

## MOLECULAR BREEDING FOR WHEAT FUSARIUM HEAD BLIGHT RESISTANCE IN CHINA

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**Abstract:** Wheat Fusarium head blight (FHB) may cause serious losses in grain yield and quality in China. More than 7 million hectares which approximately accounts for 25% of the total areas in China is infected by the disease. The cultivation of wheat varieties with resistance to Fusarium head blight is recognized as one of the most important components to diminish losses due to this disease. Chinese wheat breeders have commenced the research on FHB since 1950s. Wheat cultivars with improved FHB resistance were developed through conventional breeding. Some famous resistant varieties such as Sumai 3, Yangmai 158 and Ning 7840 were released from Jiangsu Academy of Agricultural Sciences, these varieties were widely applied in wheat production and breeding programs. Significant achievements concerning molecular mapping and marker assisted selection have been made in the past decade. The major QTL on chromosome 3BS was identified and located in the same region on chromosome 3BS in Sumai 3, Ning 894037, Wangshuibai, and Chinese Spring. Using SSR marker in this QTL region for assisted selection, some lines with the same resistance to FHB were obtained. New STS markers and SSCP markers were developed and will be tested for the efficiency of MAS. However, further achievements are still hindered by a number of constraints. More FHB resistance genetic resources from landrace in middle to lower reaches of Yangtze River are necessary to be used for improving FHB resistant. The genetic mechanism of the varieties contributing the resistance to improved cultivars is needed to be understood. Development of functional markers for FHB is discussed.

**Keywords:** wheat, Fusarium head blight, Scab, Disease resistance, Molecular breeding

### Wheat Fusarium head blight in China

*Fusarium* head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae*) is an important disease in China as well as in other wheat-growing regions of the world where rainfall frequently occurs during flowering time through early grain filling. Traditionally, the FHB epidemics occurred mainly in the middle to lower reaches of Yangtze River, including Jiangsu, Zhejiang, Anhui, Hubei, and Shanghai in central China, and northeast spring wheat growing area in China. In which areas, there were 7 severe epidemics and 10 moderate epidemics of FHB in 1951-1990 (Yao and Lu 2000). Recently, wheat varieties with moderate resistance to FHB have been released to wheat production so that the frequency of severe epidemic of FHB was lower than before. There were 2 severe epidemics and 7 moderate epidemics in 1991-2007. However, severe epidemics often appeared in parts of this region and caused great yield losses in wheat. In 2003, the proportion of scabbed spikes was 60-80% in the middle to lower reaches of Yangtze River of Jiangsu, Anhui and Hubei, which caused more than 20% yield losses in such areas (Zhu et al. 2003). In 2006, epidemic of FHB caused yield losses in an area of 266, 7 00 hectares in the north of Hubei province (Xu and Li 2007).

Since 1985, there appeared a general tendency of epidemic expansion to the north, southwest and northwest winter wheat growing areas as the changes of climate and rotation cultivation system. The outbreaks of FHB have been reported in Henan, Shandong, Hebei, Shaanxi, Ningxia, Gansu, Qinghai, and Sichuan. In 1985, an outbreak of FHB occurred in an area of approximately 3,733,000 hectares and yield losses of 885 million kg in Henan Province (Song et al. 2000). In 1998, wheat growers in China

suffered a severe epidemic of FHB. In the regions of the Huai River and the Yellow River, this epidemic is the most severe FHB outbreak in the historical record, the proportion of scabbed spikes was more than 80% and almost nothing could be harvested in some fields of the epidemic areas. (Lu et al. 1998; Zhang 1998; Guo et al. 1999; Shi and Wang 1999; Zhu 1999; Song et al. 2000). There were five severe FHB epidemics in Sichuan Province from 1987 to 1997 (Li 1996; Liu 1997). In 2004, the epidemic area was 292, 000 hectares in Sichuan, the proportion of scabbed spike ranged from 10 to 61% (Shen et al. 2005).

FHB causes sterility, poor filling and test weight decrease, thus resulting in significant yield loss. In addition to yield loss, the deteriorated quality of scabbed grain has become an even critical issue of public concern since that the scabbed grains are contaminated by mycotoxins except for their significant lower protein content.

Agronomical practices are ineffective and not feasible for controlling the disease. The application of fungicides is popular in wheat production in China, however, this practice inevitably leads to higher investment and environmental contamination, and is only partially effective, especially under a continuance raining weather. Developing FHB resistance varieties becomes the best choice for controlling the disease and meeting well the requirements for sustainable development of agriculture.

#### **Breeding for resistance to FHB**

A nationwide network of collaboration on the resistance to wheat FHB was established in China in the mid 1970s. As a result, varieties with improved resistance to FHB were developed by using different breeding programs.

##### *Systematic Selection*

Before the nationwide network was established, Chinese wheat breeders already began to select plants or spikes with the resistance to FHB from the field where FHB occurred frequently as early as in 1950s (Yao and Lu 2000). Some moderate resistant varieties were developed from susceptible varieties by using systematic selection. For instance, moderate resistant lines Wannian 2 and Wangmai 15 were selected from Nanda 2419 in 1958, and Yangmai 1 and Wumai 1 were selected from Funo in 1968. The area of these varieties was more than 400,000 hectares in 1960s. Furthermore, Yangmai 3, with more resistance to FHB than Yangmai 1 was developed in 1983. While systematic selection has a long history in wheat breeding, some breeders still adopt this approach to select lines with improved resistance to FHB. Recently, a soft wheat variety Ningmai 13 with moderate resistance to scab was selected from Ningmai 9 (Qian et al. 2006).

##### *Intervarietal Cross*

Intervarietal cross is a main method of genetic improvement for resistance to FHB in wheat. Among the resistant cultivars developed by this method, Sumai 3 is the best resistant source with higher combining ability than the land races for important agronomic traits, and has been used with remarkable success in many FHB resistant breeding programs in China (Liu and Wang 1990; Liu et al. 1991). More than 120 varieties with FHB resistance developed in China are derived from Sumai 3 (Bai et al. 1999a). Among them, Een 1 (Iovrin10/761//sumai 3) was the most common wheat varieties in the middle-lower reaches of the Yangtze Valley during 1980s and 90s, and

Zheng 9023 (83(2)3-3/84(14)43//Xiaoyan 6//Xinong 65/3/Shan213, of which 84(14)43 was derived from Sumai 3) was the common variety occupying over 2 million hectares annually in the south of Yellow river to Huai River reaches (Hu et al. 2001). Another famous cultivar, Ning 7840, derived from Sumai 3, has the same FHB resistance as Sumai 3, but carries additional genes for resistance to other diseases, such as rusts and powdery mildew, and has better agronomic characteristics than Sumai 3 (Bai et al. 1989).

Sumai 3 was selected from transgressive segregation of the cross between Funo, a moderately susceptible Italian cultivar, and Taiwanxiaomai, a moderately susceptible land race from China (Bai et al. 1999a). It's interesting that many FHB resistant varieties produced from susceptible or moderately susceptible parents. For example, Yangmai 4 was derived from Nanda 2419/Triumph//Funo, and Zheng7459 was produced from NDFSH and SCYYM. Liu et al. (1992) found that most released varieties with FHB resistance in China were related to Mentana, Funo and Taiwanxiaomai with analyzing the pedigree of the FHB resistant varieties (Fig 1).

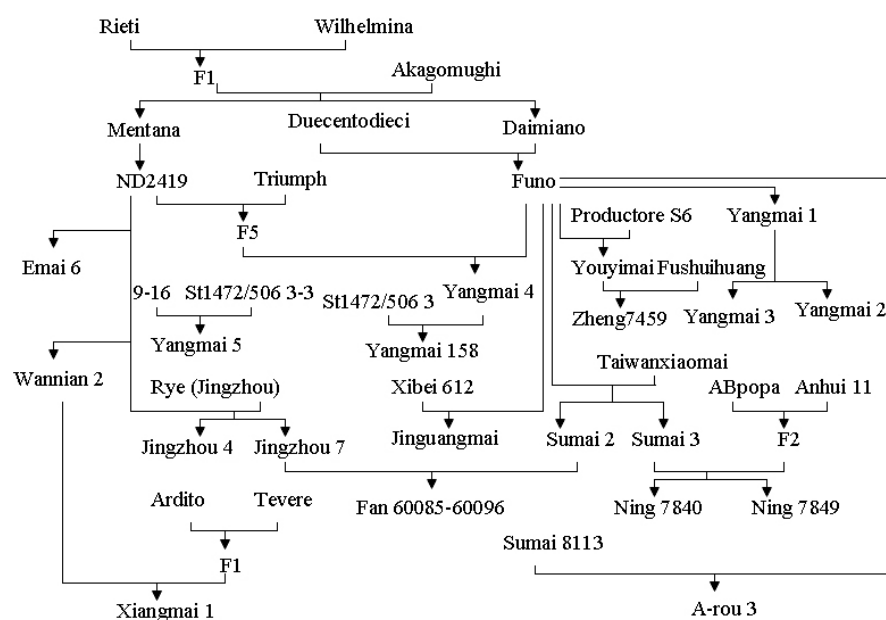


Figure 1. Pedigree of released FHB resistant varieties

#### Recurrent Selection

It's hard to improve multiple traits including agronomic characters and disease resistance simultaneously by using cross between two varieties. Wu et al. (1984) proposed a modified recurrent selection method to breed new cultivars with multiple improved characters including FHB resistance. With the aid of a dominant male-sterile gene, Ta1 (Ms2), gene pools were developed by crossing multiple parents followed by recurrent

selection. In any cycle, new sources are incorporated into the improved population to permit continued improvement, and superior genotypes will be extracted from the population to develop new cultivars. The significant achievements have been made in China. Nine lines with FHB resistance were developed, of which the resistance level of T154 was higher than that of Sumai 3 (Zhang et al. 1993). Seven resistant lines, including Futai 8711, 8829 also were developed by Fujian Academy of Agricultural Sciences (Zhang et al. 1995). To date, seven commercial wheat cultivars with FHB resistance have been released by recurrent selection and applied in wheat production (Zhuang et al. 1995; Zhang et al. 1999; Yang et al. 1997; Gan et al. 2004).

#### *Transferring Alien Resistant Genes to Wheat*

Jingzhou 1 and Jingzhou 47, with moderate resistance, were screened from the hybrid progenies of Nanda 2419 and indigenous rye (Institute of Jingzhou Agricultural Sciences, Hubei Province). In addition, the moderately resistant Jingzhou 66 was selected from a synthetic wheat material of Funo/durum and Nanda2419/rye. Liu (2002) reported that some alien addition lines with chromosomes from related species have been identified by chromosome C-banding and GISH analysis. Three addition lines with *Leymus racemosus*(*Elymus gigantors*) chromosomes, one with *Roegneria kamoji* (*Elymus tsukushiense*) chromosome and one with *Roegneria ciliaris* (*Elymus ciliare*) chromosome showed moderate to high FHB resistance in Nanjing agricultural University. At the Jiangsu Academy of Agricultural Sciences, more than 10 resistant lines involving Ning 920290 and Ning 983222 were created by sequential backcrossing of Chinese Spring with *Roegneria kamoji* and *Psathyrostachys huashanica* (unpublished).

#### **Marker assisted selection for resistance to FHB**

##### *QTL identified for resistance to FHB*

Evaluation for FHB resistance in phenotype is time and resource-intensive, and results are often confounded by environmental factors, and needs to be repeated over environments. Molecular markers may provide new resources for identifying FHB resistant genes (QTL) in breeding populations. Marker-assisted selection (MAS) will reduce, or may even eliminate, the need of phenotypic assays. QTL associated with FHB resistance were identified in Chinese varieties, especially in Sumai 3 and its derivatives. Bai et al. (1999b) identified 11 AFLP markers tightly linked to a major QTL for FHB resistance in a population of RILs derived from Ning 7840/Clark. This population was evaluated for resistance to spread within a spike in five greenhouse experiments. One major QTL explained up to 53% of phenotypic variation. This QTL was located on 3BS by using SSR markers analysis and also associated with low DON accumulation in infected kernels (Zhou et al. 2002). Other Chinese resistant sources, such as Wangshuibai, Ning 894037 and Chinese Spring were also been used in molecular mapping for FHB resistance. Wangshuibai is a landrace from Jiangsu province, Ning 894037 is a breeding line selected by somaclonal variation from Yangmai 3, a line selected from Funo. There is no close relationship in the pedigree between each two varieties of Sumai 3, Wangshuibai, Ning 894037. However, a major QTL on 3BS was located in the same chromosome region in Sumai 3, Wangshuibai and Ning 894037, which explaining 10%, 13.7% and 37.9% phenotypic variation from three

RIL populations with the same susceptible variety Alondra's as another parent (Fig 2) (Zhou et al. 2003; 2004a; Ren et al. 2003; Zhang et al. 2004). This QTL explained 42.5% of phenotypic variation in Ning 894037 by using the data from Purdue University in USA (Shen et al. 2003). The 3BS QTL in Wangshuibai was also detected from different populations: ND2419/Wangshuibai, Wangshuibai/ND571 and Wangshuibai/Wheaton (Lin et al 2004; Zhou et al. 2004b).

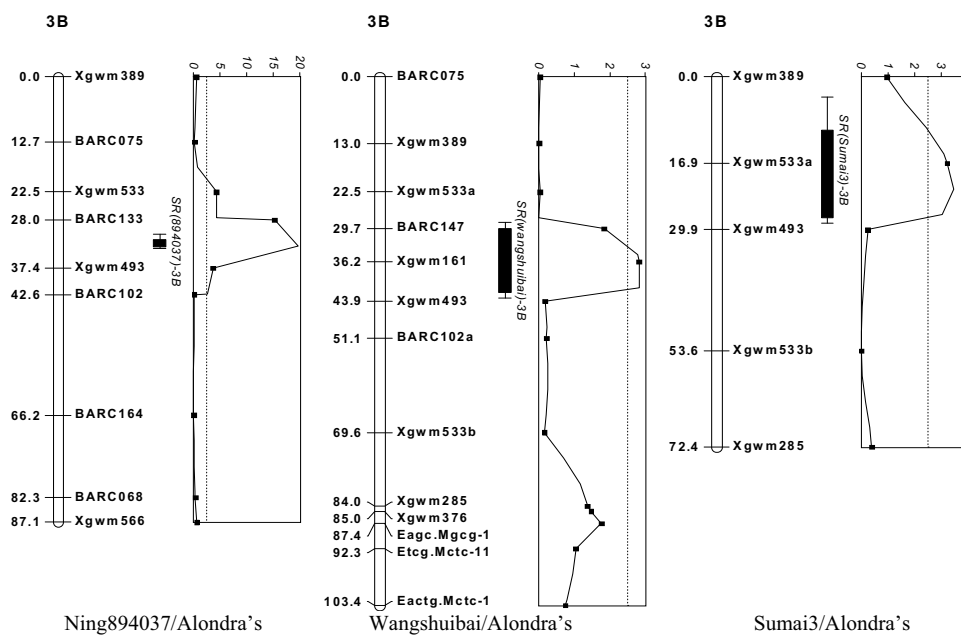


Figure 2. Analysis of QTLs for FHB resistance on 3BS chromosome in 3 RIL populations

From Chinese Spring, a landrace from southwest China with moderate resistance to FHB, we also found a major QTL on chromosome 3BS, which explained 30.2% of phenotypic variation of FHB resistance (Ma et al. 2006a). However, the banding patterns of SSR marker allele in this region in other resistant sources are different from those in Sumai 3 (Table 1) (Zhang et al. 2006).

Table 1. Haplotype of selected resistant varieties with SSR markers in 3BS QTL region

Vriety	Barc075	Gwm389	Gwm533	Barc147	Gwm493	Wmc754
Frotana	129	134	133	141	177	177,148
Zhen7495	129	136	N	123	N	161,146
Nobiokabuzu	129	151	158	125	215	202,146
Yangmai158	125	N	131	123	215	161,148
WZHHS	129	151	131	125	159	194,148
Fanshan xiaomai	129	153	131	123	215	202,147
Ningmai 6	129	N	131	125	N	177,154
Shinchungnaka	125	N	133	125	157	152,148
Sumai 3	129	153	160	123	213	198,154
Wangshuibai	129	151	158	125	215	194,146

Additional QTL associated with the resistance to spread were detected on chromosome 5A, 6A and 6B from Sumai 3 (Anderson et al. 2001), 6B from Ning 894037 and 5B, 2D, 7A and other chromosomes from Wangshuibai. Lin et al. (2006) located the QTL associated with the resistance to initial infection on chromosome 4B, 5A and 5B in Wangshuibai, the QTL on 4B and 5A explained 17.5% and 27% of phenotypic variation. Ma et al. (2006b) also mapped the QTL associated with the resistance to Deoxynivalenol accumulation and found the QTL located on chromosome 5A and 2A, which explained 12.4% and 8.5% of phenotypic variation.

#### *Marker assisted selection in practice*

Although many QTL have been identified for FHB resistance, the QTL on 3BS has a major effect on resistance. However, there are several markers in this chromosome region, so we need to know which marker or marker combination will be efficiently used in marker assisted selection for the major QTL. We used marker assisted selection at several different generations of 8 crosses which have Sumai 3 or its derived lines in our breeding program (Yu et al. 2006). The wheat leaves at early stage were collected for DNA isolation. Single marker and marker combination of Xbarc133, Xgwm493 and Xgwm533 were used for genotyping head row lines in F<sub>3</sub> and F<sub>4</sub> generation. The phenotype of FHB resistance were tested and compared with the genotype data. The single marker and marker combination have the same selective efficiency though there are little differences among them in all generations. The frequency of FHB resistance line ranged from 60% to 76.47% except for Xbarc133 has lower efficiency in F<sub>3</sub> generation than others (Table 2). It's suggested that Xgwm493 or Xgwm533 could be used for assisted selection in FHB resistant breeding program.

Table 2. Efficiency of assisted selection for FHB resistance with single marker and marker combination selection

Marker	Frequency of FHB resistant lines (%)	
	F <sub>3</sub>	F <sub>4</sub>
Xbarc133	48.15	60.00
Xgwm493	76.47	62.00
Xgwm533	66.67	66.67
Xbarc133+Xgwm493	73.33	69.23
Xbarc493+Xgwm533	68.75	61.54
Xbarc493+Xgwm533	63.64	69.23
Xbarc493+Xgwm493+Xgwm533	70.00	66.67

#### *New marker development*

To improve the efficiency of MAS for FHB resistance, more markers close to the major QTL should be developed. Sequence Tagged Site (STS) markers from wheat EST were developed and added to this region for saturating the density of 3BS QTL region. However, new STS markers are dominant markers and most of them have no polymorphism when they are used in different populations, some of them only amplify PCR fragments in susceptible cultivars (Guo et al. 2003; Liu and Anderson 2003). It's still a strong need to use more markers to saturate the chromosome interval. The genetic region containing the major QTL was saturated with Sequence Tagged Microsatellite (STM) and STS markers. A linkage map of chromosome 3B with 36 markers spanning a genetic distance of 112.4 cM was constructed. The QTL region reported by previous

research was inserted into 12 new markers. The average map distance among markers was 1.5 cM. MQM mapping indicated the major QTL explaining 45.6% of phenotypic variation was most likely in the interval of Xgwm533 – Xsts9-1 with MapQTL software. The SSR marker Xgwm533 and STM marker Xstm748tcac closely linked to the major QTL have potential for use in MAS breeding program (Zhou et al. unpublished).

Another new markers we tried to exploit to increase marker density in this QTL region are single-strand conformational polymorphism (SSCP) markers developed from wheat expressed sequence tags (ESTs) on 3BS bin 8- 0.78-1.0 (Yu et al. 2008). Sixty-nine out of 85 SSCP primer pairs amplified PCR products from the genomic DNA of 'Chinese Spring'. Thirty-four primer pairs amplified PCR products that could form clear ssDNA bands through denaturation treatment. Ten SSCP markers had polymorphisms between resistant variety Ning 7840 and susceptible variety Clark. Five of the ten polymorphic SSCP markers were located on chromosome 3B by nullitetrasome analysis. Three SSCP markers (Xsscp6, Xsscp20, and Xsscp21) were mapped into the region between Xgwm493 and Xgwm533, and possessed a higher coefficient of determination ( $R^2$ ) than Xgwm493 and Xgwm533 in molecular mapping in Ning7840/Clark RIL population. The SSCP markers, Xsscp6, Xsscp20, and Xsscp21, can be used for marker-assisted selection in FHB resistance breeding.

#### **Conclusions and future prospects**

Substantial effort of breeding wheat for FHB resistance has been carried out in China since the late of 1950's. Wheat cultivars with improved FHB resistance were developed through conventional breeding. In Jiangsu Academy of Agricultural Science, several famous varieties such as Sumai 3, Yangmai 158 and Ning 7840 were widely applied in wheat production and breeding programs. Significant achievements concerning molecular mapping and marker assisted selection have been made in the past decade. The major QTL on chromosome 3BS is a consistent QTL for FHB resistance in Sumai 3, Ning 894037, Wangshuibai, and Chinese Spring. Using SSR marker in this QTL region for assisted selection, we obtained some lines with the same resistance to FHB. New STS markers and SSCP markers were developed and will be tested for the efficiency of MAS. Above achievements provide with much more scientific basis for us to think about the restraints that hinder our further breeding achievements and what measures we should take for next step.

FHB resistant varieties released so far in China rely mostly on very limited gene sources, especially on Sumai 3. In fact, quite a number of landraces originated from middle to lower reaches of Yangtze River have stable resistance to FHB. But unfortunately these valuable varieties have never been properly used in wheat breeding for FHB resistance. Utilizing the huge gene pool for improving FHB resistance from landraces seems to be a very urgent task.

Marker analysis showed the major QTL on 3BS in Sumai 3 is from moderate resistant variety Taiwanxiaomai. What roles does another parent Funo play for the resistance in Sumai 3? Experiences of conventional breeding showed that some improved resistant varieties are not related to Taiwanxiaomai but related to Funo. Some FHB resistant varieties were produced from another old Italian variety Mentana. The genetic

mechanism of the resistance to FHB in such susceptible varieties needed to be researched for comprehensive understanding the resistance in Sumai 3.

Despite of the major QTL located on the same region in different Chinese varieties, the banding pattern in these varieties are not the same. The linkage phase between the marker and QTL alleles may differ between genotypes. It is needed to develop and use functional markers in FHB resistance breeding programs. Functional markers are 'good translators' from genomic technologies into improved crop varieties. These markers are derived from polymorphic sites within genes that causally affect phenotypic trait variation and would be the ideal tools for marker-assisted selection in wheat. However, the cloning of genes in wheat has been complicated by its allohexaploid ( $2n = 6x = 42$ ) nature and large genome size, so most of these markers could not be developed from the genes themselves. Association mapping is a new approach that can detect correlation between genotypes and phenotypes in a sample of individuals on the basis of linkage disequilibrium (LD). It has potential for identification and fine mapping of QTL and the prospects for identifying functional markers. By using population of unknown pedigree, the recombination events that have occurred over many generations are exploited for more refined mapping than is possible in conventional linkage mapping. Thus this method has potential to provide useful functional markers for marker-assisted selection in breeding programs. Such functional marker will enhance the efficiency of MAS for FHB resistance.

These

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