusarium head blight resistance in wheat. International Journal of Food Microbiology. 2007;**119**(1-2):51-53

[33] Mesterhazy A. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding. 1995;**114**(5):377-386

[34] Randhawa HS, Asif M, Pozniak C, Clarke JM, Graf RJ, Fox SL, et al. Application of molecular markers to wheat breeding in Canada. Plant Breeding. 2013;**132**:458-471

[35] Liu S, Anderson JA. Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome. 2003;**46**:817-823

[36] Liu S, Anderson JA. Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Science. 2003;**43**(3):760-766

[37] Guo P-G, Bai G-H, Shaner GE. AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theoretical and Applied Genetics [Internet]. 2003;**106**(6):1011-1017. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12671748

[38] Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, et al. Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. Nature Genetics. 2016;**48**(12):1576-1580

[39] Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC. RFLP mapping of QTL for fusarium head blight resistance in wheat. Crop Science. 1999;**39**(3):805-811

[40] Cuthbert PA, Somers DJ, Brulé-Babel A. Mapping of Fhb2 on chromosome 6BS: A gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2007;**114**(3):429-437

[41] Xue S, Li G, Jia H, Xu F, Lin F, Tang M, et al. Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2010;**121**(1):147-156

[42] Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A. Fine mapping Fhb1, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2006;**112**(8):1465-1472

[43] Xue S, Xu F, Tang M, Zhou Y, Li G, An X, et al. Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2011;**123**(6):1055-1063

[44] Cainong JC, Bockus WW, Feng Y, Chen P, Qi L, Sehgal SK, et al. Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from *Elymus tsukushiensis* into wheat. Theoretical and Applied Genetics. 2015;**128**(6):1019-1027

[45] Guo J, Zhang X, Hou Y, Cai J, Shen X, Zhou T, et al. High-density mapping of the major FHB resistance gene Fhb7 derived from *Thinopyrum ponticum* and its pyramiding with Fhb1 by marker-assisted selection. Theoretical and Applied Genetics. 2015;**128**(11):2301-2316

[46] Hovmøller MS, Sørensen CK, Walter S, Justesen AF. Diversity of *Puccinia striiformis* on cereals and grasses. Annual Review of Phytopathology [Internet]. 2011;**49**:197-217. Available from: http://www.annualreviews.org/doi/abs/10.1146/annurev-phyto-072910-095230

[47] Biffen RH. Mendel's laws of inheritance and wheat breeding. Journal of Agricultural Science [Internet]. 1905;**1**(1):4. Available from: http://www.journals.cambridge.org/abstract_S0021859600000137

[48] Yang E, Li G, Li L, Zhang Z, Yang W, Peng Y, et al. Characterization of stripe rust resistance genes in the wheat cultivar Chuanmai45. International Journal of Molecular Sciences. 2016;**17**(4):601

[49] Chen W, Wellings C, Chen X, Kang Z, Liu T. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Molecular Plant Pathology. 2014;**15**(5):433-446

[50] QayoumA.High-temperature,adult-plantresistancetostriperustofwheat.Phytopathology [Internet]. 1985;**75**(10):1121. Available from: http://www.apsnet.org/publications/phytopathology/backissues/Documents/1985Articles/Phyto75n10_1121.pdf%5Cnhttp://www.apsnet.org/publications/phytopathology/backissues/Documents/1985Articles/Phyto75n10_1121.PDF%5Cnhttp://www.apsnet.org/publications/phytop

[51] Chen X. High-temperature adult-plant resistance, key for sustainable control of stripe rust. Americas Journal of Plant Science and Biotechnology [Internet]. 2013;**4**(March):608-627. Available from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:High-Temperature+Adult-Plant+Resistance+,+Key+for+Sustainable+Control+of+Stripe+Rust#0

[52] Singh RP. Genetic association of leaf rust resistance gene Lr34 with adult plant resistance to stripe rust in bread wheat. Phytopathology. 1992;**82**(8):835-838

[53] William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D. Molecular marker mapping of leaf rust resistance gene lr46 and its association with stripe rust resistance gene yr29 in wheat. Phytopathology. 2003;**93**(2):153-159

[54] Uauy C, Brevis JC, Chen X, Khan I, Jackson L, Chicaiza O, et al. High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from *Triticum turgidum* ssp. dicoccoides is closely linked to the grain protein content locus Gpc-B1. Theoretical and Applied Genetics. 2005;**112**(1):97-105

[55] Sun Q, Wei Y, Ni Z, Xie C, Yang T. Microsatellite marker for yellow rust resistance gene Yr5 in wheat introgressed from spelt wheat. Plant Breeding. 2002;**121**(6):539-541

[56] Yan GP, Chen XM, Line RF, Wellings CR. Resistance gene-analog polymorphism markers co-segregating with the YR5 gene for resistance to wheat stripe rust. Theoretical and Applied Genetics [Internet]. 2003;**106**(4):636-643. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12595992

[57] Yaniv E, Raats D, Ronin Y, Korol AB, Grama A, Bariana H, et al. Evaluation of marker-assisted selection for the stripe rust resistance gene Yr15, introgressed from wild emmer wheat. Molecular Breeding. 2015;**35**(1):1-12

[58] Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, et al. Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theoretical and Applied Genetics. 2009;**119**(5):889-898

[59] Herrera-Foessel SA, Singh RP, Lan CX, Huerta-Espino J, Calvo-Salazar V, Bansal UK, et al. Yr60, a gene conferring moderate resistance to stripe rust in wheat. Plant Disease [Internet]. 2015;**99**(4):508-511. Available from: http://apsjournals.apsnet.org/doi/10.1094/PDIS-08-14-0796-RE

[60] Xiang C, Feng J, Wang M, Chen X, See D, Wan A, et al. Molecular mapping of stripe rust resistance gene Yr76 in winter club wheat cultivar tyee. Phytopathology. 2016;**106**(10):1186-1193

[61] Dong Z, Hegarty JM, Zhang J, Zhang W, Chao S, Chen X, et al. Validation and characterization of a QTL for adult plant resistance to stripe rust on wheat chromosome arm 6BS (Yr78). Theoretical and Applied Genetics. 2017;**130**(10):2127-2137

[62] Wan A, Zhao Z, Chen X, He Z, Jin S, Jia Q, et al. Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. Plant Disease [Internet]. 2004;**88**(8):896-904. Available from: http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS.2004.88.8.896

[63] Singh RP, Huerta-Espino J, Pfeiffer W, Figueroa-Lopez P. Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. Plant Disease. 2004;**88**(7):703-708

[64] Rosewarne GM, Singh RP, Huerta-Espino J, Herrera-Foessel SA, Forrest KL, Hayden MJ, et al. Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an avocet × pastor wheat population. Theoretical and Applied Genetics. 2012;**124**(7):1283-1294

[65] Peña RJ. Wheat for bread and other foods. In: Curtis BC, Rajaram S, Gómez Macpherson H, editors. BREAD WHEAT Improvement and Production. Rome: FAO; 2002. pp. 483-542

[66] Gale KR. Diagnostic DNA markers for quality traits in wheat. Journal of Cereal Science. 2005;**41**(2):181-192

[67] Lillemo M, Morris CF. A leucine to proline mutation in puroindoline B is frequently present in hard wheats from northern Europe. Theoretical and Applied Genetics [Internet]. 2000;**100**(7):1100-1107. Available from:. DOI: 10.1038/srep10446

[68] Bhave M, Morris CF. Molecular genetics of puroindolines and related genes: Allelic diversity in wheat and other grasses. Plant Molecular Biology. 2008;**66**(3):205-219

[69] Mohler V, Schmolke M, Paladey E, Seling S, Hartl L. Association analysis of Puroindoline-D1 and Puroindoline b-2 loci with 13 quality traits in European winter wheat (*Triticum aestivum* L.). Journal of Cereal Science [Internet]. 2012;**56**(3):623-628. DOI: 10.1016/j.jcs.2012.06.010

[70] Giroux MJ, Morris CF. A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch-surface friabilin. Theoretical and Applied Genetics. 1997;**95**(5-6):857-864

[71] Huang XQ, Röder MS. Development of SNP assays for genotyping the puroindoline b gene for grain hardness in wheat using pyrosequencing. Journal of Agricultural and Food Chemistry. 2005;**53**(6):2070-2075

[72] Morris CF, Bhave M. Reconciliation of D-genome puroindoline allele designations with current DNA sequence data. Journal of Cereal Science. 2008;**48**(2):277-287

[73] Gautier MF, Aleman ME, Guirao A, Marion D, Joudrier P. *Triticum aestivum* puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. Plant Molecular Biology. 1994;**25**(0167-4412 SB–IM):43-57

[74] Giroux MJ, Morris CF. Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 1998;**95**(11):6262-6266. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9600953&retmode=ref&cmd=prlinks%5Cnpapers3://publication/uuid/C38930D2-518C-4497-A4FA-DEAFDB1BD7D9%5Cnhttp://www.pnas.org/cgi/doi/10.1073/pnas.95.11.6262

[75] Morris CF, Lillemo M, Simeone MC, Giroux MJ, Babb SL, Kidwell KK. Prevalence of puroindoline grain hardness genotypes among historically significant North American spring and winter wheats. Crop Science. 2001;**41**(1):218-228

[76] Shewry PR. Wheat. Journal of Experimental Botany. 2009;**60**(6):1537-1553

[77] Payne PI. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annual Review of Plant Biology. 1987;**38**:141-153

[78] Barak S, Mudgil D, Khatkar BS. Biochemical and functional properties of wheat Gliadins: A review. Critical Reviews in Food Science and Nutrition. 2015;**55**(3):357-368

[79] Shewry PR, Halford NG, Belton PS, Tatham AS. The structure and properties of gluten: An elastic protein from wheat grain. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2002;**357**(1418):133-142

[80] Payne PI, Nightingale MA, Krattiger AF, Holt LM. The relationship between HMW glutenin subunit composition and the bread making quality of British grown wheat varieties. Journal of the Science of Food and Agriculture. 1987;**40**(1):51-65

[81] Butow BJ, Ma W, Gale KR, Cornish GB, Rampling L, Larroque O, et al. Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. Theoretical and Applied Genetics. 2003;**107**(8):1524-1532

[82] Liu Y, He Z, Appels R, Xia X. Functional markers in wheat: current status and future prospects. Theoretical and Applied Genetics [Internet]. 2012;**125**(1):1-10. Available from http://www.ncbi.nlm.nih.gov/pubmed/22366867

[83] Liu S, Rudd JC, Bai G, Haley SD, Ibrahim AMH, Xue Q, et al. Molecular markers linked to important genes in hard winter wheat. Crop Science [Internet]. 2014;**54**(4):1304. Available from: https://dl.sciencesocieties.org/publications/cs/abstracts/54/4/1304

[84] Pfluger L. MASWheat [Internet]. 2017. Available from: http://maswheat.ucdavis.edu/protocols/Gluten/index.htm

[85] McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Appels R, et al. Catalogue of Gene Symbols for Wheat [Internet]. 2017. Available from: https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp

[86] Zhang W, Gianibelli MC, Rampling LR, Gale KR. Characterisation and marker development for low molecular weight glutenin genes from Glu-A3 alleles of bread wheat (*Triticum aestivum* L). Theoretical and Applied Genetics [Internet]. 2004;**108**(7):1409-1419. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14727031

[87] Wang L, Li G, Peña RJ, Xia X, He Z. Development of STS markers and establishment of multiplex PCR for Glu-A3 alleles in common wheat (*Triticum aestivum* L.). Journal of Cereal Science. 2010;**51**(3):305-312

[88] Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, et al. Characterization of low-molecular-weight glutenin subunit Glu-B3 genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2009;**118**(3):525-539

[89] Zhao XL, Xia XC, He ZH, Lei ZS, Appels R, Yang Y, et al. Novel DNA variations to characterize low molecular weight glutenin Glu-D3 genes and develop STS markers in common wheat. Theoretical and Applied Genetics. 2007;**114**(3):451-460

[90] Bonafede MD, Tranquilli G, Pflüger LA, Peña RJ, Dubcovsky J. Effect of allelic variation at the Glu-3/Gli-1 loci on breadmaking quality parameters in hexaploid wheat (*Triticum aestivum* L.). Journal of Cereal Science. 2015;**62**:143-150

[91] Gupta RB, Bekes F, Wrigley CW. Prediction of Physical dough properties from glutenin subunit composition in bread wheats: Correlation studies. Cereal Chemistry. 1991;**68**:328-33

[92] He ZH, Liu L, Xia XC, Liu JJ, Pena RJ. Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread Wheats. Cereal Chemistry. 2005;**82**(4):345-350

[93] Ma W, Appels R, Bekes F, Larroque O, Morell MK, Gale KR. Genetic characterisation of dough rheological properties in a wheat doubled haploid population: Additive genetic effects and epistatic interactions. Theoretical and Applied Genetics. 2005;**111**(3):410-422

[94] Würschum T, Leiser WL, Kazman E, Longin CFH. Genetic control of protein content and sedimentation volume in European winter wheat cultivars. Theoretical and Applied Genetics. 2016;**129**(9):1685-96

[95] Krystkowiak K, Langner M, Adamski T, Salmanowicz BP, Kaczmarek Z, Krajewski P, et al. Interactions between Glu-1 and Glu-3 loci and associations of selected molecular markers with quality traits in winter wheat (*Triticum aestivum* L.) DH lines. Journal of Applied Genetics. 2017;**58**(1):37-48

[96] Zhang W, Gianibelli MC, Ma W, Rampling L, Gale KR. Identification of SNPs and development of allele-specific PCR markers for γ-gliadin alleles in *Triticum aestivum*. Theoretical and Applied Genetics [Internet]. 2003;**107**(1):130-138. Available from: http://link.springer.com/10.1007/s00122-003-1223-2

[97] Liu S, Chao S, Anderson JA. New DNA markers for high molecular weight glutenin subunits in wheat. Theoretical and Applied Genetics. 2008;**118**(1):177-183

[98] Ragupathy R, Naeem HA, Reimer E, Lukow OM, Sapirstein HD, Cloutier S. Evolutionary origin of the segmental duplication encompassing the wheat GLU-B1 locus encoding the overexpressed Bx7 (Bx7OE) high molecular weight glutenin subunit. Theoretical and Applied Genetics. 2008;**116**(2):283-296

[99] Ishikawa G, Nakamura T. A new co-dominant PCR-based marker to identify the high-molecular-weight glutenin subunit combination. Wheat Information Service. 2007;**103**:1-3

[100] Mago R, Spielmeyer W, Lawrence GJ, Lagudah ES, Ellis JG, Pryor A. Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theoretical and Applied Genetics. 2002;**104**(8):1317-1324

[101] Graybosch RA. Uneasy unions: Quality effects of rye chromatin transfers to wheat. Journal of Cereal Science. 2001;**33**:3-16

[102] Mohler V, Albrecht T, Mrva K, Schweizer G, Hartl L. Genetic analysis of falling number in three bi-parental common winter wheat populations. Plant Breeding. 2014;**133**(4): 448-453

[103] Furtado A, Bundock PC, Banks PM, Fox G, Yin X, Henry RJ. A novel highly differentially expressed gene in wheat endosperm associated with bread quality. Scientific Reports. 2015;**5**(April):1-14

[104] Guzmán C, Xiao Y, Crossa J, González-Santoyo H, Huerta J, Singh R, et al. Sources of the highly expressed wheat bread making (wbm) gene in CIMMYT spring wheat germplasm and its effect on processing and bread-making quality. Euphytica. 2016;**209**: 689-692

[105] Distelfeld A, Uauy C, Fahima T, Dubcovsky J. Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. The New Phytologist. 2006;**169**(4):753-763

[106] Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA. "Perfect" markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. Theoretical and Applied Genetics. 2002;**105**(6-7):1038-1042