DIVERSITY OF STRIPE RUST RESISTANCE GENES IN SOUTHWESTERN CHINA WHEAT AND DISCOVERY OF NEW RESISTANCE LOCI BY ASSOCIATION MAPPING

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A Thesis Submitted in Fulfillment of the Requirements for the

Degree of Doctor of Philosophy in Crop Science

Suranaree University of Technology

Academic Year 2015

ความหลากหลายของยีนต้านทานโรคราสนิมลายของข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีนและการค้นหาตำแหน่งควบคุมความต้านทานใหม่ด้วยวิธีทำแผนที่ความสัมพันธ์

นายเทียนชิง เฉิน

วิทยานิพนธ์นี้สำหรับการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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ปีการศึกษา 2558

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เทียนชิง เฉิน : ความหลากหลายของยีนต้านทานโรคราสนิมลายของข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีนและการค้นหาตำแหน่งควบคุมความต้านทานใหม่ด้วยวิธีทำแผนที่ความสัมพันธ์ (DIVERSITY OF STRIPE RUST RESISTANCE GENES IN SOUTHWESTERN CHINA WHEAT AND DISCOVERY OF NEW RESISTANCE LOCI BY ASSOCIATION MAPPING) อาจารย์ที่ปรึกษา : ศาสตราจารย์ ดร.ปิยะดา อลิฌาณ์ ตันตสวัสดิ์, 170 หน้า.

งานนี้ทำการทดสอบความต้านทานโรคราสนิมลายในข้าวสาลีสายพันธุ์ดีที่รวบรวมไว้จำนวน 140 accessions ซึ่งเหมาะสมสำหรับทำแผนที่ความสัมพันธ์ (association mapping; AM) ในระยะต้นกล้าและในสภาพไร่ (ระยะต้นโตเต็มวัย) เพื่อประเมินความต้านทานโรคราสนิมลายของข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีน และค้นหายีนต้านทานใหม่สำหรับปรับปรุงพันธุ์ข้าวสาลี และทำการศึกษารูปแบบดีเอ็นเอโดยใช้เครื่องหมาย DArT-seq จำนวน 30,485 เครื่องหมาย และเครื่องหมายโมเลกุลสำหรับตรวจสอบยีนต้านทานโรคราสนิมลาย (*Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 และ *Yr*29) จำนวน 7 เครื่องหมาย วิเคราะห์ความหลากหลายทางพันธุกรรมของข้าวสาลี 140 accessions โดยใช้ข้อมูลจีโนไทป์จากเครื่องหมาย DArT-seq ด้วยวิธี principal coordinate analysis (PCoA) และ cluster analysis นอกจากนี้ ศึกษา linkage disequilibrium (LD) ของแต่ละโครโมโซม และทำแผนที่ความสัมพันธ์โดยใช้โมเดลที่พิจารณาโครงสร้างประชากรต่างกัน 2 โมเดลคือ single factor analysis (SFA) model และ Q model ผลการทดลองแสดงว่าระดับความต้านทานโรคราสนิมลายในพันธุ์จากกุ้ยโจวสูงกว่าพันธุ์จากซีฉวนและพันธุ์จากแหล่งอื่นทั้งที่ระยะต้นกล้าและต้นโตเต็มวัย พันธุ์ส่วนใหญ่มียีนต้านทาน *Yr*26 และส่วนน้อยมียีน *Yr*29, *Yr*10, *Yr*18 และ *Yr*15 ยีน *Yr*26 มีสหสัมพันธ์เชิงบวกกับความต้านทานที่กุ้ยโจว แต่ไม่มีสหสัมพันธ์ที่ซีฉวน (เมียนยาง) โดยพบข้าวสาลีจำนวน 29 พันธุ์ที่มียีนต้านทานโรคราสนิมลายมากกว่า 1 ยีน การวิเคราะห์ PCoA ในระดับทั้งจีโนมและระดับโครโมโซม 6 AS ทำให้แบ่งข้าวสาลีออกได้เป็น 2 กลุ่ม กลุ่ม I ประกอบด้วย สายพันธุ์ non-T6VS/6ALจากแหล่งต่าง ๆ ในขณะที่กลุ่ม II ประกอบด้วย สายพันธุ์ T6VS/6AL ซึ่งส่วนใหญ่มียีน *Yr26* และ *Pm21* ผลจากเดนโดรแกรม (dendrogram) แสดงการแบ่งกลุ่มประชากรข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีนที่ไม่ชัดเจน แต่พบมีการจัดกลุ่มของ accessions ตามประวัติพันธุ์หรือแหล่งที่มา ส่วน LD analysis พบ LD ส่วนใหญ่บนโครโมโซม 6A และ 1B นอกจากนี้ AM พบเครื่องหมายที่สัมพันธ์กับความต้านทานโรคราสนิมลายด้วย SFA model จำนวนมากกว่า Q model โดยสรุปผลการทดลองเหล่านี้บ่งชี้ว่า พบยีนต้านทาน *Yr*26 มากที่สุดในข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีน แต่ยีนนี้เริ่มสูญเสียความต้านทานในซีฉวน และยีนต้านทาน

โรคราสนิมลายจากการศึกษานี้มีความหลากหลายต่ำ อย่างไรก็ตามข้อมูลความหลากหลายทางพันธุกรรมและความต้านทานโรคของข้าวสาลีในภาคตะวันตกเฉียงใต้ของประเทศสาธารณรัฐประชาชนจีนที่ได้จากการศึกษาครั้งนี้จะสามารถช่วยในการคัดเลือกพ่อแม่พันธุ์สำหรับการผสมพันธุ์ข้าวสาลีในอนาคต

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2558 ลายมือชื่ออาจารย์ที่ปรึกษา_____

TIANQING CHEN : DIVERSITY OF STRIPE RUST RESISTANCE GENES IN SOUTHWESTERN CHINA WHEAT AND DISCOVERY OF NEW RESISTANCE LOCI BY ASSOCIATION MAPPING. THESIS ADVISOR : PROF. PIYADA ALISHA TANTASAWAT, Ph.D., 170 PP.

COMMON WHEAT/STRIPE RUST/MOLECULAR DETECTION/ GENETIC DIVERSITY/ASSOCIATION MAPPING

To evaluate stripe rust resistance of Southwestern China wheat and discover new resistance genes for wheat breeding, a collection of 140 elite wheat accessions suitable for association mapping (AM) has been tested for stripe rust resistance at the seedling stage and under field conditions (adult plant stage). The collection was also profiled with 30,485 DArT-seq markers and 7 molecular markers for detection of stripe rust resistance genes (*Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 and *Yr*29). Genetic diversity of 140 wheat accessions were also analyzed with genotype data from DArT-seq profiling by principal coordinate analysis (PCoA) and cluster analysis. Furthermore, linkage disequilibrium (LD) was studied for each chromosome. Finally, AM was conducted with two models based on population structure considerations, a single factor analysis (SFA) model and a Q model. The results showed that the stripe rust resistance levels of Guizhou cultivars were higher than those of Sichuan cultivars and other regional cultivars at both the seedling stage and the adult plant stage. Most cultivars carried the resistance gene *Yr*26, and fewer cultivars carried *Yr*29, *Yr*10, *Yr*18 and *Yr*15. *Yr*26 was positively correlated with the resistance of accessions evaluated in Guizhou but not in Sichuan (Mianyang). There were 29 cultivars that carried more than one stripe rust resistance gene. PCoA at

both the whole-genome level and the chromosome 6AS level identified two groups of wheat varieties. Group I was composed of non-T6VS/6AL lines of different origins, while Group II was composed of T6VS/6AL lines and most of these carried the *Yr26* and *Pm21* genes. A dendrogram revealed that the population stratification of Southwestern China wheat was not obvious, but accessions were clustered together based on their pedigree or origin. LD analysis showed that a large extent of LD was observed on 6A and 1B. Finally, AM detected more markers associated with stripe rust resistance using the SFA model than using the Q model. In conclusion, these results indicate that all stage resistance gene *Yr*26 is prevalent in Southwestern China wheat, while it begins to lose its resistance in Sichuan. In addition, stripe rust resistance genes screened in this study are not diverse. However, the information on the genetic diversity and disease resistance of Southwestern China wheat obtained from this study will facilitate the selection of parents for future hybridization.

School of Crop Production Technology Student's Signature_____

Academic Year 2015 Advisor's Signature_____

ACKNOWLEDGEMENTS

This research could not have been accomplished without the support of the National Natural Science Funds of China (31160281, 31260321). I would like to express my sincerest appreciation and deepest gratitude to all the following individuals:

Thesis advisor, Prof. Dr. Piyada Alisha Tantasawat, for providing me with the great opportunity to pursue my graduate research. Her kindest support, untired guidance, patience, understanding, and numerous hours spent on editing this thesis and other papers are invaluable treasures for me forever.

Thesis co-advisors: Dr. Liyi Zhang for her profound knowledge and experiences shared, understanding, and appreciation. I will also express thanks to Dr. Hugo Volkaert, for his help in the field of association mapping.

Mr. Qingcai He, my supervisors, sincerely thanks for his permission, support and patience during my absence. My colleagues, for understanding and shouldering my job obligations. Friends Mr. Wendong Dai, Mr. Gang Shen, Ms. Li Liu, Ms. Ping Zhang and other friends without being mentioned here.

Last but not the least, I'd like to devote my appreciation to my parents, for their inspirations. Heart-felt thanks go to my wife Bangli Peng for her understanding during my absence for Ph. D. studies.

Tianqing Chen

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LIST OF ABBREVIATIONS

RFLP	=	Restriction fragment length polymorphisms
AFLP	=	Amplified fragment length polymorphisms
SSR	=	Simple-sequence repeats
SCAR	=	Sequence characterized amplified regions
SNP	=	Single nucleotide polymorphism
DArT	=	Diversity arrays technology
NGS	=	Next-generation sequencing
GBS	=	Genotyping by sequencing
LD	=	Linkage disequilibrium
GWAS	=	Genome-wide association studies
PIC	=	Polymorphism information content
GS Dice	=	genetic similarity coefficient
CYR32	=	Chinese stripe (yellow) rust race 32
PCoA	=	Principal-coordinates analysis
UPGMA	=	Unweighted pair group method with arithmetic mean
IT	=	Infection type
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CHAPTER Ⅰ

INTRODUCTION

1.1 Introduction

Wheat (*Triticum aestivum* L.) is an important cereal with the widest distribution, largest planting area and trade volume in the world. It is a major food source for human. More than 35% of the world population is fed by wheat. At present, the global wheat acreage is about 219 million hectares and total production is 716 million tonnes (3.3 tons/ha), which corresponds to 30.3% and 25.8% of the global cereal planting area and production (FAO, 2013). Comparing with other cereals, the cultivated area of wheat in China is the third largest, less than rice and maize. In 2013, the harvested area of wheat is 24.12 million hectares and total production is 121.93 million tonnes in China (5 tons/ha), which takes the proportion of 11.0% and 17.0% of the world, respectively (CPIIN, 2013). Therefore, wheat plays a key role in food security and life quality in China.

China is not only the largest country for wheat production but also the largest epidemic region for wheat stripe rust disease (Wan et al., 2007). There is about 20 million hectares wheat acreage (＞80%) affected by stripe rust and it can spread inter-regionally, mainly from west to east and from south to north. Winter-wheat growing regions in the north-west, south-west, and north, and spring-wheat growing

regions in the north-west are the major epidemic areas. Since 1950s, there have been 8 pandemics of wheat stripe rust in China which caused huge losses to wheat production. Among the 8 pandemics, the 4 that happened in 1950, 1964, 1990 and 2002 were more severe. There were more than 13.33 million hectares infected wheat in 1950 and 1964, 7.33 million hectares infected wheat in 1990 and 2002, which finally depressed the wheat production by 6.0, 3.2, 1.8, and 1.3 million tonnes in turn (Wan et al., 2003; Wan et al., 2004).

Moreover, climate warming in wheat growing season could shorten the development duration of the pathogen, lengthen the damage period, accelerate the development of population growth, increase one more breeding generation than normal, make the occurrence boundary move northward, elevate the limit of altitude, expand the geographic range and significantly aggravate the severity (Huo et al., 2012). Hotspots of wheat stripe rust are mainly located in South-Eastern Gansu and North-Western Sichuan, and these areas constitute the inoculum base, center of diversity, and major over-summering areas of the pathogen (Zeng and Luo, 2006). Successful control in over-summering areas is the key to achieve sustainable management of stripe rust in China. Some measures, for example, getting rid of the volunteer wheat or readjusting the structure of plant, are planned to control the propagation of the stripe rust in the over-summering areas, but they are difficult to accomplish (Guo et al., 2010).

Extensive studies on the epidemiology and management of stripe rust have been carried out since the widespread occurrence of the disease in the 1950s. Pathogen

variability and race virulence have been monitored continuously, and the resistance genetics and background of Chinese wheat cultivars had been analyzed. About 67 stripe rust resistance genes (*Yr*1 to *Yr*67) have been designated and mapped to various wheat chromosomes (McIntosh et al., 2014). Most of the genes are race-specific and cultivars carrying some of them have played important roles in wheat breeding (Cao et al., 2001).

Although wheat cultivation measures and the application of fungicides can reduce yield losses from wheat stripe rust, resistant cultivars are the most economic, effective and ecological approach to control the disease (Wu and Niu, 2000).

So far, a series of resistant cultivars have been inputted into the production and yield losses from wheat stripe rust are relieved. But wheat production of China is being confronted with another problem, the stripe rust resistant cultivars lose their resistance in a few years after being released. The pathogen causing wheat stripe rust has the character of high frequency of genetic variation, so new virulence races appear constantly. The released cultivars usually have the common genetic background in China, so single and vertical resistance make them susceptible to new virulence races (Line and Chen, 1995).

Application, diversification and deployment of various resistance genes are the essential way to achieve the durable resistance of cultivars. Excellent wheat stripe rust resistance resources and resistance genes are the basis for breeding resistance to stripe rust. Among the officially named stripe rust resistance genes, only *Yr*5, *Yr*10, *Yr*15, *Yr*24 and *Yr*26 confer resistance to the race CYR32 (Jia et al., 2003; Yang et al., 2003;

Wan et al., 2004). Hence, it is essential to identify new stripe rust resistance genes, preferably with closely associated molecular markers for marker–assisted selection (MAS). Though there are some of references reporting that a stripe rust resistance gene or QTL has been mapped on chromosome, but it is usually an allele of a known stripe rust resistance gene (Li et al., 2006). Southwest winter wheat region of China is the severely afflicted area of stripe rust, where a lot of stripe rust resistant cultivars and lines are bred by conventional breeding and distant hybridization, such as Guinong21 (Cheng et al., 2006), Guinong22 (Li et al., 2011), Guinong775 (Han et al., 2012), Chuanmai42 (Li et al., 2006), Chuanmai107 (Zhu et al., 2010), Mianmai37 (Zhou et al., 2009), YLP series (Han et al., 2008), etc. But the genetics of the resistance and stripe rust resistance genes underlying Southwestern China wheat are not well studied. Therefore, the objective of this study was to explore the stripe rust resistance gene form the wheat cultivars and breeding lines in the southwest of China.

1.2 Significance of the study

In this study, commercial cultivars and breeding lines from Southwestern China were evaluated for stripe rust resistance at seedling stage with predominant *Pst* race in China and at adult plant stage with different environments (in Sichuan or Guizhou for two years). The stripe resistance genes, which were widely used or still are effective in China, were also estimated in these cultivars (lines) by molecular detection. Whole genome profiling were carried out with DArT-seq technique and genetic diversity and population structure of Southwestern China wheat were also analyzed. Finally,

genotype and phenotype data were combined with population structure data to conduct association mapping for stripe resistance. The results of the study will provide wheat breeder valuable information about stripe rust resistance of the Southwestern China wheat, which will be helpful for wheat resistance breeding. The results of genetic diversity analysis will be useful in conservation and assessment for superior germplasm resources of Southwestern China and also can guide parent selection in hybridized breeding. Association mapping for stripe rust resistance may discover some new stripe rust resistance genes or QTLs, or improve resolution of known stripe rust resistance genes on a genetic map, which will be promising in MAS for resistant cultivars.

1.3 Research objectives

1. To identify the stripe rust resistance of Southwestern China wheat cultivars or lines.

2. To estimate the diversity of stripe rust resistance genes in Southwestern China wheat by molecular detection.

3. To assess the genetic diversity of the Southwestern China wheat with DArT-seq genotyping method.

4. To discover new stripe rust resistance genes or QTLs

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CHAPTER II

REVIEW OF LITERATURE

2.1 General information about wheat

Botanically, wheat belongs to the grass family, *Pooideae* sub-family, *Triticum* genus. There are 30 species in *Triticum* genus. According to the chromosome number, *Triticum* can be classified into diploid wheat, tetraploid wheat and hexapliod wheat (Jin, 1996). Common wheat (*Triticum aestivum* L. em. Thell. 2n=6x=42) belongs to hexaploid wheat and is an allopolyploid species which arose under cultivation 8,000 years ago. Its genome is made by the juxtaposition of three simple genomes named A, B and D also called homoeologues. DNA is organized into 21 pairs of chromosomes, seven pairs belonging to each of the A, B, and D genomes. Within each diploid genome, the chromosomes are designated from 1 to 7 (Fig. 2.1) (Zhang, 2006). The size of each diploid genome varies between 4,500 and 6,000 mega bases (Mb) depending on the species (Bennett and Leitch, 1995). The common wheat genome size was thus estimated to be 16,974 Mb (Bennett and Smith, 1991) which represents 4, 40 and 130 times more than the human, rice and *Arabidopsis thaliana* genomes, respectively.

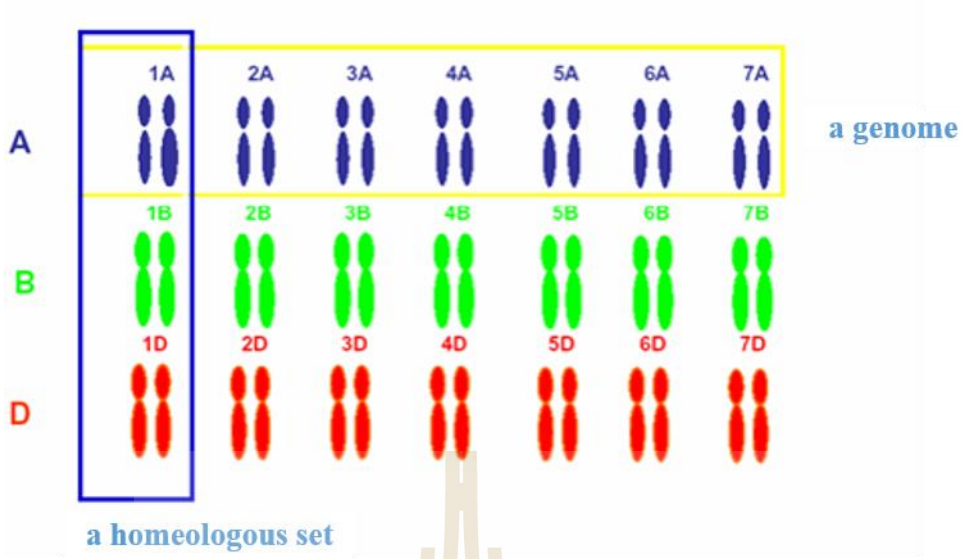


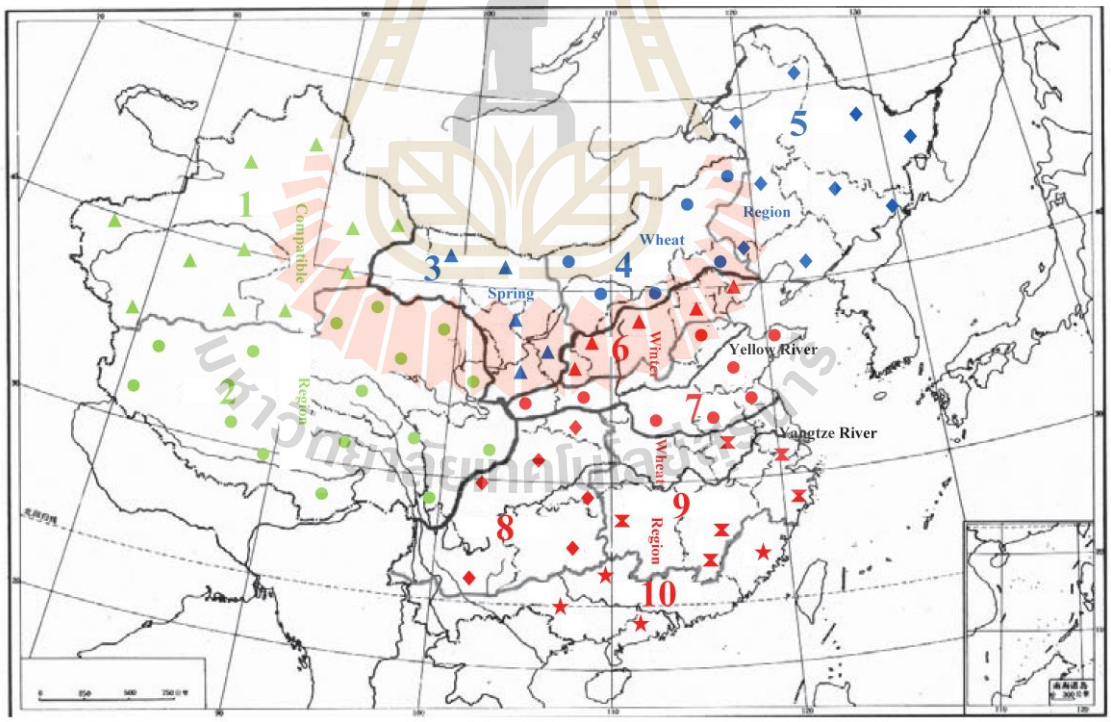
Fig. 2.1 Chromosome structure of *T. aestivum* L.

Due to strong adaptability, common wheat is widely distributed, from the Arctic Circle to the Equator. But because it is more adaptable to humid and cold climate, it is mainly cultivated in Eurasia and North America. The main countries for wheat production are China, USA, India, Russia, Kazakhstan, Canada, Australia, Turkey and Pakistan (Zhao, 2014).

Common wheat is the first staple grain crops in the world. The grain of wheat contains abundant carbohydrates, proteins, fats, vitamins and minerals, which are beneficial to human body. Special chemical composition and unique gluten make the wheat be processed as various cooked foods. Wheat also has the character of easy processability and resistant storage. At present, wheat is the raw material of staple foods, non-staple foods and the main stored grain in most countries. Besides, wheat bran is a perfectly concentrated feed and wheat straw is a good raw material for weaving and papermaking (Wu, 1990).

2.2 Wheat production in China

The wheat growing area in China can be divided into 3 main regions and 10 sub-regions according to geographic location, varieties grown and cultivation environments. The 3 main regions are spring wheat region, winter wheat region and compatible region for spring and winter wheat. The winter wheat region is the main wheat-producing area, which is constituted by north winter wheat region, Huang-Huai winter wheat region, middle-lower Yangtze winter wheat region, southwest winter wheat region and south winter wheat region (Fig. 2.2). Major provinces with large wheat cultivated areas are Henan, Shandong, Hebei, Anhui, Jiangsu, Sichuan, Shaanxi, Hubei, Xinjiang and Shanxi (Jin, 1996).



1. Xinjiang winter-spring wheat region; 2. Qinghai-Tibet winter-spring wheat region; 3. Northwest spring wheat region; 4. North spring wheat region; 5. Northeast spring wheat region; 6. North winter wheat region; 7. Huang-Huai winter wheat region; 8. southwest winter wheat region; 9. Middle-lower Yangtze winter wheat region; 10. South winter wheat region.

Fig. 2.2 Chinese wheat planting regionalization

From 1949 to 2009, the wheat cultivated areas fluctuated in a range of 21.33-30.67 million hectares. After a continued decline for the last 7 years, now wheat cultivated area is stable at 24 million hectares in China. The wheat yield has been over 4500 kg/ha since 2006. The wheat total production and yield have been growing for the past 6 years since 2009 (Zhao, 2014). According to the latest data from FAO, the harvested area of wheat is 24.12 million hectares and the total production is 121.93 million tonnes in China, which accounts for 11.0% and 17.0% of the world in 2013, respectively.

2.3 Wheat stripe rust

Stripe (yellow) rust is primarily a foliar fungal disease of wheat, which is caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*). *Pst* also can infect spike and stem tissues. If the pathogen infects spike, it will cause extensive quality and grain yield loss (Anahid et al., 2013). Infection can occur anytime from the one-leaf stage to plant maturity provided that plants are still green (Chen, 2005). Water droplets on the leaves and temperatures of 0 to 25°C are required for spore germination. Penetration is through the stomata. The latent period may vary from 11 days with mean daily temperatures of 15°C, to perhaps 180 days with near freezing temperatures (USDA, 2014). Temperatures of 10-15°C, relative humidity of 100% are optimal for spore germination, penetration and production of new, wind-dispersed spores (HGCA, 2015). Usually, symptoms appear about 1 week after infection, and sporulation starts about 2 weeks after infection, under optimum temperature conditions (Fig. 2.3). When wheat is infected, the fungus forms tiny, yellow- to orange-colored rust pustules on its leaf, called uredia. Each uredium contains thousands of uredospores. A single uredospore is

too small to be seen with the naked eye, but spores on mass are yellow- to orange-colored and powdery. Disease cycle may be repeated many times in one season.

a. One week b. Two weeks

Fig. 2.3 Symptoms of wheat stripe rust

During late summer, the dark teliospores may be produced. These can germinate to produce yet another spore type, the basidiospore (Fig. 2.4). The teliospores seem to have no function in the disease cycle but they may contribute to the development of new races through sexual recombination (HGCA, 2015).

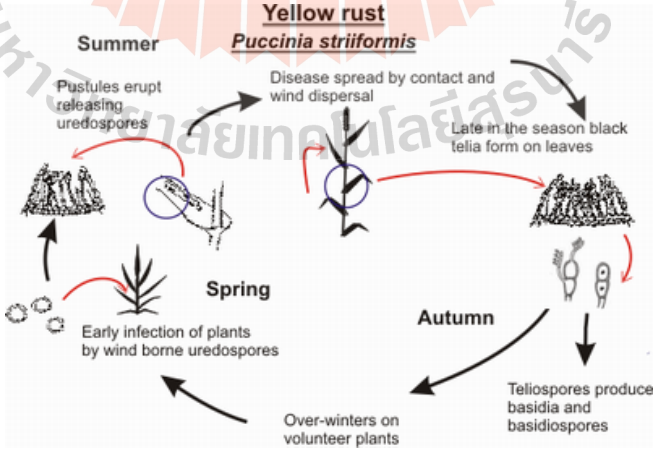


Fig. 2.4 Life cycle of *Puccinia striiformis* f. sp. *tritici*

Hot summers and dry seasons are most threatening to stripe rust survival. Survival between crop seasons is by dormant mycelium and uredinia on infected volunteer wheat in most areas. In mountainous tropical and subtropical areas stripe rust can survive by moving up and down the mountain on seeded wheat (USDA, 2014).

2.4 Epidemic of stripe rust in China

In some parts of Northwest China, the environments also suit for over-summering of the pathogen. All of these regions (southern and eastern Gansu) are the major centers of stripe rust diversity, which also provide inoculum and new races for other regions in China. Furthermore, almost all Chinese races and pathotypes were first detected in these regions. Historically, every resistance "breakdown" event occurred first in these regions (Wu et al., 1993).

Among the stripe rust epidemic systems in China, the main (South Gansu-Northwest Sichuan) epidemic system is very clear (Chen et al., 2007). After the summer (July and August), the volunteer wheat seedlings of mountainous area in these regions may produce huge amounts of spores. West winds prevail during the fall in China, so that the winter-wheat-growing areas of the middle-lower reaches of the Huai and Yangtzi rivers ("HY reaches") and the middle-lower reaches of Yellow and Huai rivers ("YH reaches") may receive spores originating from the inoculum sources of over-summer areas (Gansu-Northwest Sichuan). In the winter-wheat-growing areas of the HY reaches, stripe rust can overwinter in infected leaf tissue and disease can even progress slowly during winter. The overwintering inoculum can initiate epidemics in these areas during spring. In the areas of the YH reaches and north-central China (i.e.,

northern areas of Yellow River reaches), the rust does not overwinter well due to the low temperature, and hence the amount of early-spring inoculum is often quite low. But a huge amount of exogenous inoculum from the HY reaches can invade the more northern area carried by southern winds if the spring outbreak in the south is early and severe, which leads wheat stripe rust pandemics (Zeng and Luo, 2006). In China, all wheat-growing areas within the geographic ranges of 97 to 135°E and 22 to 53°N are embedded in this one epidemic system (Fig. 2.5), while Xinjiang and Tibet belong to other epidemic systems that are far smaller and less important (Zeng and Luo, 2006).

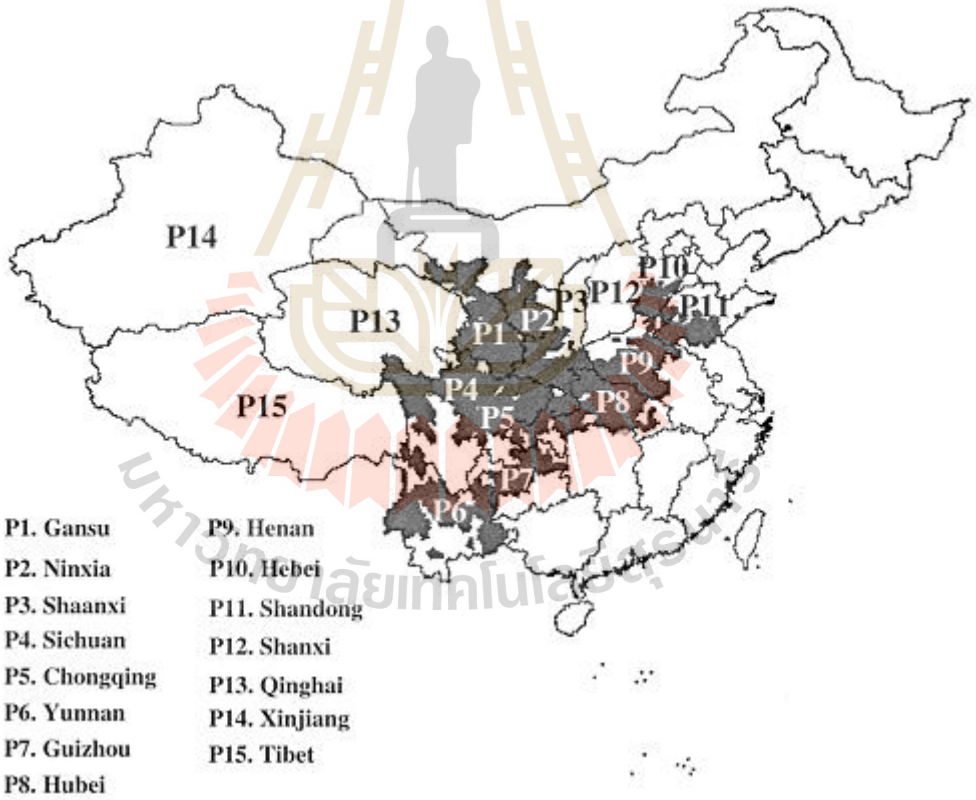


Fig. 2.5 Distribution of the wheat stripe rust epidemic (shaded area) in China in the 2001–2002 growing season (Wan et al., 2004).

In Southwest China, annual climatic conditions, geographic characteristics, and cropping systems are favorable for stripe rust every year. In some parts of Sichuan and Yunnan provinces, the environment and cropping systems provide ideal conditions for the survival, mutation, and development of races of the stripe rust pathogen (Li et al., 1997). Guizhou province is located on the east of the Yunnan-Guizhou Plateau, characterized by sub-tropic monsoon moist climatic region. As the lower temperature in summer and vertical distribution of the wheat in part of Guizhou, it is also suitable for the pathogen over-summering. Therefore, Guizhou is one of the main regions for wheat stripe rust epidemic (Zuo et al., 2011).

2.5 Evolution of the physiological races of *Pst* in China

Since the discovery of CYR1 in 1957, 33 Chinese physiological races of *Pst* have been formally named. A new physiological race appears every 1.6 years on average, which shows a high frequency of *Pst* race variation (Zhan et al., 2011). It is widely accepted that virulence variation of *Pst* is mainly achieved by the pathway of gene mutation and heterokaryosis at asexual stage (Li and Zeng, 2002). It was reported that a new heterocaryotic strain named You Ⅱ was screened out by mixed inoculation of the CYR20 and CYR28 (Kang et al., 1993). Later, it was confirmed that You Ⅱ was generated from recombination of CYR20 and CYR28 by SSR analysis (Zhan et al., 2011). Though *Pst* races have high frequency of genetic variation, growing single resistant cultivar in a large area can accelerate the variation of *Pst* races by directional selection, cause preponderant races group increasing and result in emergence of new pathogenic races with a more wide virulence spectrum (Lin et al., 2010).

Sexual generation of *Pst* race has been discovered in China recently. It was reported that wheat Mingxian 169 inoculated using aeciospores from the rust-infected leaves of *Berberis shensiana* collected from fields, produced uredia typical of stripe rust (Zhao et al., 2011). Therefore, it demonstrated that barberries (*Berberis* spp.) not only serve as alternate hosts for stripe rust pathogen of wheat in China, but the pathogen of wheat stripe rust can also perform its sexual stage on *Berberis* spp. This finding provides a more satisfactory interpretation for high level of genetic diversity and virulence variation.

At present, commercial cultivars in China resistant to main epidemic race of *Pst*, usually contain one all-stage resistance gene following the gene-for-gene interaction model. Owing to the specificity, their resistance to stripe rust is frequently overcome by new races of the pathogen (Chen and Moore, 2002). Therefore, many commercial cultivars lost their resistance to stripe rust in the past 50 years. The main virulence races of *Pst* and the representative cultivars they overcame in different stages are shown in Table 2.1 (Dong and Xu, 2009).

Table 2.1 Main virulence races of *Pst* and the representative cultivars they overcame

Year	Race	Representative cultivar
1955-1956	CYR1	Bima 1, Xibei 54
1960-1962	CYR8, CYR10	Yupi, Gansu 96
1962	CYR13, CYR16	Nanda 2419
1969	CYR17, CYR18, CYR19	Zaoyangmai, Beijing 8, Abbondanza
1976	CYR21, 22, 23, 24, 25, 26, 27	Youpi 2, Taishan 1, Fengchan 3, Afu
1985	CYR28, CYR29, pathogenic group for Lovrin	Lovrin series and Lovrin derived cultivars
1995-	CYR 30, 31, 32, 33, pathogenic group for Hybrid 46	Fan 6, Mianyang series, Shuiyuan series

2.6 Stripe rust resistance breeding in China

The breeding for stripe rust resistance in wheat has been carried out since 1950s. Collected or introduced germplasms were also screened and identified for stripe rust resistance. The effective ways for stripe rust breeding are (i) introducing resistant varieties that can be utilized directly for the production of China, (ii) crossbreeding using exotic resistant resource as one parent, (iii) mutation breeding and distant hybridization (Li and Zeng, 2002).

The *Yr*9 derivatives, including Lovrin 10, Lovrin 13, Aurora, Kavkaz, Neuzucht, Predgornaya, and other 1BL/1RS genotypes in their pedigrees, have been used in breeding programs throughout the country since the 1960s. Cultivars with *Yr*9 include the Yumai series (e.g., Yumai 10, Yumai 21, Yumai 36, Luozhen 1, and Yunong 118) in Henan Province, the Lumai series (e.g., Lumai 1, Lumai 14, and Lumai 15) in Shandong, the Jimai series (e.g., Jimai 24) in Hebei, the Jinmai series (e.g., Jinmai 49) in Shanxi, and the Ermai series (e.g., Ermai 1) in Hubei (Wan et al., 2004).

The other cultivars include those developed from Fan 6, containing the *Yr*H46. Fan 6 has been used since the 1970s and Miannong 4, Mianyang 11, Mianyang 19, Mianyang 25, Mianyang 26, Mianyang 28, Mianyang 29, Chuanmai 22, Chuanmai 25, Chuanmai 26, Chuanmai 28, Chuanmai 29, Chuanyu 12, and Chuanyu 24 were bred. Most of these cultivars became susceptible to races CYR31 and CYR32, which were firstly detected in the early 1990s in western China (Gansu, Sichuan, and Qinghai) and subsequently spread to other regions (Wan et al., 2004).

At present, resistant resource of Guinong series (*Yr*Gn21, *Yr*Gn775) and 92R series (*Yr*26) are widely used in breeding program in China. Many cultivars have been bred by applying these materials as a crossing parent. Cultivars with pedigree of

Guinong series include Longjian 9343, Tianxuan 43, Linmai 33, Linmai 34, Mianmai 39-Mianmai 47, Chuanmai 44, Chuanmai 49 and Chuanmai 50. Cultivars with pedigree of 92R series include Lantian 24, Lantian 17, Zhongliang 29, Neimai 9, Neimai 10 and Neimai 11 (Cao et al., 2011).

2.7 Types of resistance to the wheat stripe rust

Race-specific resistance (vertical resistance) and non-race-specific resistance (horizontal resistance) are two major types of resistance to wheat stripe rust. All-stage resistance (ASR), also being called seedling resistance, is generally race-specific and qualitatively inherited and shows hypersensitive response when pathogen infects the host. The variety with all stage resistance expresses an immunity or high resistance to the pathogen but loses resistance when physiological races change, which leads to instability or unsustainability of disease resistance (He et al., 2011).

High-temperature adult plant (HTAP) resistance, slow rust or partial resistance, collectively called adult plant resistance (APR), is non-race-specific or lower race-specific but durable, and is often quantitatively inherited (He et al., 2011). Adult plant resistance to stripe rust is expressed only at the adult plant stage and is thought to be attributed to the additive effect of several minor genes (Lu et al., 2009). The presence of adult plant resistance is characterized by reducing the rate of disease development resulting from longer latent periods, lessening infection frequencies, decreasing uredinial size, and reducing quantities of spore production, because of the frequent failure of haustorium formation rather than immunity or necrotic response to pathogen (Dehghani et al., 2002; Niks and Rubiales, 2002). Adult plant resistance to rust (slow-rusting) was identified in spring bread wheat (*Triticum aestivum* L.)

germplasms of CIMMYT (Singh and Rajaram, 1993), where such resistance has been a major target for selection for over 30 years. Singh et al. (2000) developed wheat lines with near-immune levels of adult-plant resistance based on 4–5 slow-rusting genes that have small to intermediate, but cumulative effects.

2.8 Methods utilized for discovering resistance genes

2.8.1 Qualitative trait mapping

The number and identity of stripe rust resistance genes in wheat cultivars can be conclusively determined by genetic analysis. In this method, the cultivar being studied is crossed with a susceptible parent and the F_1 plants are selfed to obtain F_2 populations, or are backcrossed to the susceptible parent to obtain BC_1F_1 plants. The F_2 or BC_1F_1 plants are then selfed to obtain F_3 or BC_1F_2 families, respectively. The number of segregating resistance genes can then be determined by inoculating the F_3 and BC_1F_2 families with specific rust races in seedling tests, and also evaluating the segregating families for adult-plant resistance in field tests using a representative mixture of rust races. Evaluation of resistance based on segregation of F_3 and BC_1F_2 families is more reliable than just using F_2 plants, because more than a single plant is evaluated for infection type and severity. F_3 and BC_1F_2 families can also be tested simultaneously with different races. Segregation ratios obtained with different races can be used for identifying the number and dominant/recessive nature of resistance genes (Kolmer, 1996).

Bulked segregant analysis is a rapid procedure for identifying markers linking to the gene. The method involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool, or

bulk, the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. Two pools contrasting for a trait (e.g., resistant and susceptible to a particular disease) are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked (Michelmore et al., 1991).

Aneuploid analysis can be used to preliminarily and physically map a gene on a chromosome or chromosome arm through the location of its linked markers. Different types of aneuploid stocks including a series of nullitetrasomic and ditelosomic lines have been developed in wheat and are available for molecular genetic studies (Sears, 1966). These genetic stocks are powerful tools for locating a molecular marker to a specific chromosome (Anderson et al., 1992).

These methods have been widely used to identify stripe resistance gene in wheat. To map the stripe rust resistance gene in Chinese wheat cultivar Chuanmai 42, a cross between Chuanmai 42 and a susceptible line Taichung 29 was made and 819 pairs of wheat SSR primers were screened on the two parents and the resistant and susceptible bulks. Genetic analysis indicated that the stripe rust resistance in Chuanmai 42 was conferred by a single dominant gene, temporarily designated *Yr*CH42, located close to the centromere of chromosome 1B and flanked by nine SSR markers (Li et al., 2006). To study the stripe rust resistance gene in Australian wheat cultivar Rubric, Rubric was crossed with Avocet 'S'. Genetic analysis of the F_3 population indicated monogenic inheritance of resistance. Bulked segregant analysis placed *Yr*Rub distal to the microsatellite marker barc75 in chromosome 3BS (Bansal et al., 2010). To study the genetics of the spring wheat cultivar IDO377s' resistance, IDO377s was crossed with 'Avocet Susceptible' (AvS). The results of bulk segregant

analysis showed that eight primer pairs generating strong and repeatable polymorphic bands could differentiate the AvS and the susceptible bulk (SB) from IDO377s and resistant bulk (RB). The stripe rust resistance gene of wheat cultivar IDO377s was identified to be flanked by two RGAP markers with a distance of 4.4 and 5.5 cM. Aneuploid analysis further located the flanking RGAP markers on long arm of the chromosome 2B with nulli-tetrasomic lines and ditelosomic lines of 'Chinese Spring' (Cheng and Chen, 2010).

2.8.2 Quantitative trait mapping

Adult plant resistance to stripe rust is a complex trait, often quantitatively inherited and thought to be conferred by the additive effect of several minor genes. It is also influenced by multiple quantitative trait loci (QTLs), their interaction, the environment, and the interaction between QTL and environment. To more effectively deploy resistance based on diverse slow-rusting genes, it is important to determine their chromosomal locations and develop diagnostic markers for marker-assisted selection (MAS). Conventional QTL mapping and association mapping are the two commonly used tools for dissecting complex traits.

2.8.2.1 Conventional QTL mapping

The conventional QTL mapping also known as linkage analysis mapping. Its principle is that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allow them being analyzed in the progeny. QTL mapping can be completed by two steps-constructing linkage maps and conducting QTL analysis–to identify genomic regions associated with traits.

The principle for linkage map construction is that markers that are close

together or tightly-linked will be transmitted together from parent to progeny more frequently than markers that are located further apart. The frequency of recombinant genotypes can be used to calculate recombination fractions, which may be used to infer the genetic distance between markers. By analyzing the segregation of markers, the relative order and distances between markers will be determined. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome. The three main steps of linkage map construction are: (1) production of a mapping population; (2) identification of polymorphism and (3) linkage analysis of markers (Collard et al., 2005).

QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotypic groups according to the presence or absence of a particular marker locus, and to determine whether significant differences exist between locus and the trait being measured. Three widely-used methods for detecting QTLs are single-marker analysis, simple interval mapping (SIM) and composite interval mapping (CIM) (Collard et al., 2005).

Some linkage analysis studies have been conducted to map the QTL for wheat stripe rust resistance. To elucidate the genetic basis of the resistance of the Italian common wheat cultivars Libellula and Strampelli, F_3 populations were developed from crosses between the two cultivars and susceptible cultivar Huixianhong. The F_3 lines were evaluated for disease severity at three locations for 3 years. Joint- and single-environment analyses by composite interval mapping identified five QTLs in Libellula for reduced stripe rust severity across 4 environments. Three QTLs were also detected in Strampelli across 5 environments. (Lu et al., 2009).

To identify major QTL for HTAP resistance to stripe rust in the spring wheat cultivar 'Louise', the mapping population consisted of 188 recombinant inbred lines (RIL) from a cross Louise × 'Penawawa' was developed. $F_{5:6}$ lines were evaluated for stripe rust reaction at five locations in the US Pacific Northwest in 2007 and 2008. The parents and RIL population were also evaluated with 295 polymorphic simple sequence repeat. One major QTL, associated with HTAP resistance in Louise, was detected on chromosome 2BS within a 16.9 cM region flanked by Xwmc474 and Xgwm148 (Carter et al., 2009). To elucidate the genetic basis of the resistance, a mapping population of 178 RILs was developed from a cross between Pioneer 26R61 and the susceptible cultivar AGS 2000. A genetic map with 895 markers covering all 21 chromosomes was used for QTL analysis. One major QTL (*Yr*R61) was detected, explaining up to 56.0% of the mean phenotypic variation, flanked by markers Xbarc124 and Xgwm359, and assigned to the distal of the 2AS (Hao et al., 2011).

2.8.2.2 Association mapping

Linkage disequilibrium (LD) is the nonrandom combination of alleles at two genetic loci, which in random mating populations is mostly generated by mutation and genetic drift, and decays by recombination. Therefore, LD will be observed between two loci if they are in tight linkage or if the haplotype is recent (Breseghello and Sorrells, 2006a).

Association mapping, also known as linkage disequilibrium (LD) mapping, which is a method that relies on linkage disequilibrium to study the relationship between phenotypic variation and genetic polymorphisms, has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level (Zhu et al.,

2008). As a new alternative to traditional linkage analysis, association mapping offers three advantages; (i) increased mapping resolution, (ii) reduced research time, and (iii) greater allele numbers. Since its introduction to plants, association mapping has continued to gain favorability in genetic research because of advances in high throughput genomic technologies, interests in identifying novel and superior alleles, and improvements in statistical methods (Yu and Buckler, 2006).

Based on the scale and focus of a particular study, association mapping generally falls into two broad categories (Fig. 2.6); (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits, and (ii) genome-wide association mapping, or genome scan, which surveys genetic variation in the whole genome to find signals of association for various complex traits (Zhu et al., 2008).

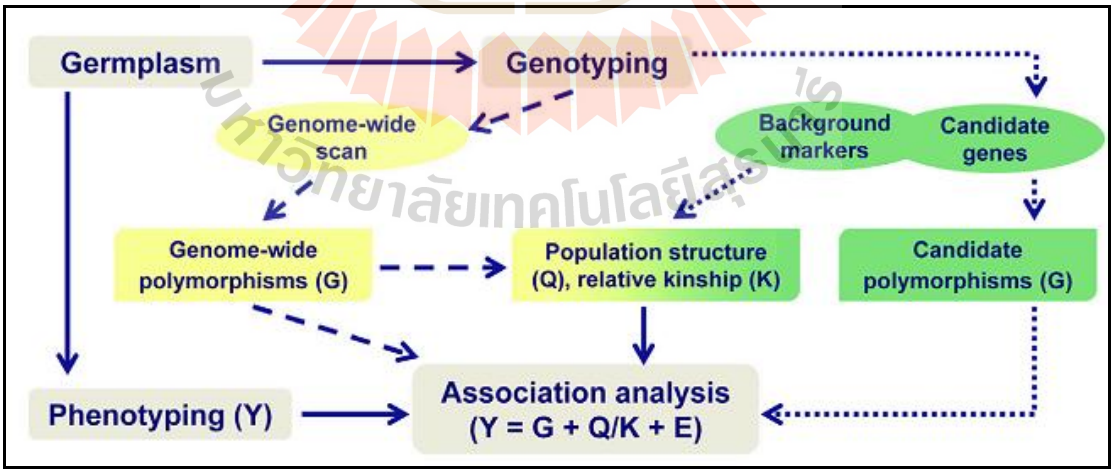


Fig. 2.6 Schematic diagram and contrast of genome-wide association mapping and candidate-gene association mapping

Population structure is universal among plants. It can arise naturally in the form of geographical origins, local adaptation and breeding history of assembled genotypes (Yu and Buckler, 2006). For association mapping, a given sample may fall into one of these defined by population structure associated with local adaptation or diversifying selection and familial relatedness from recent co-ancestry. Spurious associations between genotype and trait due to population sub-structure is widely recognized as a serious obstacle to association mapping.

To better deal with highly structured populations, Pritchard et al. (2000) developed approach of structured association (SA). SA analysis first uses a set of random markers to estimate population structure (Q) and then incorporates this estimate into further statistical analysis. Modification of SA with logistic regression has been used in previous association studies, and a general linear model version (GLM) of this method is implemented in the software TASSEL (Zhu et al., 2008). However, SA incorporating only population structure information in the analysis is not good enough itself when highly structured population with some degree of related individuals was used in the association mapping (Abdurakhmonov and Abdukarimov, 2008).

Hence, recently, Yu et al. (2006) developed new methodology, a mixed linear model (MLM). In this method, random markers are used to estimate Q and a relative kinship matrix (K), which are then fit into a mixed-model framework to test for marker-trait associations. To perform MLM: (1) Q-matrix is generated using STRUCTURE, (2) the pairwise relatedness coefficients between individuals of a mapping population (K-matrix) measured using SpAGeDi software, and (3) then both Q-and K-matrices are used in association mapping to control spurious associations.

Although computationally intensive, MLM approach found to be effective in removing the confounding effects of the population in association mapping.

Recently, association mapping has been used to study the genetics of complex traits in agricultural crops such as rice, maize, and barley. Maccaferri et al. (2011) reported that a collection of 189 elite durum wheat accessions were used to dissect the genetic basis of drought-adaptive traits and grain yield (GY) in 15 environments highly different for water availability during the crop cycle. The results showed that several significant experiment-wise marker-trait associations were detected across five or more environments. To understand the genetics of the kernel size and milling quality, Breseghello and Sorrells (2006b) performed association mapping on a selected sample of 95 cultivars of soft winter wheat. Through a mixed-effects model, association of 62 SSR loci on chromosomes 2D, 5A, and 5B with kernel morphology and milling quality was obtained. To map the *Stagonospora nodorum* glume blotch (SNG) resistance, Tommasini et al. (2007) used the linkage and association to investigate the associations between markers in the region of QSng.sfr-3BS and SNG resistance. In agreement with linkage analyses, association mapping by a least squares general linear model (GLM) at marker loci in the region of QSng.sfr-3BS revealed the highest association with SNG resistance for SUN2-3B ($p<0.05$). This indicated that the association mapping population had a marker resolution potential at least 390-fold higher compared to the RIL population.

2.9 Genomic technology

Numerous DNA-based genetic marker analysis methods have been developed over the last two decades. These include restriction fragment length polymorphism

(RFLP), simple-sequence repeats (SSR), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). Among them, SSR is the most widely used method. The numerous advantages of the microsatellites have been well-documented, such as highly polymorphic rate, co-dominant markers with a Mendelian inheritance and high reproducibility (Zhang, 2006).

Recently, diversity arrays technology (DArT) markers were developed to discover and score genetic polymorphic markers in the whole genome. This technology is a sequence-independent and high-throughput method (Wenzl et al., 2004). Genetic marker analysis through diversity arrays offers a low-cost high-throughput, robust system with minimal DNA sample requirement capable of providing comprehensive genome coverage even in organisms without any DNA sequence information.

This technology is assaying for the presence (or amount) of a specific DNA fragment in a representation derived from the total genomic DNA of an organism or a population of organisms. Two different approaches are presented: the first involves contrasting two representations on a single array while the second involves contrasting a representation with a reference DNA fragment common to all elements of the array. Both two approaches include these steps: (1) generating representations; (2) cloning and amplification of the fragments from representations; (3) printing and processing of diversity panels; (4) fluorescent labelling of representations; (5) hybridisation and washing; (6) scanning, image analysis and data manipulations (Jaccoud et al., 2001).

DArT markers have been applied to several species. Not only the technology has been used to create high-density genetic maps (Semagn et al., 2006) and for association studies (Crossa et al., 2007), but it is also expanding into the study of

genetic diversity and population genetics (Stodart et al., 2008; White et al., 2008). It was reported that when AFLP, SSR, and DArT markers were compared for estimation of genetic diversity in landrace cultivars of bread wheat, DArT markers were more suitable (Stodart et al., 2008).

Single nucleotide polymorphisms (SNP), proposed as third-generation markers, are the main source of genetic variation in plant and animal genomes (Lander, 1996). The decreasing cost, along with the rapid progress in next-generation sequencing (NGS), and related bioinformatics computing resources has facilitated large-scale discovery of SNPs in crops. Genotyping by sequencing (GBS) methods, combining a reduction of genome complexity using restriction enzymes with sequencing using NGS techniques, offer a greatly simplified library production procedure that is more amenable for use on large numbers of individuals/lines (Elshire et al., 2011). Recently, a GBS method combining DArT with NGS, called DArT-seq™, has been developed. The technique, which involves genome complexity reduction, can detect both SNPs and silico DArTs using cost-effective and efficient strategies (Raman et al., 2014). DArT-seq™ has been used in crop studies over the previous two years. It was used to perform genome-wide association mapping of root traits in a panel of Japonica rice accessions, and 9727 DArTs and 6717 SNPs were obtained (Courtois et al., 2013). Additionally, a total of 27,748 markers were also developed to explore the genetic diversity of the U.S. collection of a new oilseed crop of lesquerella and related species using the DArT-seq platform (Cruz et al., 2013).

2.10 Genetic diversity of wheat

Genetic diversity refers to the total number of genetic characteristics in the

genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment (Wikipedia, 2015). Knowledge of the genetic diversity within a germplasm collection is the basis for selection of crossing parents, establishing heterotic groups and has a significant impact on the improvement of crops. Therefore, assessment of the extent and nature of genetic variation in bread wheat is important to breeding and genetic resource conservation programs (Zhang et al., 2011). For this purpose, a number of diversity studies were undertaken in wheat using data on a variety of morphological traits that were subjected to D2 analysis and clustering in the last century (Lee and Kaltsikes, 1973).

However, there are inherent problems with the use of data on morphological traits, the latter being limited in number and greatly influenced by the environment and by genotype × environment interactions. Molecular markers, therefore, provide a satisfactory alternative because they are almost unlimited in number and are not influenced by the environment. Microsatellites or simple sequence repeats (SSRs) are abundant, ubiquitous in presence, hypervariable in nature and have high polymorphic information content (PIC) (Gupta et al., 1996). Due to these properties, a lot of research were reported to use the microsatellites for studying genetic variability based on DNA polymorphism in wheat (Prasad et al., 2000; Reif et al., 2005; Fu and Somers, 2009). Recently, high-throughput genotyping platforms have been available for wheat. DArT or DArT-seq markers have been widely used in genetic diversity analysis (Cruz et al., 2013; Nielsen et al., 2014; Yu et al., 2014).

Some appropriate methods, cluster analysis, principal component analysis and factor analysis, for genetic diversity identification, parental selection, tracing the pathway to evolution of crops, center of origin and diversity, and study interaction between the environment are currently available (Khodadadi et al., 2011). Various algorithms have been used in studying of genetic diversity in cluster analysis, of which, UPGMA methods are the most popular approaches. UPGMA is the most valid method in accordance with the relationship of family (Mohammadi and Prasanna, 2003). There are lots of related reports using these methods. In order to help establish heterotic groups, cluster analysis and principal-coordinates analysis (PCoA) were used to identify the genetic diversity of Chinese northern wheat cultivars (lines). By PCoA, all lines fell into one of two major groups reflecting 1RS/1BL type (1RS/1BL and non-1RS/1BL). Cluster analysis based on the UPGMA suggested the existence of two subgroups within the non-1RS/1BL group and four subgroups within the 1RS/1BL group (Zhang et al., 2011). To summarize the relationships among all lesquerella accessions, cluster analysis was performed on Dice similarity values with the SAHN procedure using the UPGMA method. It indicated that the different accessions were successfully classified based on species, by geographical source, and breeding status (Cruz et al., 2013). To analyze the genetic diversity within the European bread wheat population, the relatedness of the genotypes was investigated using both Bayesian clustering approach and principal component analysis (PCA). Two major subgroups of wheat varieties, GrI and GrII, were identified by both of the two approaches (Nielsen et al., 2014).

2.11 Mapped stripe rust resistance genes or QTLs

Many stripe rust resistance genes have been designated on wheat chromosomes during the past years and its linked molecular markers are also confirmed. Closely linked markers can provide a powerful tool for resistance genes pyramiding and MAS in wheat breeding programs (Narvel et al., 2001; Karakousis et al., 2003). Dong and Xu reported (2009) that 43 stripe resistance genes (*Yr*) on 40 loci were formally named, as shown in Table S2.1. There were 3 and 2 alleles on the locus of *Yr*3 and *Yr*4, respectively. Among them, *Yr*11-*Yr*14, *Yr*16, *Yr*18, *Yr*29, *Yr*30, *Yr*36 and *Yr*39 were adult plant resistance (APR) genes, and the remaining *Yr* genes were all stage resistance genes. Except for *Yr*11-*Yr*14, all of *Yr* genes were mapped on wheat chromosomes. So far, stripe rust resistance genes from *Yr*41 to *Yr*67 also have been mapped and designated (Table S2.2). Besides, several stripe rust resistance genes have been temporarily designated (Table S2.3). QTLs for adult plant resistance to stripe rust, which were detected by conventional QTL mapping methods, have also been summarized (Table S2.4) (Maccaferri et al., 2015). These genes or QTLs and their flanking markers, and selected common wheat lines with the genes should be valuable for diversifying resistance genes used in breeding wheat cultivars with stripe rust resistance.

2.12 Reference

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CHAPTER Ⅲ

THE DIVERSITY OF STRIPE RUST RESISTANCE GENES IN SOUTHWESTERN CHINA WHEAT

3.1 Abstract

In this study, it was aimed to investigate the resistance to stripe rust and the combination of 6 resistance genes (*Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 and *Yr*29) among a collection of 140 common wheat cultivars, mainly from Southwest China. Their resistance to stripe rust was evaluated by natural infection at 3 sites in Guizhou (Guiyang and Hezhang) and Sichuan (Mianyang) provinces at adult plant stage from 2012 to 2014. The results showed that 49 cultivars (lines) were resistant at all sites and years. Among them, 39 cultivars were from Guizhou and 7 from Sichuan. Meanwhile, these accessions were tested by 7 molecular markers linked to stripe rust resistance genes. Most cultivars carried the resistance gene *Yr*26 (48.6%), and fewer cultivars carried *Yr*29 (12.1%), *Yr*10 (7.9%), *Yr*18 (6.4%) and *Yr*15 (0.0%). There were 10 types of *Yr* gene combinations among 29 cultivars in our collection. Among which, the gene combination of *Yr*9+*Yr*26 and *Yr*26+*Yr*29 were the most frequent. Our results indicate that all stage resistance gene *Yr*26 is prevalent in Southwestern China wheat, and stripe rust resistance genes are not diverse. Therefore, wheat breeders in this region should pay more attention to discovery and application of new resistance genes, as well as polygene pyramiding in the future.

3.2 Introduction

Wheat stripe rust is a type of leaf disease caused by a fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), which is usually found in northwest, southwest, north and north Huai river winter wheat region and northwest spring wheat region of China. Among these regions, Sichuan, Yunnan, Guizhou and Tibet winter wheat regions are most seriously infected (Yang and Wu, 1990; Ma et al., 1999). It may reduce wheat production by 20 to 30% in epidemic years but 50% or more in disastrous years (Li and Shang, 1989; Ma et al., 2013).

Due to high variation of *Pst* races, many race specific resistant germplasms and their derivations lost their resistance to new race, leading to epidemics of wheat stripe rust. *Yr*9 was originated from rye. It was mapped on chromosome 1BL/1RS (Francis et al., 1995). Accompany with the introduction of Lovrin series (1BL/1RS translocation lines), *Yr*9 was widely used in wheat breeding in China before the emergence of race CYR32. *Yr*10 was firstly discovered in common wheat PI178383 (Macer, 1975). It was then located on 1BS, with 2.0 cM distance to the *Rg*1 locus (Metzger and Silbaugh, 1970). It received wide attention for its resistance to Chinese yellow rust races, but has not been utilized. *Yr*15 was firstly reported as a dominant resistant gene to yellow rust in wild emmer wheat and was shown to be highly resistant to more than 20 stripe rust races from six countries (Gerechter-Amitai and Stubbs, 1970). The *Yr*15 gene was then introgressed from *T. dicoccoides*, accession G-25, to cultivated tetraploid and hexaploid wheat (Grama and Gerechter-Amitai, 1974), and was mapped on chromosome 1BS using cytogenetic analysis (McIntosh and Silk, 1996). R55 possessed resistance to stripe rust at all growth stages and its gene was mapped on 1BS. It was formally named as *Yr*26, which was probably derived from *Triticum turgidum*

cv.80-1, the original wheat parent used in the cross with *H. villosa* for the development of the 6VS/6AL translocation lines (Ma et al., 2001). Since the appearance of Chinese yellow rust race 32 (CYR32), it was widely used in wheat breeding in southwestern China (Han et al., 2010).

In recent years, geneticists and plant breeders have emphasized the importance of developing and deploying cultivars that carry slow rusting or adult plant resistance based on quantitatively inherited multiple genes. *Yr*18 is most widely used slow rusting gene, which was proved to be the same gene as *Lr*34 and *Pm*38 on 7D (Lillemo et al., 2008; Krattinger et al., 2009). It had a high frequency in wheat landraces of China and has kept its resistance for 70 years (He et al., 2011a). *Yr*29, also being a slow rusting gene, was mapped on chromosome 1BL and closely linked with *Lr*46 (William et al., 2003). Both *Yr*18/*Lr*34/*Pm*38 and *Yr*29/*Lr*46 are accompanied by leaf tip necrosis, so it can be used as a phenotype marker for field selection. It was also reported that yield loss might reach 31 to 52% of total yield when the multi resistance locus *Yr*18/*Lr*34/*Pm*38 existed alone in a cultivar but would be less than 10% when it combined with 3 or 4 minor effect genes (Singh et al., 2000). Since 1960s, CIMMYT has bred a series of adult resistant cultivars, which have been used commercially (Singh and Rajaram, 1992).

In the last decade, resistance genes from related plants were introduced into common wheat by distant hybridization and a series of resistant cultivars (lines) were bred, such as Guinong 21 (Cheng et al., 2008), Guinong 22 (Li et al., 2011), Guinong 775 (Han et al., 2012), Chuanmai 107 (Zhu et al., 2010), Chuanmai 42 (Zhang et al., 2006) etc. In this study, a collection mainly consisting of wheat cultivars (lines) from Southwest China were scored for resistance to stripe rust under different environments.

Besides, the stripe rust resistance genes possibly carried by the Southwestern China wheat were also estimated using a molecular maker detection method. The specific objectives of our study were to: (i) obtain the information on the distribution of all stage resistance genes (*Yr*9, *Yr*10, *Yr*15 and *Yr*26) and adult plant resistance genes (*Yr*18, *Yr*29) in Southwestern China wheat; (ii) comprehensively evaluate the durable resistance of Southwestern China wheat.

3.3 Materials and Methods

3.3.1 Plant materials

The 140 wheat (*Triticum aestivum* L.) cultivars (lines) included in our study represent diverse cultivars utilized in winter wheat regions of Southwest China. Among them, 75 cultivars were collected from Guizhou province, 42 from Sichuan Province, 5 from Jiangsu province and other 18 from Northern part of China (Hubei, Henan, Shanxi province and Beijing) (Table S 3.1). Besides, the cultivar Avocet S and near-isogenic lines (Avocet S *Yr*10 NIL, Avocet S *Yr*15 NIL and Avocet S *Yr*18 NIL), which were used in molecular detection as a positive and negative check, were provided by Plant Protection Institute of the CAAS (Chinese Academy of Agricultural Sciences).

3.3.2 Resistance identification for wheat stripe rust

The experiment was laid out for 2 seasons: during 2012 to 2013 at the plots of Guiyang (Guizhou province), Hezhang (Guizhou province) and during 2013 to 2014 at plots of Guiyang (Guizhou province), Mianyang (Sichuan province). All field evaluations were conducted with RCBD design in a three-repeat trial. Approximately 100 seeds of each line were sown in 60 cm wide paired-row plots, 1 m in length, with

30-cm row spacing and a 50-cm pathway between plots. Each plot was surrounded by the susceptible wheat cultivar ‘Mingxian 169’ as a spreader to ensure an effective disease spread. Disease reaction was recorded generally after the stem elongation stage. All of sites were recorded 3 times. The scores for the time when stripe rust reaction of the susceptible check reached the highest disease severity were used in analysis. The host response (infection types) to stripe rust followed a 0-to-4 scale (Li and Zeng, 2002). Infection types 0, 0;, 1, 2, 3, 4 were characterized as immune (IM), nearly immune (NIM), highly resistant (HR), moderately resistant (MR), moderately susceptible (MS), and highly susceptible (HS), respectively (Li and Zeng, 2002).

3.3.3 DNA extraction and *Yr* gene detection

Genomic DNA from the 140 different genotypes was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) procedure (Saghai-Maroof et al., 1984). Plant material was harvested at the seedling stage and freeze dried. After precipitation of the DNA with isopropanol, the DNA pellet was transferred into a new 1.5 ml microfuge tube, and the DNA was washed two times with cold 75% ethanol and air dried. The final DNA was diluted with TE buffer (pH 8.0) to a concentration of 20 ng DNA per µL.

Seven markers used for detecting the *Yr* genes in this study are listed in Table 3.1. The PCR reaction was performed in a PTC200 Peltier Thermal Cycler in a volume of 20 µl containing 1.0 U Taq DNA polymerase, 2 µl of 10×buffer (50 mM KCl, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, pH 8.3), 200 µM of each dNTP, 6 pM of each primer and 50–100 ng of template DNA. The PCR conditions were as follows: denaturation at 94℃ for 4 min, followed by 35 cycles of 94℃ for 1 min, 50–61℃ (depending on primers) for 1 min, 72℃ for 1 min and a final extension for 10 min at 72℃. PCR

products were mixed with 4 µl of the formamide loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cynol, pH 8.0) and heated at 94℃ for 5 min. Each sample of 5–7µl was loaded on 6% denaturing polyacrylamide gels and run at 80 W for approximately 1.5 h and then resolved by the silver staining method as described by (Bassam et al., 1991).

Table 3.1 Molecular markers for stripe rust resistance genes

Yr **Gene**	**Primer**	**Primer sequence (5'→3')**	**Genetic distance (cM)**	**Amplicon length (bp)**	**Reference**
*Yr*9	AF1/AF4	GGAGACATCATGAAACATTTG CTGTTGTTGGGCAGAAAG	Rye specific	+1500	(Francis et al., 1995)
*Yr*10	SC200	CTGCAGAGTGACATCATACA TCGAACTAGTAGATGCTGGC	0.5	+200	(Shao et al., 2001)
*Yr*15	Barc8	GCGGGAATCATGCATAGGAAAACAGAA GCGGGGGCGAAACATACACATAAAAACA	Completely linked	+250	(Peng et al., 2000)
*Yr*18	csLV34	GTTGGTTAAGACTGGTGATGG TGCTTGCTATTGCTGAATAGT	0.4	+150	(Lagudah et al., 2006)
*Yr*26	Gwm11	GGATAGTCAGACAATTCTTGTG GTGAATTGTGTCTTGTATGCTTCC	1.9	+193	(Ma et al., 2001)
	We173	GGGACAAGGGGAGTTGAAGC GAGAGTTCCAAGCAGAACAC	1.4	+551	(Wang et al., 2008)
*Yr*29	Wmc44	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	5.6	+242	(William et al., 2003)

Note: The presence of band + indicates that a cultivar might carry the resistant allele. For *Yr*26, 2 user-friendly markers were available.

3.4 Results

3.4.1 Evaluation of stripe rust resistance of Southwestern China Wheat

Through natural disease infestation, 140 cultivars were evaluated for stripe rust resistance for 2 years at different sites. Due to bad germination, 116 and 139 cultivars were scored for infection type at Hezhang site in 2013 and at Mianyang site in 2014. As shown in Table S3.2, there were 66, 10 and 64 cultivars that were immune, highly to moderately resistant and highly to moderately susceptible to stripe rust, respectively at Guiyang site in 2013. Similarly, there were 59, 16 and 41 cultivars that were immune, highly to moderately resistant and susceptible to stripe rust, respectively at Hezhang site in 2013. However, the numbers of immune, highly to moderately resistant and highly to moderately susceptible cultivars were 69, 43 and 28, respectively at Guiyang site, 12, 67 and 60, respectively at Mianyang site in 2014. Based on the data of 2 years evaluation, there were 49 cultivars that were resistant to the stripe rust at all environments, among which, there were 39, 7 and 3 cultivars from Guizhou, Sichuan and other provinces (52.0%, 16.7% and 13.0% of total cultivars, respectively). Among the 49 cultivars, 7 cultivars were immune to stripe rust at all environments, which were 0308, Guinong18, Guinong 19, Guinong 28, TP3, Guixie 1 and Guixie 3. In contrast, there were 6 cultivars that were highly susceptible to stripe rust at all environments, which were Mianyang 26, Miannong 4, Qianmai 14, Qian2032-8, Fengyou 92212 and Xiaoyan 54.

A comparison of resistance of 140 cultivars to wheat stripe rust at different sites in 2013 and 2014 was performed as shown in Figure 3.1. There were 47%, 51% and 49% cultivars immune to stripe rust at Guiyang and Hezhang sites in 2013 and Guiyang site in 2014, respectively, however, only 9% cultivars were immune to stripe

rust at Mianyang site in 2014. Meanwhile, the percentage of cultivars with resistant level was also different in different environments. Eighty percent of cultivars were immune or resistant at Guiyang site in 2014 and 65% of cultivars did at Hezhang site in 2013. Lower percentages of cultivars were immune or resistant at Guiyang site in 2013 and Mianyang site in 2014, which were 54% and 57%, respectively. It showed that stripe rust was more serious at Guiyang site in 2013 and Mianyang site in 2014.

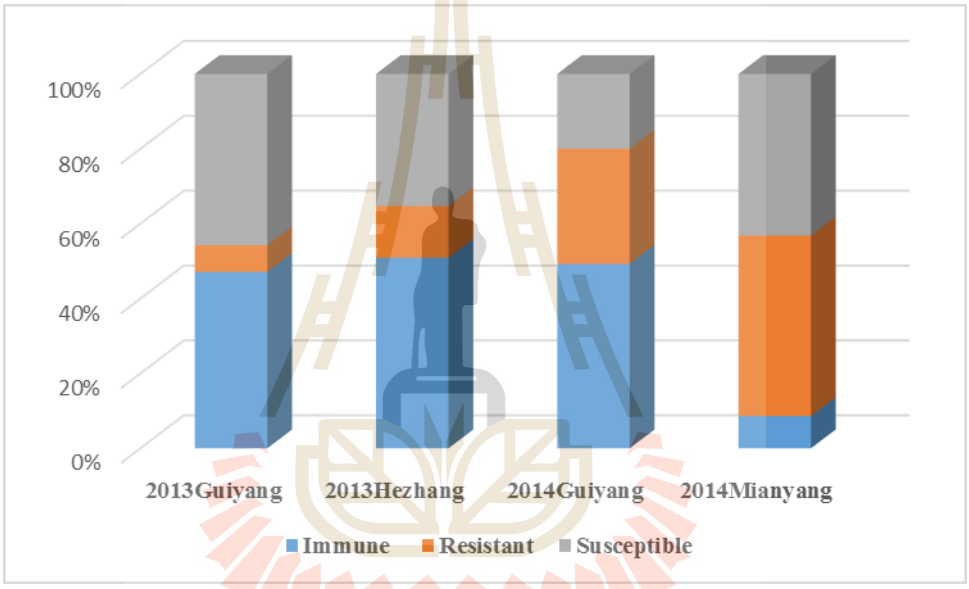


Fig. 3.1 Resistance to wheat stripe rust in different environments. Cultivars with the infection type 1 or 2 were classified as resistant but those with the infection type 3 or 4 were classified as susceptible. Only the infection types 0 or 0; were classified as immune.

A comparison of resistance of the cultivars with different origins at different sites in 2013 and 2014 was also performed. The results are shown in Table 3.2. In light epidemic environments (Hezhang site in 2013 and Guiyang site in 2014), there were

66% and 83% of Guizhou cultivars that were resistant in the field evaluations, respectively. Meanwhile, there were 67% and 83% of Sichuan cultivars that were resistant, respectively. But in serious epidemic environments (Guiyang site in 2013 and Mianyang site in 2014), there were 68% and 65% Guizhou cultivars that were resistant, respectively. By contrast, there were 45% Sichuan cultivars that were resistant at both sites. At Guiyang and Hezhang site in 2013 and Guiyang site in 2014, there were 29%, 40% and 71% of other regional cultivars that were resistant to stripe rust, which were lower than the cultivars from Guizhou and Sichuan. Nevertheless, there were 50% of other regional cultivars resistant to stripe rust at Mianyang site in 2014, which was higher than Sichuan cultivars.

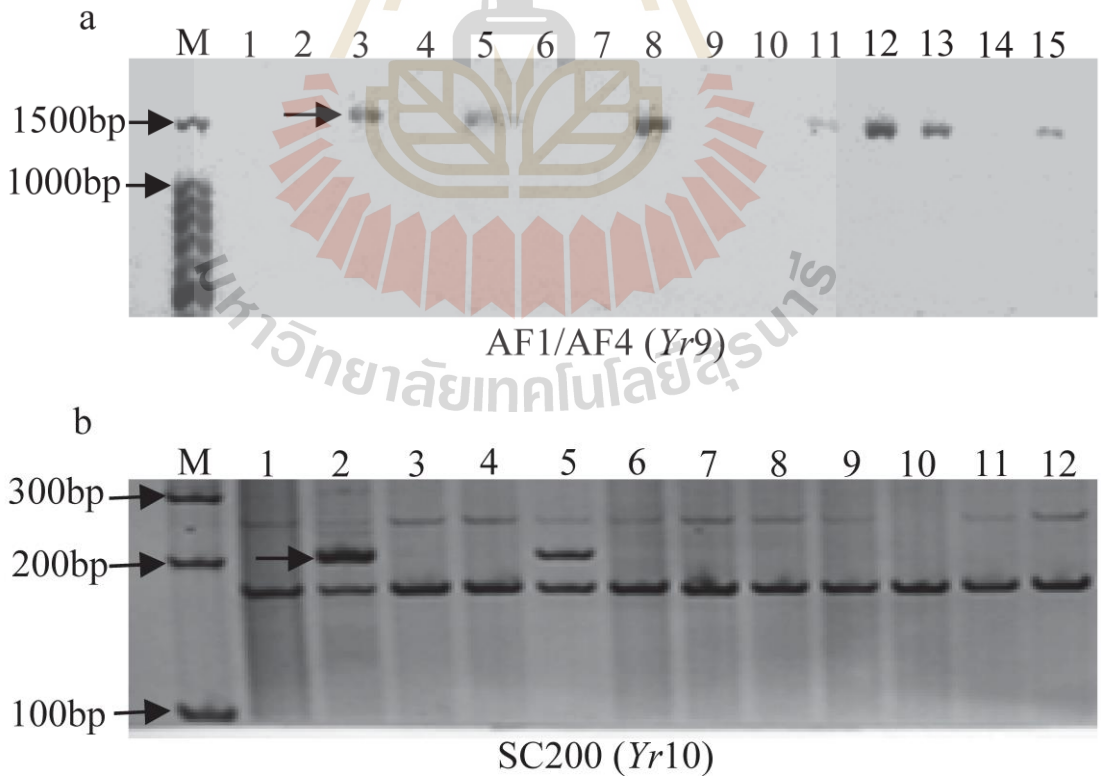
Table 3.2 Resistance of the cultivars from different origins to wheat stripe rust

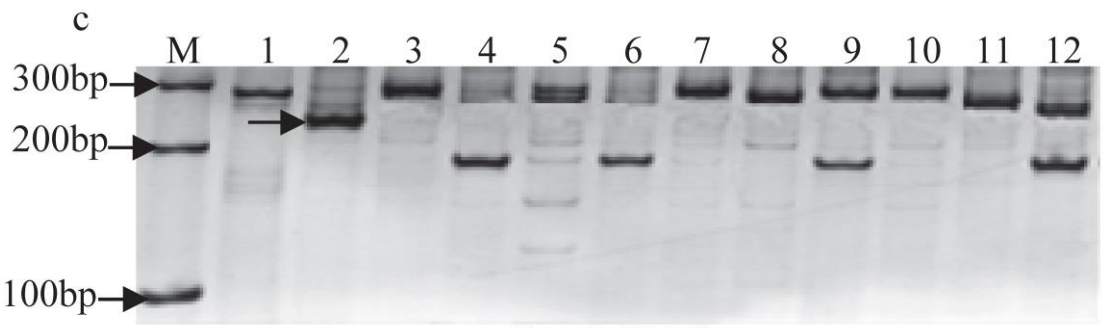
Year	Site	Number of resistant cultivars (percentage)		
		Guizhou cultivars	Sichuan cultivars	Other regional cultivars
2013	Guiyang	51 (68%)	18 (45%)	7 (29%)
2013	Hezhang	47 (66%)	20 (67%)	4 (40%)
2014	Guiyang	62 (83%)	33 (83%)	17 (71%)
2014	Mianyang	49 (65%)	18 (45%)	12 (50%)

3.4.2 Distribution of the stripe rust resistance genes in Southwestern China wheat

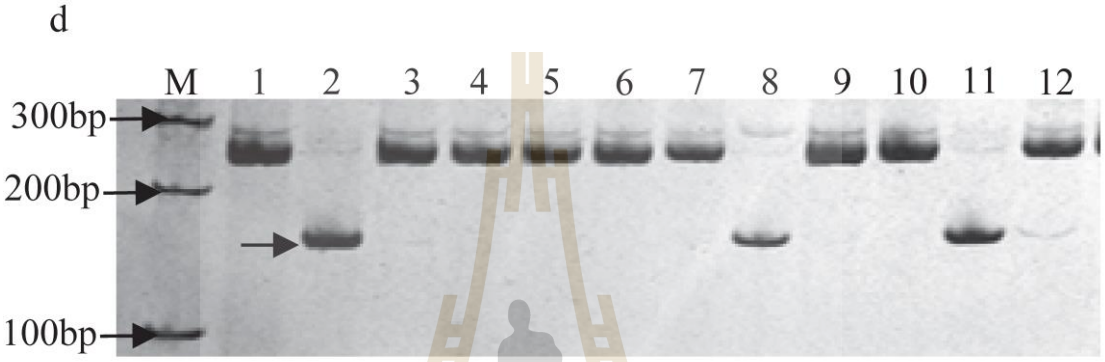
In this study, the 140 cultivars were also screened for the stripe rust resistance gene *Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 and *Yr*29 by the closely linked molecular markers (Figure 3.2). The results are shown in Table S3.2 and Table 3.3. Distribution of the 6 stripe rust resistance genes in the Southwestern China wheat from the highest to the

lowest were *Yr*26 (48.6%), *Yr*9 (37.9%), *Yr*29 (12.1%), *Yr*10 (7.9%), *Yr*18 (6.4%) and *Yr*15 was not detected. For the 75 Guizhou cultivars, *Yr*26 was the most abundant stripe rust resistance gene, accounting for 61.3%, and *Yr*18 was the rarest one, accounting for 5.3%. For the 42 Sichuan cultivars, *Yr*9 was the most abundant, accounting for 51.2%, but *Yr*10 and *Yr*18 were not detected. For the 23 other regional cultivars, *Yr*26 and *Yr*9 were the most frequent, both accounting for 25%, and *Yr*29 was the rarest, accounting for 8.3%. Distribution of a certain stripe rust resistance genes in the cultivars of different origins was also analyzed. It showed that *Yr*26 and *Yr*29 were the most abundant in Guizhou cultivars; *Yr*9 was the most abundant in Sichuan cultivars; and the rest stripe rust resistant genes were the most abundant in other regional cultivars.



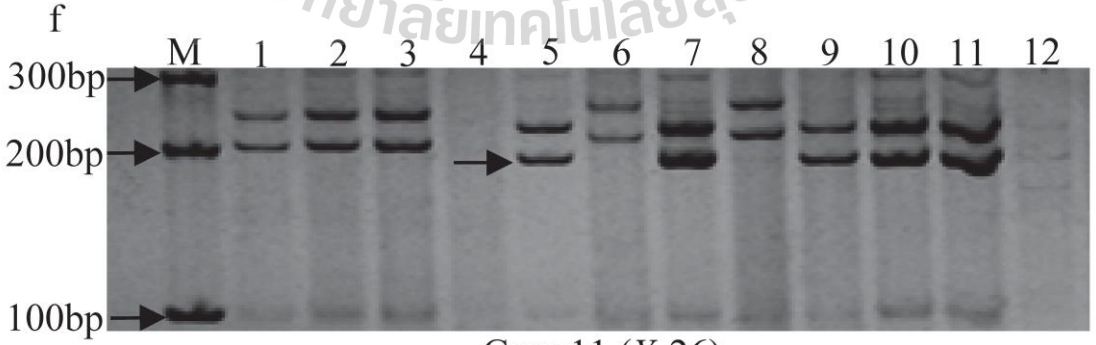


Barc8 (*Yr*15)



csLV34 (*Yr*18)

We173 (*Yr*26)



Gwm11 (*Yr*26)

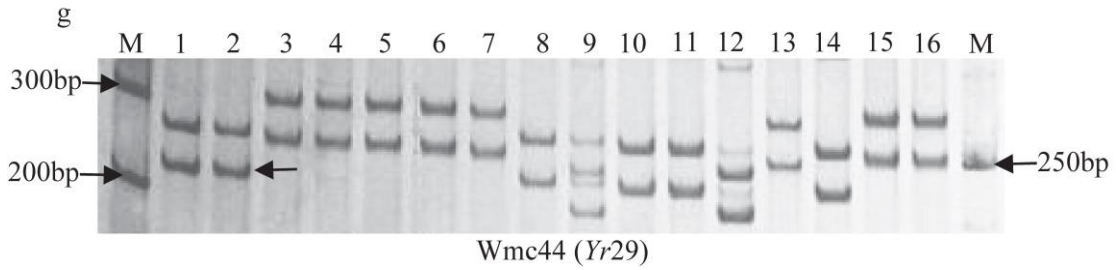


Fig. 3.2 Screening results using the markers linked to the stripe rust resistance genes in some cultivars. In electrophoretogram a, M is marker, and cultivars (lines) with the code from 1 to 15 are AVS, Anmai 7, Bi2007-1, Bi2007-7, Bimai 18, 0308, Chinese Spring, Mianmai 42, 9712, Mingxian 169, Guimai 12, Fengyou 1, Fengyou 6, Guiyu 17 and Chuanmai 44, respectively. In electrophoretogram b, c, d, e and f, M is marker, 1 is AVS and 3 to 12 are Anmai 7, Bi2007-1, Bi2007-7, Bimai 18, 0308, Chinese Spring, Mianmai 42, 9712, Mingxian 169 and Guimai12, repectively. Wheat accession with code 2 is AVS/6*_Yr_10、AVS/6*_Yr_15 and AVS/6*_Yr_18, respectively, in electrophoretogram b, c and d, but is Qianmai 16 in electrophoretogram e and f. In electrophoretogram g, M is marker, and cultivars (lines) with the code from 1 to 16 are Xingyu 7, Fengyou 9, Guinong 18, Guinong 19, Guinong 25, Guinong 28, Baimian 3, Xikemai 2, Guinong 21-1, Chuanmai 60, P5-1, P7-9, Jieyanpu 2, P7-26, TG and TP2, respectively. The bands, which the arrows point to in all electrophoretograms, indicate sequences with 1500 bp, 200 bp, 250 bp, 150 bp, 551 bp, 193 bp and 242 bp length (positive alleles for *Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 and *Yr*29), respectively.

Table 3.3 Genotyping results of 6 stripe rust resistance genes in 140 wheat cultivars (lines)

Origins	Number of cultivars (percentage)					
	***Yr*9**	***Yr*10**	***Yr*15**	***Yr*18**	***Yr*26**	***Yr*29**
Guizhou	26 (34.7%)	6 (8.0%)	0 (0%)	4 (5.3%)	46 (61.3%)	13 (17.3%)
Sichuan	21 (51.2%)	0 (0.0%)	0 (0%)	0 (0.0%)	16 (39.0%)	2 (4.9%)
Other regions	6 (25.0%)	5 (20.8%)	0 (0%)	5 (20.8%)	6 (25.0%)	2 (8.3%)
Total	53 (37.9%)	11 (7.9%)	0 (0%)	9 (6.4%)	68 (48.6%)	17 (12.1%)

3.4.3 Relationship of the stripe rust resistance and Yr genes

In order to understand the relationship between stripe rust resistance and *Yr* genes of Southwestern China wheat, Pearson correlation coefficient was calculated with field evaluation data of 140 cultivars in 4 environments and results are shown in Table 3.4. In the 3 environments (Guiyang site in 2013 and 2014, Hezhang site in 2013), *Yr*26 showed a highly significant correlation with stripe rust resistance and the correlation coefficients were 0.58, 0.41 and 0.50 respectively, however, no significant correlation was observed at Mianyang site in 2014. Besides, negative correlation between stripe rust resistance and *Yr* genes was also found, for instance, *Yr*9 showed significantly negative correlation with stripe rust resistance in three environments (Guiyang site in 2013 and 2014, Hezhang site in 2013).

Table 3.4 Correlation analysis of resistance genes and disease resistance

Year	Site	*Yr*9	*Yr*10	*Yr*18	*Yr*26	*Yr*29
2013	Guiyang	-0.23*	-0.16	0.01	0.58**	0.08
2013	Hezhang	-0.31**	0	0.00	0.50**	0.12
2014	Guiyang	-0.24*	0.08	0.06	0.41**	0.08
2014	Mianyang	0.01	-0.01	0.05	0.06	-0.07

Note: Significant level: * $p<0.05$; ** $p<0.01$

3.4.4 Analysis of stripe rust resistant loci combination

According to number of detected *Yr* genes by molecular markers, 140 cultivars were divided into 5 types. There were 26, 85, 26, 2 and 1 cultivars with 0, 1, 2, 3 and 4 *Yr* genes, respectively. For one gene type, there were 39, 5, 2, 36 and 3 cultivars with single *Yr*9, *Yr*10, *Yr*18, *Yr*26 and *Yr*29, respectively. For the two genes, three genes and four genes types, each *Yr* gene combination was shown in Figure 3.3. There were total 10 gene combinations or 29 wheat cultivars belonging to combinations carrying from 2 genes to 4 genes. When these 29 wheat cultivars were considered, 2-gene combinations were the most frequent, which can be further divided into 7 classes and included 26 wheat cultivars, accounting for 90%. Among the 2-gene combinations, *Yr*9+*Yr*26 and *Yr*26+*Yr*29 were the most frequent. Besides, there were 2 cultivars and 1 cultivar belonging to the 3-gene combination and 4-gene combination, accounting for 7% and 3%, respectively.

Combination	*Yr*9	*Yr*10	*Yr*18	*Yr*26	*Yr*29	Number	Total	Frequency
2 genes						3	26	90%
						7		
						2		
						1		
						2		
						2		
						9		
3 genes						1	2	7%
						1		
4 genes						1	1	3%
Total						29	29	100%

Fig 3.3 Distribution of resistance gene combinations to wheat stripe rust. Different colors represented the different stripe rust resistance loci a cultivar is carrying and a certain gene combination refers to the cultivars carrying the same number of *Yr* genes, which were also consistent by type. Number refers to how many cultivars were included in a certain combination.

To compare different types of resistance gene combinations, cultivar infection types at 4 environments were summarized, excluding the cultivars from three genes and four genes types because of small sample size (Fig 3.4). Cultivars pyramided with more *Yr* genes were resistant to stripe rust at more environments on average.

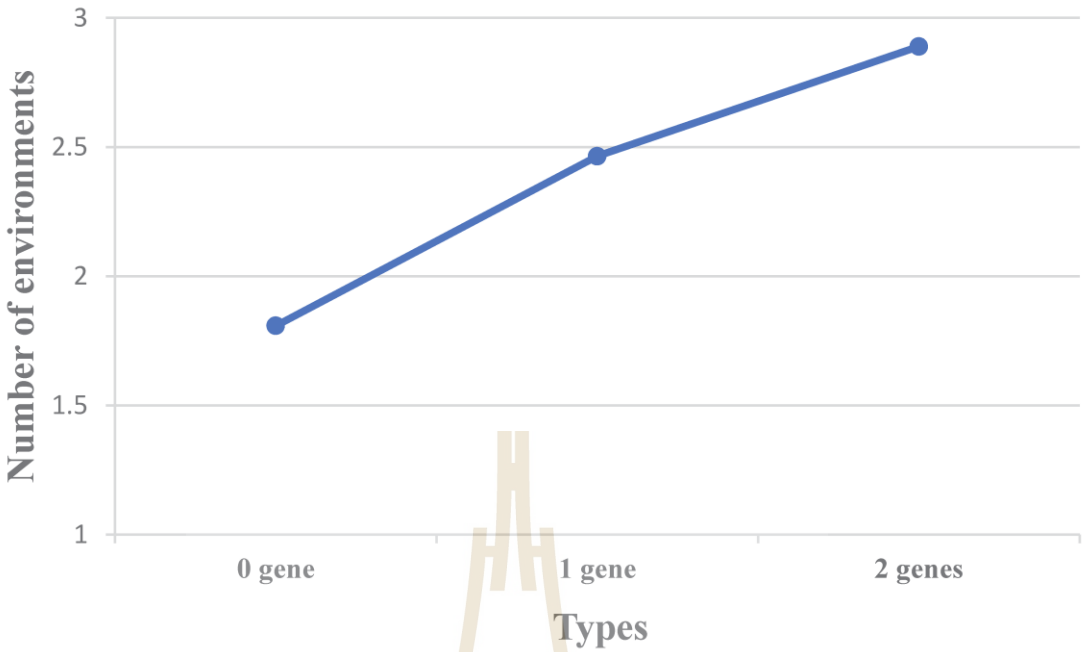


Fig 3.4 Average number of environments that cultivars with different types resistance gene combinations were resistant to stripe rust. Cultivars were field evaluated for stripe rust resistance at 4 environments in total. For 26 zero-gene type cultivars, it appears to be resistant at 1.8 environments on average. But for one-gene type and two-genes type cultivars, it appears to be resistant at 2.5 environments and 2.9 environments on average, respectively.

Besides, we also compared the *Yr* gene combinations of cultivars which were immune or highly susceptible at 4 environments (Figure 3.4). For the 7 immune cultivars, all carried the gene *Yr*26 except the cultivar Guixie3. Among the 6 *Yr*26 carriers, 4 cultivars only carried the *Yr*26, and the rest all carried 2 *Yr* genes with the combination of *Yr*26+X (X represented *Yr*9 or *Yr*29). For the 6 highly susceptible cultivars, none was detected with the *Yr*26, and 2 cultivars were not detected with any of the 5 *Yr* genes. Moreover, among the 6 cultivars, there were 3 cultivars that were detected with *Yr*9 and 1 cultivar with *Yr*10.

Cultivars	Resistance gene					Infection type			
	*Yr*9	*Yr*10	*Yr*18	*Yr*26	*Yr*29	2013GY	2013HZ	2014GY	2014MY
Guixie3						0	0	0	0
0308						0	0	0	0
Guinong18						0	0	0	0
Guinong19						0	0	0	0
Guinong28						0	0	0	0
TP3						0	0	0	0
Guixie1						0	0	0	0
Qianmai14						4	4	4	4
Qian2032-8						4	4	4	4
Fengyou92212						4	4	4	4
Xiaoyan54						4	4	4	4
Miannong4						4	4	4	4
Mianyang26						4	4	4	4

Fig 3.5 Comparison of the resistance gene combinations between wheat cultivars immune to stripe rust and those highly susceptible to it. Different colors represented the different stripe rust resistance loci a cultivar is carrying.

3.5 Discussion

3.5.1 Resistance of wheat cultivars to Southwestern China Pst races

Virulence of *Pst* races appeared to be variable in different provinces of China. When thirty nine near-isogenic lines with single wheat stripe rust resistance gene and materials containing known wheat stripe rust resistance genes were used for the virulence identification and virulence frequency analysis of 124 isolates of Chinese wheat stripe rust collected from Sichuan, Yunnan, Guizhou and Gansu, it was found that Sichuan region had the highest virulence, Guizhou region had the lowest virulence, and Yunnan region had the middle one (Zheng, 2009). Similar results were also shown in this study. There were 47.1%, 49.3% and 50.9% of total cultivars immune to local stripe rust races in Guizhou (Guiyang site for two years and Hezhang site in 2013) but only 8.6% of total cultivars that did in Sichuan (Mianyang site in 2014). Therefore, it

proved that stripe rust races in Sichuan were more virulent than Guizhou. Moreover, we also found that the results of field evaluation of stripe rust resistance for 140 wheat cultivars were not consistent at the same site in two years. For example, there were 54.3% of cultivars being at least moderately resistant to local stripe rust races at Guiyang site in 2013 but 80% of cultivars doing so in 2014. Therefore, in addition to the population structure of stripe rust races, pathogen quantity, temperature or moisture of diseased period might also play a role in severity of the disease.

By comparing resistance of the cultivars with different origins, it was found that Guizhou cultivars had the same degree of resistance with Sichuan cultivars in lighter disease environments (at Hezhang site in 2013 and Guiyang site in 2014) but were more resistant in serious disease environments (at Guiyang site in 2013 and Mianyang site in 2014), which proved that durable resistance of Guizhou cultivars was better than Sichuan cultivars. It might be due to lacking of non-race specific resistance genes (slow rusting genes) in Sichuan cultivars, which was confirmed by resistant gene screening. The stripe rust resistance for other regional (Jiangsu and North China) cultivars was lower, especially in 2013 with the ratio of 29.2% and 40% cultivars being resistant at Guiyang site and Hezhang site, respectively. By contrast, Han et al. (2010), who used similar origin cultivars, reported that 70% of studied cultivars were not resistant to current epidemic races. However, other regional cultivars were found to have a higher ratio of resistant cultivars (50%) in the serious disease environment (Mianyang site in 2014), which might be caused by the high temperature induced resistance (high-temperature adult-plant resistance HTAP) in some cultivars at Mianyang site in 2014 (Uauy et al., 2005).

3.5.2 Distribution of stripe rust resistant genes

Stripe rust resistance genes are not evenly distributed in Southwestern China wheat. *Yr*26 was used as the main resistant source in breeding programs in Southwest China. Meanwhile, *Yr*9 also played an important role but all growth stage genes *Yr*10, *Yr*15 and adult plant resistant genes *Yr*18, *Yr*29 were neglected in wheat breeding programs of Southwest China. By contrast, Xue et al. (2014) reported that 24 (32.4%) and 5 (6.8%) cultivars were detected to carry *Yr*9 and *Yr*26 but no cultivars were detected with *Yr*10, *Yr*15 and *Yr*18, when 74 Chinese elite wheat were studied using molecular markers. Zeng et al. (2014) studied 494 Chinese leading cultivars or breeding lines based on stripe rust reactions and molecular markers, who found that *Yr*9, *Yr*18, and *Yr*26 were confirmed in 134 (29.4%), 10 (2%), and 15 (3%) entries, respectively, and no entry had *Yr*10 or *Yr*15. All three studies indicate that *Yr*9 have been widely used in breeding programs in China due to its resistance to most popular stripe rust races since 1970s (Li et al., 2006b) but *Yr*10, *Yr*15 and *Yr*18 still fail to raise concern of breeders. Different from other two studies using cultivars from all parts of China, we detected more cultivars with *Yr*26, which implies that *Yr*26 were over-used in Southwest China, due to widely utilizing 6VS/6AL translocation lines and Guinong series in Southwest winter wheat region of China (He et al., 2011b).When cultivars of different origins were analyzed separately, it showed that, *Yr*26 was the predominant resistant gene in Guizhou, however, breeders of which also paid attention to *Yr*9 and *Yr*29. Wan et al. (2011) also reported that *Yr*26 was most abundant (38%) when 108 resistant wheat germplasms of Guizhou were used to detect *Yr*10, *Yr*15, *Yr*18 and *Yr*26. However, less diversity of resistant genes was observed in Sichuan cultivars (no cultivar was detected with *Yr*10, *Yr*15 and *Yr*18) while the ratio of *Yr*26 and *Yr*29 were

lower comparing to Guizhou cultivars. These results could explain why resistance of Sichuan cultivars fluctuated strongly in different environments. Wu et al. (2007) also reported lower ratio of *Yr*10 and *Yr*15 in 72 wheat lines of Sichuan province trial and proposed that *Yr*26 was still the main resistant source used in Sichuan wheat breeding. But in other regional cultivars, the ratio of *Yr*26 was far below those in Guizhou or Sichuan cultivars. It implied that most parts of those regions were not usually the hard-hit for stripe rust, so no close attention was paid to the resistance gene *Yr*26. But adult plant resistant genes *Yr*18 or other unknown HTAP genes, which were non-race specific, were better utilized in these regions. Therefore, if new races that could overcome *Yr*26 emerged, many other regional cultivars still can maintain the non-race specific resistance, which was proved by the evaluation results at Mianyang site in 2014.

Based on the correlation analysis, the presence or absence of *Yr*26 was significantly and positively correlated with resistance of cultivars in 3 environments (Guiyang and Hezhang site in 2013, and Guiyang site in 2014) but not at Mianyang site (Sichuan province). It might be due to the appearance of the new virulent mutant of stripe rust, currently nominated CH42 (V26) or avr*Yr*10/24/26/ch42 in different research (Liu et al., 2010; Liu et al., 2012; Kuang et al., 2013). This new pathotype could lead to the disability of *Yr*24/26 and was also virulent to*Yr*10 and *Yr*Ch42 (Liu et al., 2010). However, this new pathotype was avirulent to *Yr*9 but could infect 38 of tested 39 commercial wheat cultivars registered in Sichuan (Kuang et al., 2013). It was also reported that when 1,014 stripe rust samples collected from 14 provinces during the year of 2010-2011 were phenotyped, V26 pathotype was mainly distributed in Sichuan, Gansu and Yunnan provinces (Liu et al., 2012). Correlation analysis also

showed that *Yr*9 was negatively and significantly correlated with stripe rust resistance of cultivars at the 3 environments in Guizhou. CYR32 is a predominant race in Guizhou (Zuo et al., 2011), which can infect the cultivars with *Yr*9 (Wan et al., 2004). *Yr*9 is mapped on 1RS for 1BL/1RS wheat, meanwhile, *Yr*26 is on 1BS for normal wheat (1BL/1BS) (Ma et al., 2001; Wang et al., 2008). It was very difficult to recombine between 1BS and 1RS during the process of meiosis (Zhou et al., 2004). In this study, *Yr*9 and *Yr*26 were also significantly and negatively related (data not shown). Therefore, the cultivars carrying *Yr*9, which usually did not carry *Yr*26, were susceptible in Guizhou. In our study, *Yr*10 were not significantly correlated with stripe rust resistance at all environments. But unpublished data showed that, among the 11 cultivars positive for SC200, 8 cultivars were susceptible to CYR32, which is not consistent with the previous research reporting that CYR32 is avirulent to *Yr*10 (Li et al., 2006a). Similar results showing that cultivars with the $Yr10_{CG}$ gene displayed varying infection types from susceptible to fully resistant was also reported recently (Yuan et al., 2012). Therefore, whether positive alleles in the 8 cultivars are *Yr*10 or expression level of the gene in different backgrounds affects the resistance level remains to be investigated. *Yr*18 and *Yr*29 are both mini-effect APR genes, cultivars with which do not appear to be immune or have necrosis response but slow disease development at adult plant stage (He et al., 2011a). Therefore, few APR genes cannot make a cultivar resistant under high disease pressure, which lead to non-significant correlation between an APR gene and resistance to stripe rust.

3.5.3 *Yr* genes pyramiding and its effect to the resistance to Southwestern China Pst races

Gene combination analysis showed that there were only 29 cultivars carrying

two resistance genes or more in 140 Southwestern China wheat. Among them, the main cultivars were the ones with *Yr*9+*Yr*26 and *Yr*26+*Yr*29. It indicated that all growth stage resistant genes *Yr*9 and *Yr*26 were the main resistance genes and adult plant resistant gene *Yr*29 was gradually used for pyramiding in Southwestern China wheat. The resistance of cultivars with different types of resistance gene combinations showed the trend that the more *Yr* genes were pyramided together, the better resistance the cultivar would possess. But it is not always the case. For the Yiguang series, Yiguang-1, Yiguang-3 and Yiguang-4, which were detected to carry 3, 3 and 4 *Yr* genes by molecular markers, were susceptible at 2, 1 and 1 environments (Table S3.2). Yiguang-1 with the gene combination of *Yr*9+*Yr*10+*Yr*29, was susceptible under high disease pressure (at Guiyang in 2013 and Mianyang in 2014). Besides, Yiguang-1 also were susceptible to CYR32 (unpublished data), which implied that it might carry a disabled *Yr*10 homologue (Yuan et al., 2012). *Yr*9 has been disabled since the emergence of CYR32 in China (Wan et al., 2004). Meanwhile, *Yr*29 is a minor effect *Yr* gene (William et al., 2003). Therefore, Yiguang-1 without functional main-effect *Yr* gene might lead to its susceptibility at the two environments. Yiguang-3 and Yiguang-4, with gene combination of *Yr*18+*Yr*26+*Yr*29, *Yr*9+*Yr*10+*Yr*26+*Yr*29, were moderately susceptible and highly susceptible at Mianyang but immune or highly resistant at other 3 environments. Single *Yr*10 or *Yr*26 was disabled to V26. While, single *Yr*9 is disabled to prevalent *Pst* race but resistant to the V26. *Yr*9 combined with *Yr*10 and *Yr*26 in Yiguang-4 showed weaker resistance than Yiguang-3 (combining *Yr*18 with *Yr*26) at Mianyang site, where the V26 race might exist at a certain frequency. Similar results were also shown in Chuanmai 44 and Chuanmai 50, both of which were with gene combination of *Yr*9+*Yr*26 (Table S3.2). It implies that stripe rust

resistance is a complex process, which might exist the gene interaction between *Yr* genes or closely related to the genetic background. Besides, *Yr*9 and *Yr*26 are significantly and negatively correlated due to chromosome position, therefore, it need a further verification of the *Yr*9 (*Yr*26 has been verified by the test of CYR32, data not shown) by stripe rust reaction especially in cultivars with the *Yr*9+*Yr*26. Though *Yr*26 was still the most efficient resistant source in Guizhou, it could not ignore the fact that V26 were spreading and might be a prevalent race due to the release of single *Yr*26-carrying cultivars in Guizhou in the future. Therefore, breeding for resistant cultivars in Guizhou should pay more attention to the possibility of pyramiding the *Yr*9 with *Yr*26 and evaluation of their effects on stripe rust in Sichuan.

The gene combination of 7 cultivars which were immune in 4 environments were analyzed. The results showed that 6 cultivars carried *Yr*26, of which 4 cultivars were detected to have only one resistant gene (*Yr*26). Only the cultivar TP3 showed a combination *Yr*26+*Yr*9. Therefore, the results might implied that there might be other stripe rust resistant genes or combinations, which can overcome the new race V26 and predominance races of Guizhou and Sichuan. It would make sense to dissect the resistance genes or gene combinations in these cultivars in the future. We also noted that Guixie3 and Xiaoyan54 both were detected with *Yr*10, but one was immune while the other was highly susceptible to stripe rust at all four environments. CYR32 test showed that both of which were susceptible (unpublished data). We deduced that both of the two cultivars carried a disabled *Yr*10 homologue. It implies that there are other stripe rust resistance genes in Guixie3. Similar to Guixie3, there were other 13 cultivars that were resistant at all 4 environments but were not detected with the 6 *Yr* genes or only detected with *Yr*9, such as Qian9939-5, Guinong 19-4, Guinong 21,

P13-3, Anmai 7, etc. (Table 3.2), which need to be further studied.

3.5.4 Prospects of molecular marker selection

Molecular marker is widely utilized to screen to stripe rust resistance gene. Marker assisted selection taking the advantage of the no requirement of pedigree information, is less likely to be affected by environment comparing to field evaluation and more precise than gene postulation methods. However it needs some prerequisites and has a bottleneck in application. Two markers flanking the target gene at both sides should be within the distance of 2-5 cM for molecular selection, however, single marker should be within a distance of 2 cM to the target gene (He et al., 2011b). Meeting this condition also cannot guarantee the highly precise level for molecular selection. Zeng et al. (2014) utilized two rye chromosome specific markers (AF1/AF4 and H20) to screen the *Yr*9 and then verified results by CYR23 and found that, among the 127 cultivars positive for both markers, 120 cultivars did carry the *Yr*9. In our study, SC200, which is 0.5 cM to the *Yr*10, was used for screening the *Yr*10. Only 11 cultivars were positive for SC200. Among the 11 cultivars, Xiaoyan54 and Huaimai 18 were susceptible at 4 environments and 3 environments in field evaluation, respectively. Unpublished data showed that among the 11 cultivars, 8 cultivars were susceptible to CYR32. It indicates that SC200 cannot discriminate the *Yr*10 from *Yr*10 homologues. Similarly, Mingxian169 were positive for csLV34 in our study, which was also reported by Yuan et al. (2012) and Zeng et al. (2014). Yuan et al. (2012) isolated *Yr*18 cDNA from Mingxian 169, sequenced its full-length and found that the coding sequence of Mingxian 169 has a 99% identity with the $Yr18_{RH}$ gene from the wheat Chinese Spring, but has a C-to-T point mutation (Leu913Phe) in exon 17. Besides, Fengyou 8 and Xingyu 823 bred by the same institute were both positive for

We173 and Gwm11 but only resistant at Guiyang site in 2014. Unpublished data also showed that both of them were resistant to CYR32. Some resistance genes were reported to be resistant to CYR32 at the *Yr*26 locus, such as *Yr*26, *Yr*Ch42 and *Yr*Gn22, which originated from 92R series, Chuanmai 42 series and Guinong series. But it is still controversial now whether they are the same gene or alleles (Li et al., 2006a; He et al., 2011b). It was reported that *Yr*Ch42 had lost its resistance in Sichuan, meanwhile, this process for *Yr*26 and *Yr*Gn22 was slower (He et al., 2011b). Though the pedigree of the two cultivars cannot be traced back, we may deduce that both cultivars use a parent with *Yr*26 allele, which can be detected by the We173 and Gwm11 but has a different resistance spectrum in field evaluation (only being resistant in Guiyang in 2014, meanwhile, most *Yr*26 were resistant in Guizhou for two years). Therefore, precise molecular detecting of *Yr* genes should be combined with specific *Pst* race test or pedigree analysis.

3.6 Conclusion

The results of our study showed that Guizhou cultivars have better resistance than Sichuan cultivars. Widely utilized stripe rust resistance genes were *Yr*9 and *Yr*26 in Southwest China wheat. Gene combination analysis showed that *Yr*9+*Yr*26 and *Yr*26+*Yr*29 were the most frequent. Adult plant resistance (slow rusting) genes were scarce in Southwest China wheat, especially in Sichuan cultivars, and more attention should be paid to pyramid all growth stage genes and adult plant rusting genes. These results not only lead to the discovering of some stripe rust resistant cultivars but also providing theoretical foundation for wheat resistant breeding in China.

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CHAPTER IV

POPULATION STRUCTURE OF WHEAT RESISTANT GERMPLASMS IN SOUTHWESTERN CHINA REVEALED USING THE DART-SEQ TECHNIQUE

4.1 Abstract

Knowledge of genetic diversity is essential for the effective use of genetic resources in breeding programs. Since the development of molecular markers, they have been extensively used in the analysis of genetic diversity of crops. Recently, a new technique called DArT-seq™, has been developed, which can detect both single nucleotide polymorphisms (SNP) and diversity arrays technology (DArT) markers and has been used in crop studies. In this study, a collection of 138 wheat germplasms, mainly from Southwestern China, were analysed using 30,485 markers (6,486 SNPs and 23,999 DArTs) from the whole genome. Two groups of wheat varieties (Group I and Group II) were identified using principal-coordinates analysis (PCoA) of the whole-genome level and the chromosome 6AS level. Group I was composed of non-T6VS/6AL lines of different origins, while Group II was composed of T6VS/6AL lines and most of these carried the *Yr26* and *Pm21* genes and were resistant to both stripe rust and powdery mildew. The neighbour-joining cluster dendrogram was also constructed, which was tested by Bootstrap. It showed that the population

stratification was not obvious for wheat accessions. Many small subgroups were clustered with high confidence level based on their origin and pedigree. Our data provide valuable information on the genetic diversity of wheat in Southwestern China, which will facilitate crossing selection to maximise the genetic diversity across whole genomes based on disease resistance complementation.

4.2 Introduction

Bread wheat (*Triticum aestivum* L.), an economically important cereal, is cultivated worldwide. Of the nearly 600 Mt of wheat harvested worldwide, about 80% is used as human food (Zhang et al., 2011). Wheat-breeding programs around the world are working towards improved grain yields of better quality, disease-resistance and agronomic performance. Knowledge of the genetic diversity within a germplasm collection forms the basis for selection of parents and establishing heterotic groups, and has a significant impact on the improvement of crops. Therefore, assessment of the extent and nature of genetic variation in bread wheat is important for breeding and genetic resource conservation programs (Zhang et al., 2011). Since the development of first- and second-generation molecular markers, they have been extensively used in the analysis of genetic diversity in common wheat; for example, restriction fragment length polymorphisms (RFLPs) (Siedler et al., 1994; Autrique et al., 1996), amplified fragment length polymorphisms (AFLP) (Barrett and Kidwell, 1998; Soleimani et al., 2002) and simple-sequence repeats (SSR) (Plaschke et al., 1995; Prasad et al., 2000).

Compared to the previously mentioned molecular marker systems, single

nucleotide polymorphisms (SNP), proposed as third-generation markers, are the main source of genetic variation in plant and animal genomes (Lander, 1996). Due to the complexity of genetic characteristics in common wheat, such as allohexaploidy (2n=42) and a huge genome (17 Gb) with more than 80% repeat sequences, the major challenge is the high cost of discovery, development and genotyping of large numbers of SNPs (Belova et al., 2013). The decreasing cost, along with the rapid progress in next-generation sequencing (NGS), and related bioinformatics computing resources has facilitated large-scale discovery of SNPs in crops. Genotyping by sequencing (GBS) methods, combining a reduction of genome complexity using restriction enzymes with sequencing using NGS techniques, offer a greatly simplified library production procedure that is more amenable for use on large numbers of individuals/lines (Elshire et al., 2011). Diversity arrays technology (DArT) is a microarray hybridisation-based technique for whole-genome profiling (Wenzl et al., 2004). Recently, a GBS method combining DArT with NGS, called DArT-seq™, has been developed. The technique, which involves genome complexity reduction, can detect both SNPs and DArTs using cost-effective and efficient strategies (Raman et al., 2014).

Recently, SNP markers have been widely used in molecular genetics of major crops, such as maize (Huang et al., 2009) and rice (Yan et al., 2009). For common wheat, a genome-wide set of 1,536 SNPs was used to study linkage disequilibrium (LD) and population structure in a panel of 478 spring and winter wheat cultivars from 17 populations across the United States and Mexico (Chao et al., 2010). Recently,

9,000 SNPs were also used in a worldwide sample of 2,994 accessions of hexaploid wheat, including landraces and modern cultivars, to uncover multiple targets of selection for improvement (Cavanagh et al., 2013). DArT-seq™ has been developed and used in crop studies over the previous two years. It was used to perform genome-wide association mapping of root traits in a panel of Japonica rice accessions, and 9,727 DArTs and 6,717 SNPs were obtained (Courtois et al., 2013). Additionally, a total of 27,748 markers were also developed to explore the genetic diversity of the U.S. collection of a new oilseed crop of lesquerella and related species using the DArT-seq platform (Von Mark et al., 2013).

Wheat stripe rust and powdery mildew are major diseases that affect wheat production in China, especially in the southwestern region (Ma et al., 1999; Huo et al., 2002). Currently, the main resistant genes against stripe rust and powdery mildew are *Yr26* and *Pm21* (Han et al., 2010; Zhan et al., 2010), respectively, which are derived from the Guinong (GN) serials and 92R serials in the region. Both of the two serials are *T. aestivum-H. villosa* 6VS/6AL translocation lines developed by crossing *T. turgidum* and *H. villosa* (Chen et al., 2001; Li et al., 2011). The stripe rust resistance gene *Yr26* was derived from *T. turgidum* and mapped to chromosome 1BS (Ma et al., 2001). Since the appearance of Chinese stripe (yellow) rust race 32 (CYR32), it has been widely used in wheat breeding in Southwestern China (Han et al., 2010). The powdery mildew resistance gene *Pm*21, located on chromosome 6VS of T6VS/6AL, confers durable and broad-spectrum resistance to wheat powdery mildew (Zhan et al., 2010). *Pm21* is a key gene resource for powdery mildew resistance breeding globally

(Cao et al., 2010).

In this study, a collection consisting mainly of wheat resistance resources from Southwestern China was genotyped from whole genomes using the DArT-seq™ technique. The main objectives were to identify the population structure, explore the distribution of the resistance genes *Yr26* and *Pm21* and investigate their genetic diversity. This knowledge is critical for genome-wide association studies (GWAS) of disease resistance in Southwestern China wheat and to understand the role of selection and breeding in the distribution of genetic variation across the wheat genome.

4.3 Materials and Methods

4.3.1 Plant materials

The 138 wheat (*Triticum aestivum* L.) cultivars (lines) included in this study represent diverse cultivars utilised in winter wheat regions of Southwestern China. Of these, 115 cultivars were collected from Southwestern China (75 from Guizhou province, 40 from Sichuan province) and 23 from other regions in China (Jiangsu, Hubei, Henan, Shanxi and Beijing). A complete listing of these genotypes is provided in Table S 3.1.

4.3.2 DNA extraction and resistance gene detection

Plant material was harvested at the seedling stage and genomic DNA was extracted from young leaf tissue from a single plant of each genotype using the protocol recommended by Triticarte Pty. Ltd. (James, 2005).

Two markers were used to scan for resistance genes in this study, including the

co-segregating sequence characterized amplified regions (SCAR) marker (SCAR1265: forward, 5'-CACTCTCCTCAAACCTTGCAAG-3', reverse, 5'-CACTCTCCTCCACTA ACAGAGG-3') for the powdery mildew resistance gene *Pm*21 and the SSR marker (Gwm11: forward, 5'-GGATAGTCAGACAATTCTTGTG-3', reverse, 5'-GTGAATTGT GTCTTGTATGCTTCC-3') closely linked to the strip rust resistance gene *Yr26*. The PCR reactions were performed according to the optimised conditions for *Pm21* (Liu et al., 1999) and *Yr26* (Ma et al., 2001).

4.3.3 Whole genome genotyping

Genotyping was conducted at Diversity Arrays Technology Pty Ltd. (DArT P/L), Australia, using the DArT-seq™ method. The DArT-seq™ technology (abbreviated as DArT-seq) was optimised for wheat by selecting the most appropriate complexity-reduction method (*Pst*I-*Mse*I restriction enzymes). DNA fragments digested using restriction enzymes were ligated with *Pst*I adaptors and unique barcodes, then amplified using PCR. All amplicons were pooled and sequenced in a single lane on an Illumina Hiseq2000 instrument. DArT P/L analysed all sequences and provided scores of markers originating from the sequence polymorphism data as present/absent (present=1 vs. absent=0). The sequences were trimmed at 69 bp (5 bp of the restriction site plus 64 bases with a minimum Q score of 10). A proprietary analytical pipeline developed by DArT P/L was used to produce DArT score tables and SNP tables. The remaining 69-bp sequences were aligned to the wheat genome preassembly chromosomes using Bowtie v0.12 (Langmead et al., 2009) with a maximum of three mismatches to recover the position of the restriction site for the

DArT markers and the position of the polymorphism(s) within the 69-bp sequences for the SNPs. The same sequences were then aligned to the pseudomolecules using BLAST (e-value<1.0 e-20) to assess whether additional sequences could be positioned. The sequences that had only one hit on the preassembly chromosomes or had more than one hit but with a difference of at least 1.0 e-5 between the first and the second hits were retained for further analysis. Marker position(s) on contig(s) were identified using the best alignment of marker/tag to an existing model genome. Call rates were measured for all SNPs and DArTs and markers with call rates below 80% were discarded. The allele frequencies of the remaining markers were then calculated and markers for which the minor allele had a frequency below 2.5% were discarded.

4.3.4 Statistical analyses

The polymorphism information content (PIC) values were calculated for each SNP and DArT marker using the formula $PIC = 1-\sum(Pi)^2$, where Pi is the proportion of the population carrying the *i*th allele. A binary matrix was produced from the SNP and DArT table by scoring as 1 or 0 for the presence or absence of a specific mutation, respectively. Consistent 0/1 data matrices were used as input for genetic diversity and population structure analysis. NTSYS-pc (version 2.21) analysis software was used to perform principal-coordinates analysis (PCoA) using a matrix based on the Dice genetic similarity coefficient (GS), which was preferred over the simple matching coefficient because DArT and SNP are dominant marker systems. The Dice coefficient (sij) measures the asymmetric information on binary variables and is computed according to the following formula: sij=2a/(a+b+c), where a=number of bands present

in both individuals (i and j), b=number of bands present in i and absent in j, c= number of bands present j and absent in i (Dice, 1945). The software DARwin 6 was employed to determine the level of dissimilarity between accessions, and a dendrogram was constructed based on neighbour-joining algorithm using the Dice genetic similarity coefficient (Perrier and Jacquemoud-Collet, 2006). The significance of each node was evaluated by bootstrapping data over a locus for 1,000 replications of the original matrix.

4.4 Results

4.4.1 DArT-seq genotyping

The GBS method yielded 32,763 markers (8,764 SNPs and 23,999 DArTs). To estimate the quality of each marker, the index of call rate was used. When the call rate of a marker was greater than 0.8, it was deemed to be informative, according to the DArT P/L instructions. A total of 30,485 markers (6,486 SNPs and 23,999 DArTs) with a call rate greater than 0.8 were used to examine population structure and genetic diversity. The average heterozygosity, calculated from the SNP markers, was low (1.2%), as expected for loci from autogamous plants. The average PIC value for SNPs was 0.28 and the median was 0.26; the average value for DArT markers was 0.33 and the median was 0.37. The pattern of distribution for SNP PICs was almost symmetrical and the highest frequency (17%) of this marker had a value in the range of 0.20 to 0.25. For DArT markers, the distribution of PIC values was asymmetrical and skewed towards the higher values and most (32%) had a PIC value in the range of 0.45 to 0.50 (Fig. 4.1).

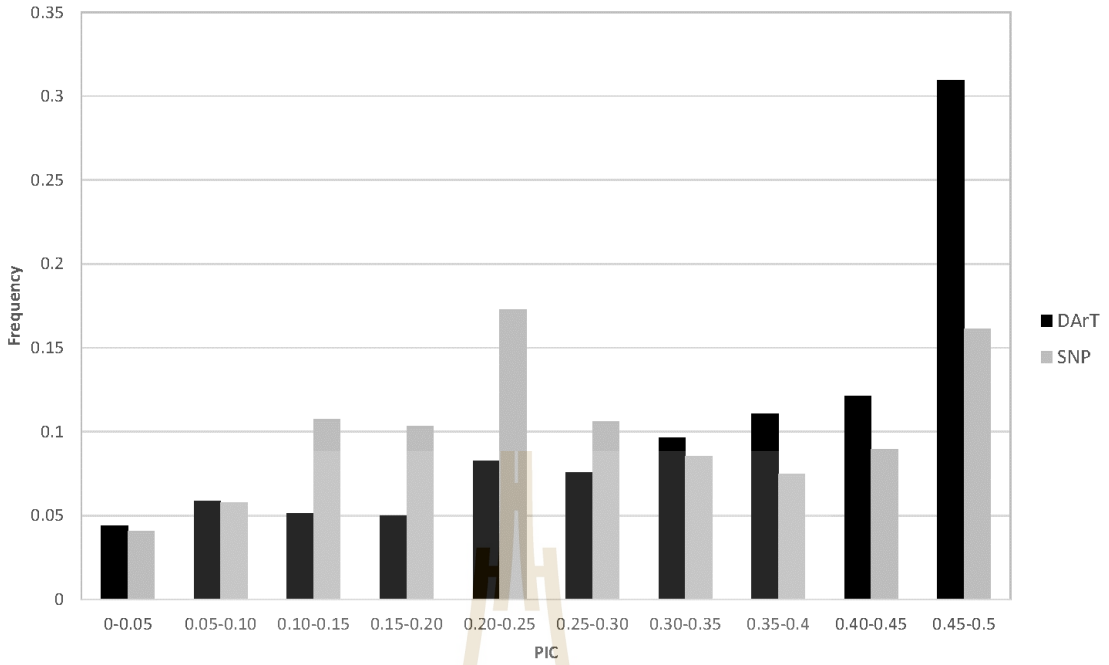

Fig. 4.1 Distribution of PIC values for DArT and SNP markers

The marker sequences were aligned against the sequenced scaffolds of the wheat genome model. 19,942 markers (5,645 SNPs and 14,296 DArT) with a call rate>0.8 were mapped on the wheat genome preassembly chromosomes, of which 10,605, 4,101, 2,070 and 3,162 markers had one, two, three or more than three copies on the whole genome, respectively. The 10,605 (2,392 SNPs and 8,213 DArTs) one-copy markers were unevenly distributed in the whole genome (Table 4.1). Of the seven homologous groups of common wheat chromosomes, group 2 harboured the largest number (2,369), while group 4 had the least (949). Of the three genomes, genome B had the largest number (4,886), followed by genome A (3,842) and genome D (1,877). For each chromosome, 2B harboured the largest number (1,101), while 3D had the least (151).

Table 4.1 Distribution of one-copy markers on the whole wheat genome

Genome	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Total
A	460	780	376	501	316	769	640	3,842
B	671	1,101	1,033	296	830	444	511	4,886
D	228	488	151	152	240	290	328	1,877
Total	1,359	2,369	1,560	949	1,386	1,503	1,479	10,605

4.4.2 Population structure at the whole-genome level

PCoA analysis was used to examine the population structure of the collection. PCoA analysis is based on the decomposition of any multidimensional distance metric (in this case d). A two-dimensional scatter plot (Fig. 4.2) shows that the first (PCO-1) and second (PCO-2) principal coordinates accounted for 12.3% and 6.0% of the variation, respectively. Two groups were distinguished. The left-hand group (I) is highly clustered with no obvious separation of cultivars (lines) originating from different geographic regions. In Group I, 92 cultivars were present, with most cultivars from Sichuan and other regions in China, as well as some Guizhou cultivars. In contrast, the right-hand group (II) was spread widely along both the PCo-1 and PCo-2 axes. Forty-eight cultivars were in this group, mainly from Guizhou province.

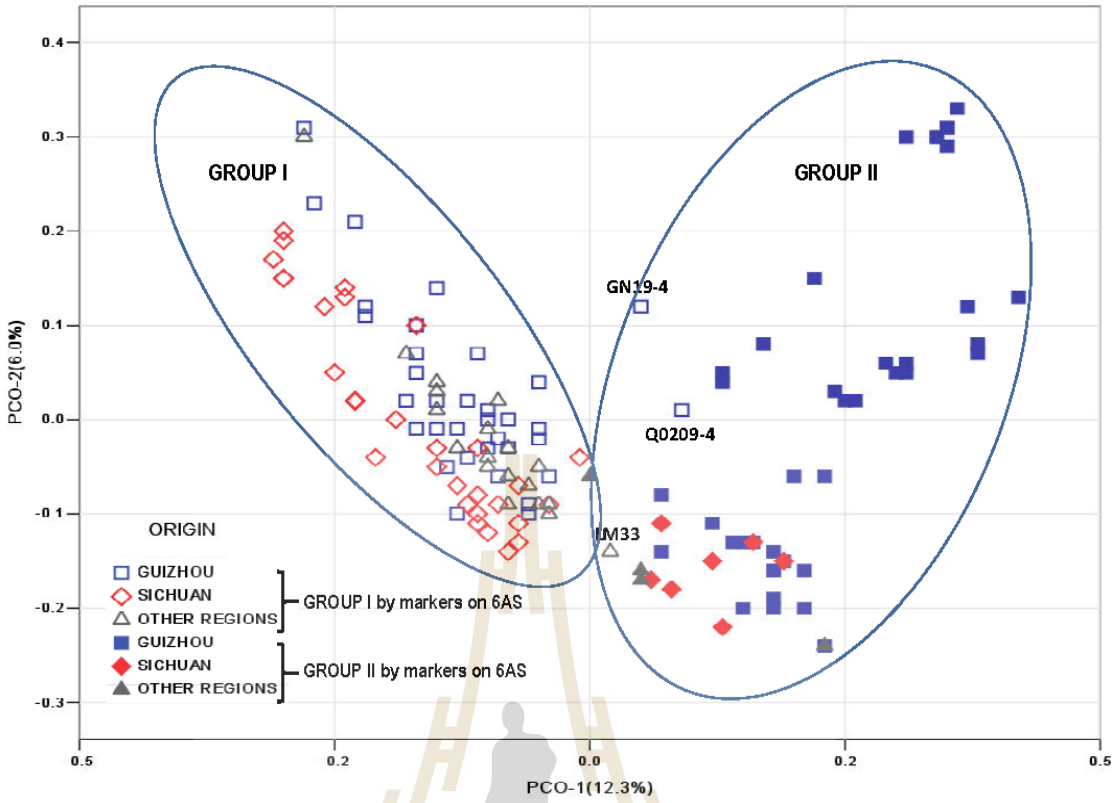

Fig. 4.2 Population structure based on PCoA using data from 30,485 informative markers at the whole-genome level. Two groups are present. The cultivars are indicated by different colours. Cultivars in blue, red and grey were from Guizhou, Sichuan and other regions, respectively. Interestingly, Group I and Group II correspond to the two groups formed in the PCoA analysis based on genotype data from 6AS markers (Fig. 4.4). Cultivars are designated by hollow and solid symbols.

4.4.3 Population structure based on presence or absence of resistance genes

To investigate the distribution of stripe rust and powdery mildew resistance genes in the southwestern region of China, markers Gwm11 (*Yr26*) and SCAR1265 (*Pm21*) were scanned in our collection. About 50% of cultivars (lines) amplified the specific allele (193bp) for the *Yr26* gene (Gwm11) resistant to stripe rust and 40.6% for the specific allele (1265bp) for the *Pm21* gene (SCAR1265) resistant to powdery mildew. Regarding the distribution of resistance genes in Guizhou and Sichuan provinces, the frequencies of wheat lines carrying the *Yr26* gene were 61.3% and 37.8%, respectively, and the frequencies of those carrying the *Pm21* gene were 60.0% and 23.7%, respectively. The PCoA results shown in Figure 4.3A and 4.3B are based on the presence of the *Yr26* and *Pm21* genes. As shown in Figure 4.3A, the distribution of the cultivars carrying *Yr26* was uneven, with more in Group II than in Group I. Almost all *Pm21*-carrying cultivars are in Group II, while very few *Pm21*-carrying cultivars are in Group I (Fig. 4.2 and 4.3B).

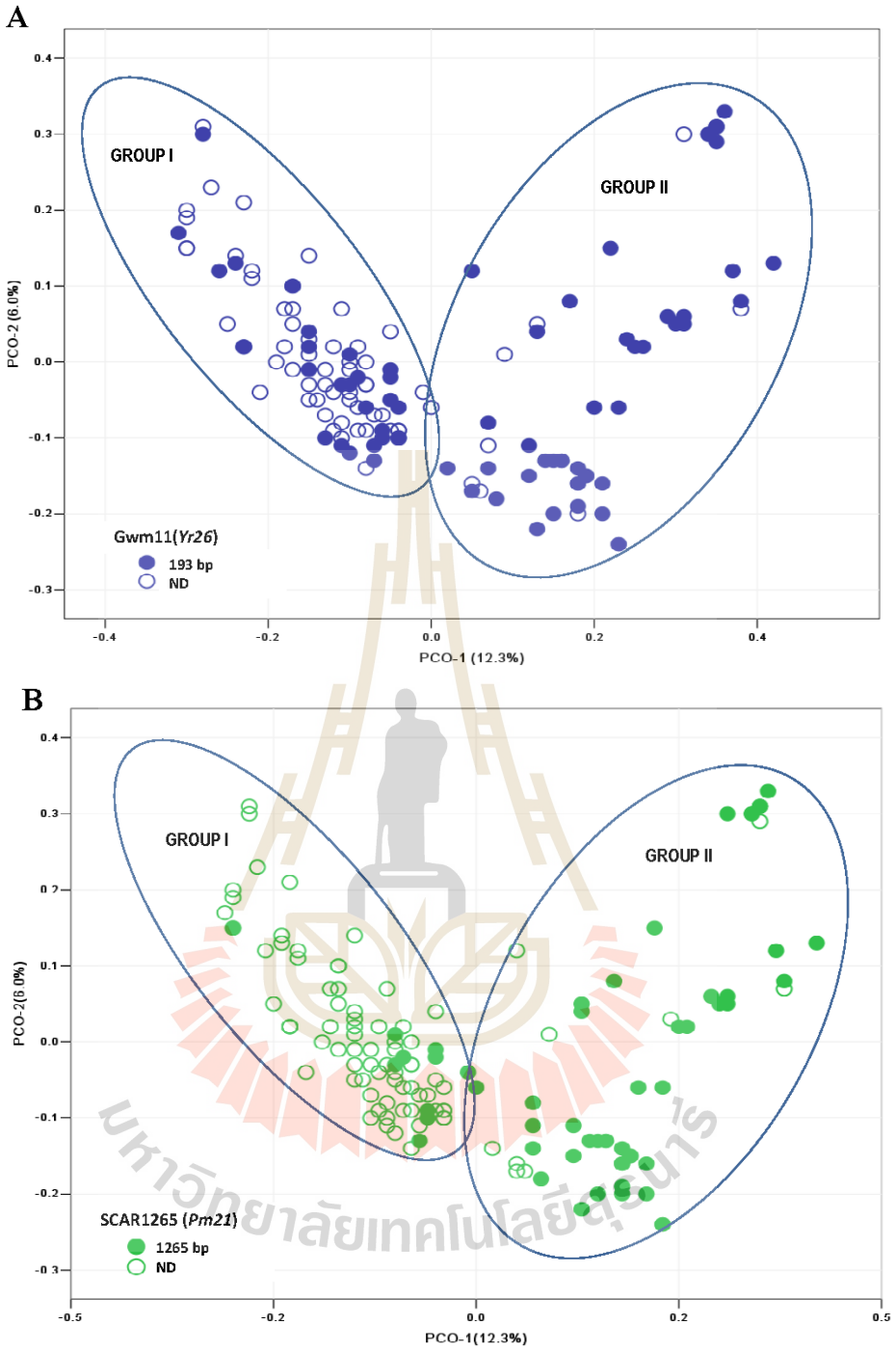

Fig. 4.3 Distribution of resistance gene carriers in different groups based on PCoA at the whole-genome level. A. Plot based on cultivars carrying or not carrying the *Yr*26 gene locus (Gwm11). B. Plot based on cultivars carrying or not carrying the *Pm*21 gene locus (SCAR1260).

4.4.4 Population structure based on chromosome 6AS

Considering that *Pm21* is derived from the T6VS/6AL lines, PCoA analysis was performed based on genotype data from the 769 one-copy markers on chromosome 6AS. A very clear separation was observed along the PCO-1 axis (Fig. 4.4). The first (PCO-1) and second (PCO-2) principal coordinates accounted for 62.4% and 6.0% of the variation, respectively. The PCoA pattern was highly consistent with the results obtained using whole genome markers (Fig. 4.2). Three exceptions were found by comparing the two figures. Cultivars GN19-4, Q0209-4 and LM33, located in Group I in Figure 4.4, were clustered together in Group II in Figure 4.2, but they were located at the edge of Group II. A significant correlation ($r=0.8$, $P<0.01$) was observed between cultivars in Group II (Fig. 4.4) and those carrying the *Pm21* gene. Combining the results of both the PCoA and correlation analysis, we deduced that Group I was the 6AS/6AL group and Group II was the T6VS/6AL group (Fig. 4.4).

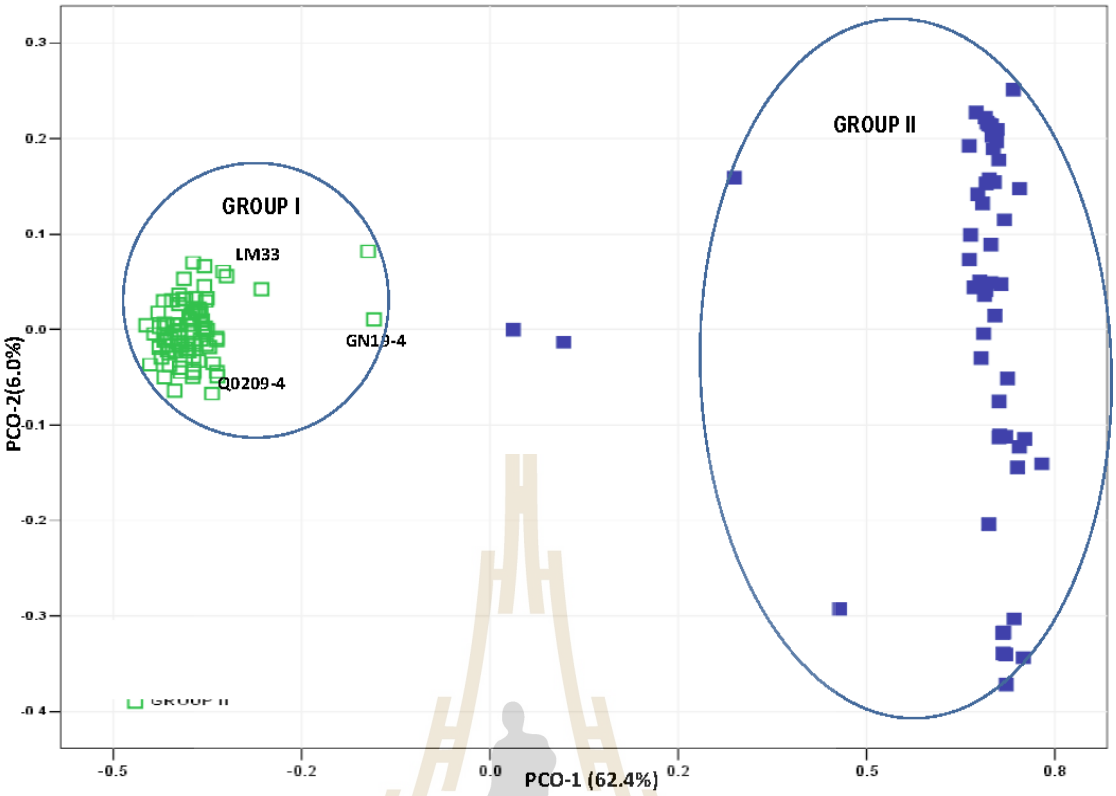

Fig. 4.4 Population structure based on PCoA using genotype data from 6AS markers. Two groups were formed. Group I is indicated by green hollow squares. Group II and other two cultivars near the origin of PCO1 are indicated by blue solid squares.

4.4.5 Genetic relationships among wheat resistant germplasms

The Dice GS was calculated using the genotyping data of 30,485 informative markers between all possible pairs of accessions. It ranged from 0.60 to 0.99, with a mean value of 0.72. The GS between cultivar MX 169 and Q0209-4 was the lowest, which indicated a distant genetic relationship, while the GS between lines P13-3 and GX4 was the highest, indicating a close genetic relationship. When examining the

cultivars based on origin, the mean GD value was 0.73, ranging from 0.61 to 0.73 for Guizhou wheat, while the mean GD value was 0.76, ranging from 0.67 to 0.99 for Sichuan wheat.

The neighbor-joining (NJ) tree is shown in Figure 4.5. Bootstrap value larger than 50 (percent) are marked on the branches. The 138 wheat cultivars formed three main groups but the node is weakly supported by the bootstrap resampling (bootstrap value less than 50, Fig. 4.5). The second and third group (GII and GIII) was composed of 34 and 16 cultivars, mainly from Guizhou. There were 88 cultivars within the first group (GI) from Sichuan, Guizhou and other regions of China. Moreover, many reliable subgroups (with bootstrap value from 50 to 100) can be distinguished in the three group (GI, G II and GIII), and cultivars were clustered together mainly based on origin or pedigree. For example, the subgroup (from GN22 to MM39, with bootstrap value of 57) was composed of lines from all regions, whereas the lines within subgroups (SG, from CM107 to CM16, from CM60 to MM41 and from MY26 to MM46, with bootstrap value of 88, 57 and 98, respectively) were primarily from Sichuan province. CN04-1/CN04-2 or YP1/YP2 in GI, both of which are sister lines, were clustered together with bootstrap value of 100, but other sister lines, such as GN21, GN22 and GN21-1, were clustered in different subgroups or groups. On the basis of available information on pedigree, two genotypes sharing no immediate common parents and from different regions of China, such as NAU06Y603/CAU23 or P13-1/EM15, were clustered together with bootstrap value of 100.

It is possible that the dendrogram obtained from the NJ was not fully

coincident with the separation of T6VS/6AL and 6AS/6AL types. The T6VS/6AL lines were mainly distributed in GII, GIII and SGIa. However, 7 lines among the 34 lines in GII and 4 lines among the 16 lines in GIII were not the T6VS/6AL type.

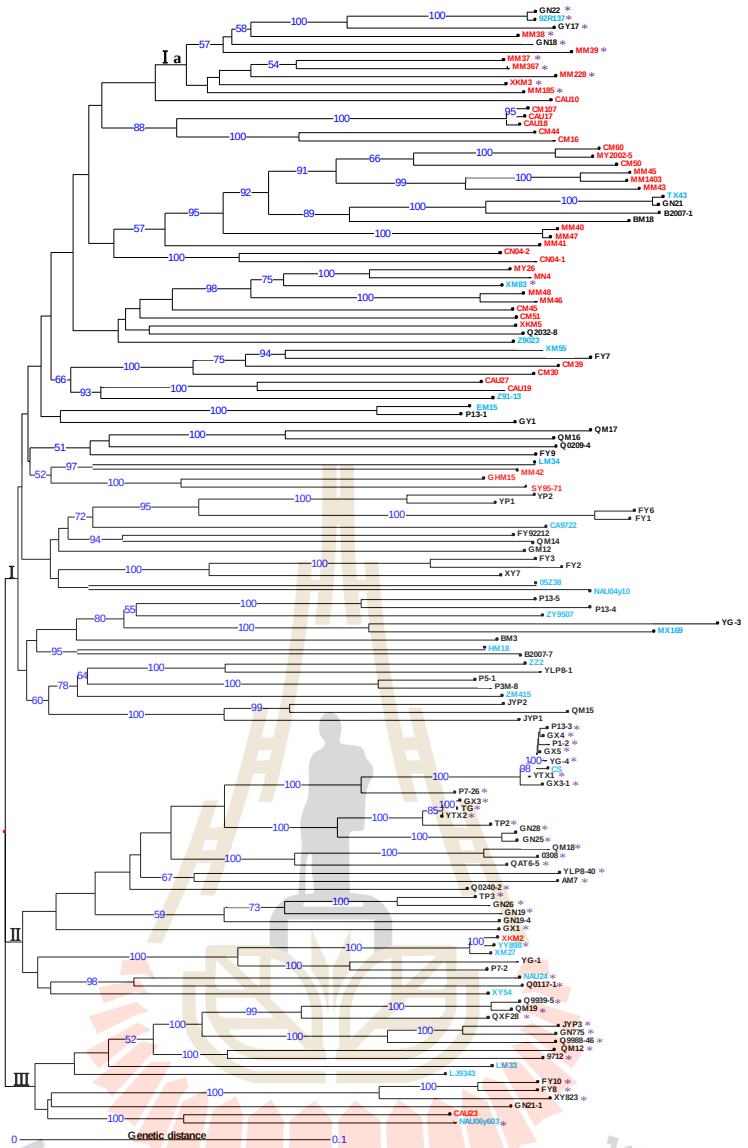

Fig. 4.5 Dendrogram showing relationships among 138 Southwestern China wheat accessions, based on a bootstrapped neighbour-joining algorithm using the Dice similarity coefficient. The values on the branches represent the bootstrap probabilities. The cultivars are indicated by different colours. Cultivars in black, red and blue are from Guizhou, Sichuan and other regions, respectively. The cultivars indicated by asterisks are 6VS/6AL translocation lines.

4.5 Discussion

4.5.1 Application of DArT-seqTM technology to population structure analysis

DArT-seq™, based on a GBS platform, was developed recently by DArT P/L and SNP and DArT markers can be developed on a large scale from the whole genome. Compared to traditional DArT technology, DArT-seq™ can reveal further polymorphic molecular markers. For example, 1,637 polymorphic markers were obtained when DArT was used to analyse the population structure of 118 northern Chinese wheat cultivars (Zhang et al., 2011). In this study, 8,764 SNPs and 23,999 DArTs were obtained using DArT-seq™ to genotype wheat from Southwestern China. We found that DArTs revealed a greater number of polymorphisms than SNPs, and the distribution of DArT PICs was asymmetrical and skewed towards the higher values, because their polymorphisms are based on SNPs and INDELs at restriction enzyme cleavage sites and restriction fragments (Wenzl et al., 2004). In the DArT-seq platform, the amount of polymorphic SNPs was less than for other GBS technologies. For instance, 20,000 polymorphic SNPs were developed using the wheat mapping population W97846×Opata85 and GBS technology (Poland et al., 2012). It was reported that 102,324 polymorphic SNPs were identified from 384 European and American wheat genotypes from two different breeding programs using GBS methods (Lado et al., 2013). The advantage of DArT-seq™ lies in lower detection costs (6,525 dollars/96 samples), which is attractive to local colleges or institutions with limited research funds.

The distribution of markers with different copy numbers showed that more than half (53.2%) had one position on the wheat genome, and two and three marker copies were present in 20.6% and 10.4%, respectively. This means that markers developed by DArT-seq were mainly located in coding regions and are mutations within functional genes. In addition, the polymorphic markers were frequently present on the B genome, but seldom occurred on the D genome. For each chromosome, the highest number of markers were on chromosome 1B, while the fewest was on chromosome 4D. This agrees with the fact that the B genome presents the highest level of polymorphism, whereas the D genome shows the lowest level among the wheat ABD genomes (Cui et al., 2014; Nielsen et al., 2014).

4.5.2 Population structure based mainly on the 6VS/6AL translocation line

Some candidate regions for differential selection could represent specific chromosomal areas that were targets of intensive breeding (Crossa et al., 2007), and could also have an impact on the separation of population subgroups. For example, it was reported that markers on chromosome 2D near the Rht8 locus had a major impact on the population structure of European hexaploid bread wheat and different Rht8 alleles were found in GrI and GrII (Nielsen et al., 2014). Zhang et al. (2011) reported that two groups (1RS/1BL translocation group and non-1RS/1BL group) were formed when the population structure of 118 wheat cultivars from northern China was analysed using PCoA and DArTs (Zhang et al., 2011). In this study, different PCoA results were observed. The 138 cultivars formed two groups based on the presence of T6VS/6AL. Several lines were scattered between the two groups and it is possible that

these lines carry 6VS segments of different sizes, because there was a low frequency of pairing and recombination between chromosome 6VS from *H. villosa* and 6AS from cultivated wheat species (Cao et al., 2011).

In this study, about 50% of lines carried the *Yr26* gene for resistance to stripe rust and 40.6% carried the *Pm21* gene for resistance to powdery mildew. Similar results were reported in other studies. Wan et al. (2011) reported that 38% of lines carried the *Yr26* gene among 108 Southwestern China wheat cultivars (Wan et al., 2011). In an investigation of the distribution of *Pm21* in Chinese winter wheat and breeding lines, Jiang et al. (2014) found that 34.4% of the lines carried this gene in the Southwestern Winter Wheat Region and 3.3% in other Chinese regions (Jiang et al., 2014). PCoA plots showed that the two resistance genes were unevenly distributed between the two groups. The majority of lines carrying the *Yr26* gene were clustered in Group II, while almost all of the *Pm21* carriers were located in the T6VS/6AL group (Group II). More cultivars from different origins were closely clustered in the non-T6VS/6AL group, which indicated a close genetic relationship due to mutual introduction. Cultivars in the T6VS/6AL group were mainly from Guizhou province and more scattered. This is because breeders in Southwestern China tend to breed varieties resistant to disease because of environmental pressures, while those in northern China prefer wheat cultivars with high yields. These results confirmed that there were more genetic differences among Guizhou cultivars, which would be beneficial for disease resistance. For example, a GN series was bred by distant crosses and is widely utilised in China. Additionally, new resistant resources, like the GX

series and YG series, have been bred in recent years. The introduction of excellent resistant genes in common wheat by distant crosses will be important in maintaining stable wheat production.

4.5.3 Genetic relationships among the wheat accessions

Broadening the genetic base is a prerequisite for increasing yield and improving resistance in wheat. Compared to wheat landrace cultivars, plant breeding has reduced the genetic diversity of elite breeding germplasms (Reif et al., 2005). A similar discovery was reported on the genetic diversity of Canadian wheat (Fu and Somers, 2009). To determine the genetic relationships and population structure of wheat in Southwestern China, the GS of the 138 cultivars (lines) was computed using 30,485 markers. The estimate of the genetic similarity (GS) coefficient between pairs of genotypes ranged from 0.60 to 0.99 with a mean GS of 0.72. This GS value can be compared with two studies reported earlier. Prasad et al. (2000) reported that 55 elite wheat originated in 29 countries representing six continents were analysed with SSR markers and mean GS coefficient value of 0.23 was obtained (Prasad et al., 2000). Ni et al. (2012) also reported similar results with 40 wheat cultivars collected from China and USA (Ni et al., 2012). It may suggest that the 138 genotypes used in this study were not diverse. This may be due to the study being confined to Guizhou and Sichuan, two adjacent provinces in Southwestern China, which share a similar ecology and core wheat parents, leading to a lack of diversity in wheat genetics. CIMMYT breeders successfully increased genetic diversity through the introgression of various novel wheat materials (Reif et al., 2005). Therefore, it appears that Southwestern China

wheat breeding programs could be improved by utilising more diverse genotypes.

Accessions were clustered into three main groups (GI, GII, and GIII) but with bootstrap value less than 50 (less trustable). Many special subgroups with large bootstrap value were observed within the three groups, reflecting the region of plant breeding and pedigree relationships. The majority of Sichuan cultivars, other regional cultivars and some Guizhou cultivars were clustered in GI. Most Sichuan cultivars were closely clustered, which indicated little genetic diversity. This clustering may be explained by the presence of some important developmental genes on the genome, which may be involved in selection or adaptation to local environments (Zhang et al., 2011). GII and GIII was mainly composed of Guizhou cultivars, which indicated that these cultivars were heterogeneous compared to cultivars in GI. Pairs of cultivars were closely related in the pedigree-based analysis, but markedly less closely related by cluster analysis using molecular marker data. Weak correlations between molecular marker data and pedigree records in research on wheat and other cultivars have been reported by several authors (Tinker et al., 1993; Barrett et al., 1998). The explanation may be that co-ancestors may exist for a pair of closely clustered cultivars, but this cannot be traced due to lack of comprehensive pedigree information. In this study, we did not obtain detailed pedigree information on cultivar parents and in the cluster analysis some cultivars with high similarity in DArT or SNP loci were placed in a group. Grouping these cultivars represents similarity of these loci and relative genetic uniformity.

In comparison to PCoA plots, most of the GII and GIII cultivars and all of the

GIa cultivars in the dendrogram were T6VS/6AL, which were not clustered into one group. One possible explanation is that, combining the NJ algorithm with bootstrap test, the dendrogram disclosed more information. Contrast population stratification is not shown in the dendrogram (bootstrap value is lower for all three groups GI, GII and GIII). But it revealed that a lot of small clusters with high confidence level existed. For the PCoA plot, it only considered the first two PCO, which could explain 68.4% genetic variation based on 6AS level but 18.3% for whole genome level. Therefore, PCoA plot revealed less genetic information and fewer groups especially at the whole genome level, while, clusters of dendrogram combining with booststrap test at whole genome level were more comprehensive and reveal the true relationship between the wheat accessions.

4.6 Conclusion

DArT-seq™, based on GBS methods, is an effective means of developing DArT and SNP markers and genotyping of the whole genome. Using principal coordinate analysis (PCoA), two groups (Group I and Group II) were recognised for 138 wheat accessions based on 30,485 markers, which is consistent with the population structure obtained by PCoA using genotype data of markers in 6AS and *Pm21* carriers (6VS/6AL translocation lines). Cultivars in Group II had better resistance to stripe rust and powdery mildew, due to the presence of *Yr26* and *Pm21*. Dendrogram with bootstrap values revealed that population stratification of Southwestern China wheat was not obvious, but accessions were clustered together

based on their pedigree or origin, and abundant genetic diversity was present in Guizhou cultivars. This information is useful for resistant wheat breeding in China.

4.7 References

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CHAPTER V

ASSOCIATION ANALYSIS FOR STRIPE RUST RESISTANCE LOCI IN WHEAT CULITIVARS AND LINES FROM SOUTHWESTERN CHINA

5.1 Abstract

Stripe rust restricts the productivity and profitability of wheat (*Triticum aestivum* L.) cultivation in Southwestern China. Continued gains can be obtained by identifying superior alleles and novel stripe rust resistance loci that can be incorporated into breeding programs. We used association mapping (AM) to identify genomic regions associated with stripe rust resistance using 140 wheat accessions mainly from Southwestern China and 5,224 polymorphic diversity arrays technology (DArT) markers. Infection types were scored both at seedling stage with CYR32 inoculation and at adult plant stage in four environments with natural inoculation. However, disease severity was scored only in the four field tests. Single factor analysis (SFA) model and Q model were both used in AM with infection type (IT) data, and Q model did with disease severity (DS) data. Population structure of the wheat accessions were estimated by principal component analysis (PCA). Genome-wide association analyses detected markers that were significantly associated with stripe rust resistance on all chromosomes using the SFA model but on 1BL, 3B and 4BL

using the Q model with IT data. The markers in association also decreased from 595 to 4. Markers associated with stripe rust resistance were also detected with DS data and the Q model, which were 6 in total and distributed on 6AS, 1BL and 3B. These genomic regions correspond to previously identified loci for stripe rust resistance but need to be further confirmed. These results demonstrate that genome-wide association mapping with Q model can exclude the false positive association and be used in identifying stripe rust resistance loci efficiently.

5.2 Introduction

Common wheat (*Triticum aestivum* L.) is globally the most widespread crop and also the second largest staple food crop in China. It is grown on over 28 million hectares with total yield of 115 million tonnes (FAO, 2015). Extreme weather events, such as frost, heat shock and drought or biotic factors (pests and diseases) impact wheat production and represent a significant risk which needs to be managed to maintain profitable production (Wan et al., 2004; Liu et al., 2007; Barlow et al., 2015). Among fungal pathogens that infect wheat, stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) has recently become a major threat to wheat production (Wellings, 2011). Stripe rust is a serious disease of wheat occurring in most areas with cool and moist weather conditions during the growing season (Chen et al., 2014). In terms of acreage affected by stripe rust, China is the largest epidemic region in the world. The disease occurs most frequently in winter wheat growing areas in Northwest, Southwest, and North China and causes huge yield losses (Wan et al., 2004). Growing resistant cultivars is the most effective, economical, and environmentally friendly method of disease control. Resistance to stripe rust can be broadly categorized as

all-stage resistance (also called seedling resistance), which can be detected at the seedling stage, and adult-plant resistance, which is expressed at later stages of plant growth (Chen, 2005). More than 60 genes for stripe rust resistance have been identified in cultivated wheat and its wild relatives (McIntosh et al., 2014). Unfortunately, most of these genes are not effective or available in China wheat resistance breeding. Only *Yr*5, *Yr*10, *Yr*15, *Yr*26, *Yr*Gn775 and *Yr*Zhong4 maintain the resistance to the stripe rust races predominant in China and could be used in breeding programs (Han et al., 2008). Since the rapid development of new *Pst* races often circumvents resistance genes, it is therefore essential that new genes for stripe rust resistance are discovered and introgressed into elite wheat germplasms to protect it against this devastating disease.

Linkage disequilibrium (LD) refers to a historically reduced (non-equilibrium) level of recombination of specific alleles at different genetic loci controlling particular variations in a population. Association mapping (AM) or linkage disequilibrium mapping, is a method that relies on the detection of linkage disequilibrium to infer a relationship between phenotypic variation and genetic polymorphisms (Flint-Garcia et al., 2003). Because LD extent in wild populations depends on a long history of recombination, it is possible to obtain finer mapping resolution with AM than with linkage studies of bi-parental mapping populations (Abdurakhmonov and Abdukarimov, 2008). But its effectiveness depends on the number of individuals studied, magnitude of LD in the investigated population and the availability of a large number of mapped markers (Mackay and Powell, 2007). However, linkage disequilibrium can also be the result of differential relatedness among subgroups, or population structure, which can greatly inflate the number of spurious marker-trait associations identified through AM

(Pritchard et al., 2000). Spurious associations can be minimized by applying general linear models in the association analysis which considers the population structure (Q matrix or PC matrix) as a fixed effect.

AM has been successfully used in mapping QTL for different traits in several plant species (Agrama et al., 2007; Björn et al., 2008; Cockram et al., 2008; Wang et al., 2008b; Kump et al., 2011). In wheat, AM has been successfully used to study agronomic traits (Yao et al., 2009; Liu et al., 2010), quality traits (Bordes et al., 2011; Reif et al., 2011), pre-harvest sprouting (Kulwal et al., 2012), seed longevity (Arif et al., 2012), aluminum resistance (Francki et al., 2010) and disease resistance (Adhikari et al., 2011; Miedaner et al., 2011; Yu et al., 2011).

Diversity arrays technology (DArT) is a microarray hybridisation-based technique for whole-genome profiling (Wenzl et al., 2004). The DArTseq is newly developed method which deploys sequencing of the DArT representations on the Next Generation Sequencing (NGS) platforms. It combines the advantages of DArT and NGS, such as high-throughput, high marker densities, tendency of covering active genes regions, independence of sequence data, and ability to detect SNPs and indels, etc. DArTseq is increasingly used in crop improvement applications, especially in high resolution mapping and detailed genetic dissection of traits (Courtois et al., 2013; Zou et al., 2014).

In this study, we evaluated 140 accessions mainly from the Southwest China winter wheat zone for resistance against *Pst*. Resistance was evaluated both at the seedling stage in controlled environments (to *Pst* races CYR32) and at the adult plant stage in multiple years and field locations in the Southwestern China (to mixtures of naturally occurring *Pst* races). Combined with data of the population structure (PC

matrix), we employed 5,224 silico DArT markers to investigate patterns of LD for each chromosome and to evaluate the possibility of detecting markers significantly associated with resistance to *Pst*. Stripe rust resistance loci discovered here can be directly selected in the future by MAS to improve the resistance of this staple crop.

5.3 Materials and methods

5.3.1 Materials

The 140 wheat (*Triticum aestivum* L.) cultivars (lines) included in our study represent diverse cultivars utilized in winter wheat regions of Southwest China (shown in Table S3.1). Among them 75 cultivars were collected from Guizhou province, 42 from Sichuan Province, 5 from Jiangsu province while an additional 18 came from the Northern part of China (Hubei, Henan, Shanxi provinces and Beijing).

5.3.2 Phenotyping

5.3.2.1 Adult-plant field conditions

Accessions were evaluated under natural pathogen presence in four field trials performed at three locations (Guiyang and Hezhang in 2013, Guiyang and Mianyang in 2014). Disease reaction was recorded in the season at the adult plant stage, when stripe rust reaction of the susceptible check Mingxian169 reached the highest disease severity. The host response (infection types; IT) to stripe rust followed a 0-to-4 scale according to the following convention: 0=immunity, no visible infection, “0;”=diffuse presence of hypersensitive flecks, no uredinia, 1=small uredinia surrounded by necrosis, 2=small or medium uredinia surrounded by chlorosis, 3= numerous uredinia of moderate size without necrosis or chlorosis, 4=large uredinia (Li and Zeng, 2002). Disease severity (DS) was also scored by visual inspection according

to percentage of leaf area covered by uredinia of *Pst* (Li and Zeng, 2002).

5.3.2.2 Single race seedling test

Seedlings of the 140 accessions were evaluated for IT response to *Pst* race CYR32, under controlled greenhouse conditions. The *Pst* race was maintained at the State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest Agriculture & Forestry University (China). CYR32 is one of the predominant and widely distributed races in the Southwestern China.

Ten seeds of each accessions were planted in a plastic seedling-raising plate (540×280×80 mm, 70 holes) filled with a mixture of 2 humus soil:1 vermiculite and then grown in a growth chamber. Inoculations were performed by brushing conidia of isolates onto the seedlings to be tested, when the first leaf is fully expanded. Inoculated seedlings were then placed in plastic-covered cages and incubated at 9℃ and 100% relative humidity (RH) for 24 h. Seedlings were then transferred into a growth chamber with identical conditions, i.e., the day/night regime of 14 h light (22,000 lx) at 17°C and 10 h of darkness at 12°C, with 70% RH. Infection types (ITs) were scored 15–16 days after inoculation. ITs were recorded using the same scale as for the field test. ITs from 0 to 2 were considered as avirulent (resistant response of the plant) while ITs 3 and 4 were considered as virulent.

5.3.3 Genotyping

Genomic DNA from the 140 different genotypes was extracted using the cetyl trimethyl ammonium bromide (CTAB) procedure (Saghai-Maroof et al., 1984). Genomic DNA of each accession was diluted to a concentration of 100 ng/µl. Genotyping was conducted at Diversity Arrays Technology Pty Ltd. (DArT P/L, Australia), using DArT-seq™. Genotyping data were detected and DArT-seq variants

with<20% missing values were retained.

5.3.4 LD for each chromosome

Only non-rare alleles (corresponding to a frequency≥0.10) were considered for the LD and association mapping, thus reducing the false positives and the LD inflation effects which have been frequently associated to the use of rare alleles. The LD analysis, carried out with the software TASSEL 3 (Bradbury et al., 2007), was performed separately for each wheat chromosome using the germplasm collection data of all the possible pairs of the silico DArTs. LD squared allele frequency correlation (r^2) estimates for all pairwise comparisons between silico DArTs were calculated.

5.3.5 Association mapping for stripe rust response

First, a single factor analysis of variance (SFA) that did not consider population structure was performed using each marker as the independent variable and comparing the mean performance of each allelic class. This was performed using the general linear model (GLM) function in TASSEL 3. Next, population structure was included as covariates in a fixed GLM (Q model). Population structure was evaluated by principal component analysis (PCA) with software DARwin 6 (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006). In both the GLM analyses, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on 1,000 permutations. ITs obtained from seedling tests with CYR32 and from field tests with different locations and years were analyzed separately. ITs, were expressed using a numeric scale as follows: avirulent phenotypes: 0~2=0; virulent phenotypes: 3~4=1. Significant associations were judged on the basis of an F-test, with P-value≤0.05 (after permutation testing). For markers that were significant, a general linear model was used to estimate the amount of phenotypic

variation explained by the QTL. The standardized effect of the marker was also calculated by dividing the difference between the two allelic classes (presence, absence) by the phenotypic standard deviation of the trait. To detect QTL for APR (adult plant resistance), disease severity for 4 environments (Guiyang and Hezhang in 2013, Guiyang and Mianyang in 2014) were also included in AM using Q model.

5.4 Results

5.4.1 Stripe rust response at the seedling stage and adult plant stage

Through inoculations with *Pst* races CYR32, 140 cultivars were evaluated for stripe rust resistance at seeding stage under controlled greenhouse conditions. Resistance scores were obtained for 136 cultivars against the *Pst* race CYR32 (Table S5.1). There were 52.2% cultivars that were resistant to CYR32 (Table 5.1). However, the resistance varied with the origin of the wheat line. Guizhou cultivars had the highest frequency of resistant cultivars (67.1%) while other regional cultivars had lower frequency of resistant cultivars.

Table 5.1 Resistance of the cultivars from different origins

origins	Resistance	Number	Percentage
Guizhou	R	49	67.1%
	S	24	32.9%
Sichuan	R	16	39.0%
	S	25	61.0%
Other regions	R	6	27.3%
	S	16	72.7%
All	R	71	52.2%
	S	65	47.8%

The infection type of the 140 cultivars in the four environments were shown in Chapter III (Table S3.2). Disease severity for all of the cultivars was also scored. Details were shown in Table S5.2.

5.4.2 Marker statistics and linkage disequilibrium

The GBS method yielded 23,999 silico DArTs. Of the silico DArT loci, 9,703 (40.4%) could not be assigned to a known map positions and 6,083 (25.3%) markers had more than one positions (copies) in the whole genome. Both sets of markers were removed. Therefore, there were 8,213 silico DArTs that could be assigned to a unique position on the wheat chromosomes. To estimate the quality of unique loci marker, the index of call rate was used. The markers with a low minor allele frequency (MAF) were excluded from analysis, thus reducing the false positive and the LD inflation effect which have been frequently associated to use of rare alleles. Finally 5,224 markers with a call rate greater than 0.8 and MAF greater than 0.1 were used in PCA, LD and AM (Table 5.2). The silico DArTs were not evenly distributed, with the most marker distribution on B genome (2,294) and the least marker distribution on D genome (1,127). As the 7 homeologue groups were concerned, the most markers distributed on group 2 (1,227), while, the least markers did on group 4 (448). To the 21 chromosomes, 2B harbored the most markers but 4D harbored the least ones.

Table 5.2 Distribution of qualified one-copy silico DArTs on the whole wheat genome

Genome	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Total
A	176	375	191	216	114	461	270	1,803
B	359	548	399	146	396	195	251	2,294
D	142	304	100	86	144	198	153	1,127
Total	677	1,227	690	448	654	854	674	5,224

LD analysis was conducted for each chromosome using the genotype data of silico DArT markers. Totally, there were 233,375 pairwise silico DArTs comparisons. Among them, 72,515 were in LD state (significance level at P value of 0.01). Due to the uneven distribution of silico DArTs in the wheat genome, the number of pairwise silico DArTs for each chromosome was diverse. Therefore, ratio of pairwise silico DArTs in LD for each chromosome was calculated and compared (Fig. 5.1). There were the highest ratio (0.76) of pairwise silico DArTs in LD on 6A. Meanwhile, 1B was also in a high level of LD, with 55% of the pairwise silico DArTs in LD. The average of LD squared allele frequency correlation (r^2) for each chromosome were also analyzed (Table 5.3). The average of LD squared allele frequency correlation (r^2) for 6A was the largest (0.40). Meanwhile, the value for 1B (0.20) and 2D (0.14) were in the second and third place, respectively.

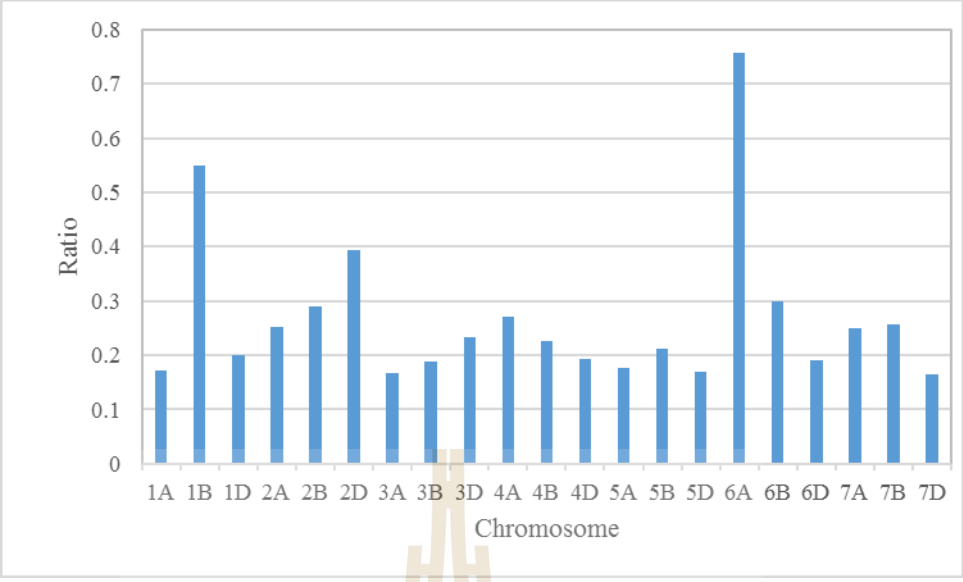

Fig. 5.1 Ratio of significant LD among all pairwise silico DArTs

Table 5.3 Average LD squared allele frequency correlation (r^2) estimates for all pairwise silico DArTs

Genome	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
A	0.05	0.08	0.05	0.07	0.05	0.40	0.07
B	0.20	0.07	0.04	0.06	0.07	0.07	0.06
D	0.07	0.14	0.09	0.04	0.05	0.06	0.05

5.4.3 Association mapping for stripe rust resistance

To discover the significant stripe rust response/marker association, 5,224 silico DArTs genotyping data and infection type data of seedling test or field tests were combined for association mapping with SFA model and the results were shown in Table S5.3 .

The most significant marker-trait associations were detected for CYR32 response (520) but the fewest (2) were detected for stripe rust response at Mianyang site. The number of silico DArTs associated with stripe rust response for each chromosome was also analyzed in the five tests (Table S5.3). Due to few marker-trait association detected at Guiyang and Mianyang site in 2014, ratio of these markers were shown in Fig. 5.2 only for the seedling test of CYR32 and field test at Guiyang and Hezhang site in 2013. For all three tests, higher ratio of markers associated with stripe rust response were revealed on 6A and 1B.

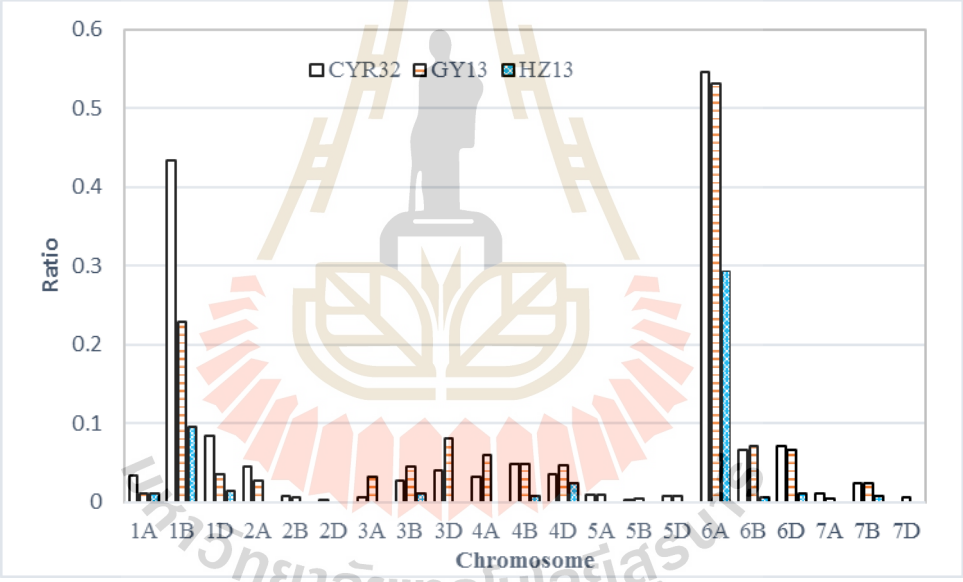

Fig. 5.2 Ratio of markers associated with stripe rust resistance in the seedling test of CYR32 and field tests at Guiyang and Hezhang sites in 2013

For association with stripe rust resistance, there were 595 markers in total that were detected on 21 wheat chromosomes in significant association. Among them, the main were distributed on 6A and 1B, the number of which were 259 and 166, with a

proportion of 43.5% and 27.9%, respectively. These markers can be further divided into 1 test type, 2 test type, 3 test type and 4 test type according to their association with stripe rust response being detected in 1, 2, 3 or 4 tests (no marker was detected in association with stripe rust response in all the 5 tests), which was shown in Table S5.4. There were 218, 196, 179 and 2 markers that were in 1 test type, 2 test type, 3 test type and 4 test type, respectively. The 4 test type markers were not evenly distributed in the whole genome. The largest part (93) distributed on 1B for 1 test type markers. However, it mainly distributed on 6A (111 and 132) for the 2 test type and 3 test type markers. The ratios of these markers on each chromosome were also diverse (Fig. 5.3). There were 56% of markers on 6A that were in association with stripe rust response. Meanwhile, the ratio is 46% for 1B. But for other chromosomes, ratio of the markers in association with stripe rust response were lower than 10%. For the 6A, the ratio of 2 test type and 3 test type markers were far above the ratio of 1 test type markers. But for the 1B, it was opposite.

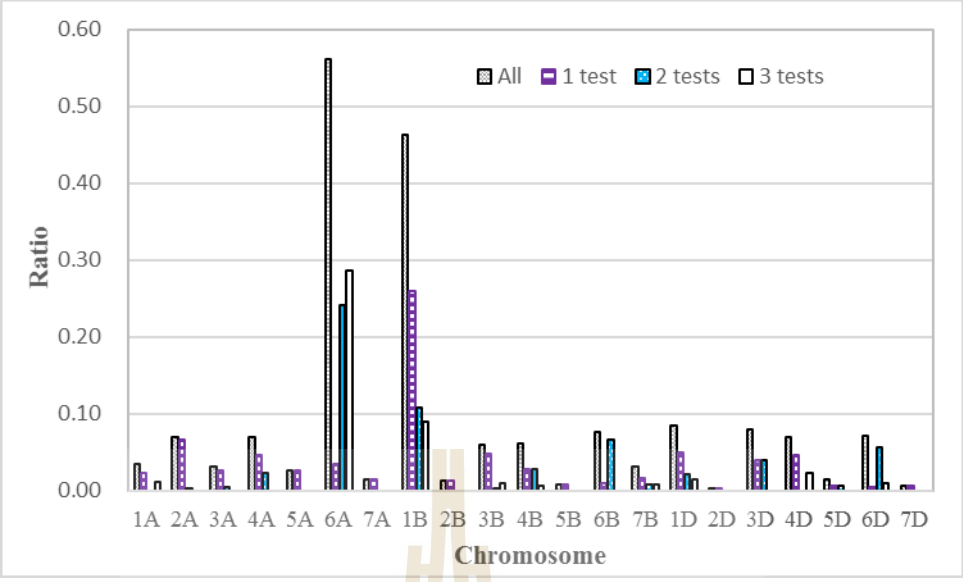

Fig. 5.3 Ratios of the markers in significant association with stripe rust resistance for each chromosome. In the Figure, 1 test, 2 test, 3 test means the three type markers which were divided into according to their association with stripe rust response being detected in 1, 2 or 3 of the 5 tests (seedling test with CYR32, field test in Guiyang, Hezhang in 2013 and field test in Guiyang, Mianyang in 2014).

To avoid the type I error (false positive) in association mapping, population structure were estimated by principal component analysis (PCA) in the study (Table S5.5) and top PC variation were combined with phenotype and genotype data for AM (Q model). PCA showed that 23.4%, 8.0%, 6.2%, 5.6% and 4.2% of the variation was explained by the top five components. We tested the sensitivity of Q model to the number (K) of principal components (PCs) used in AM with IT data. K=2, 5 or 10 were tried separately according to report of Price et al. (2008). Identical marker-trait association were found for both K=5 and 10, when P value after permutation was set at

0.01 as criteria. It means that there was a sufficient number of PCs to capture true population structure effects when K was equal to 5. Finally, top 5 PCs calculated from the relationship matrix used as the Q matrix variables were combined with genotype data and phenotype data (infection type or disease severity data) in the Q model.

Using infection type data of the 5 tests, we compare the AM result of Q model with SFA model (Table 5.4). In the Q model, only 6 marker-trait association were detected. Among them, 3, 2 and 1 were detected in seedling test of CYR32, field test at Guiyang in 2013 and 2014, respectively. For the markers associated with stripe rust response on 1BL, all were related to the two markers (1395486 and 3064366), which were both associated with stripe rust response in seedling test of CYR32 and field test at Guiyang in 2013. But one marker was detected to be in association with stripe rust response at one test for both 3B (4439854) and 4BL (4408062). The percentage explained variation accounted for by genetic loci on 1BL, 3B and 4BL ranged from 9 to 15%. Positive alleles at the significant silico DArT markers were associated with reduced ITs, which were estimated to range from 0.39 to 0.64 standard deviation units (SDU). For the 6 marker-trait associations, 5 of them were also detected in SFA model, but with larger marker-F value, marker R^2, allele effect and lower marker-p, perm-p value.

To detect micro effect QTL for stripe rust resistance, disease severity was also combined in AM with Q model. The results were shown in Table 5.5. There were 2, 3 and 1 marker that were detected to be associated with stripe rust resistance at Guiyang site in 2013, 2014 and Mianyang site in 2014, respectively. Among the 6 markers, 4 markers were on 6AS, 1 on 1BL and 1 on 3B. The marker 3064366 was also detected to be associated with stripe rust resistance in seedling test of CYR32 and field test at

Guiyang in 2013 with the infection type data. The percentage explained variation accounted for by these 6 silico DArTs loci ranged from 12 to 17%. Positive alleles at the significant silico DArT markers were associated with reduced disease severity, which was estimated to range from 0.25 to 0.35 SDU.

Totally, there were 9 silico DArTs that were associated with stripe rust resistance by Q model AM using both infection type and disease severity data. Among the 9 markers, there were 4, 2 and 2 markers on the same chromosome (6AS, 1BL and 3B, respectively). To obtain LD information of these markers on the same chromosome, LD analysis was carried out by TASSEL 3. The results showed that the marker 1395486 and 3064366 on 1BL were in highly significant LD. So did the 4 markers (1093943, 3948356, 3956745 and 1127223) on 6AS. But the markers on 3B (4394514 and 4439854) were not in LD (Table S5.6).

Table 5.4 Comparison of the 6 trait-marker associations detected using IT data in Q model with the counterparts in SFA model

Marker	Chromo.	Tests	SFA model					Q model				
			Marker-F	Marker-p	Perm-p	Marker R^2 (%)	Effect (SD units)	Marker-F	Marker-p	Perm-p	Marker R^2 (%)	Effect (SD units)
1395486	1BL	CYR32	110.47	9.39E-19	0.001	48	0.69	32.8	8.18E-08	0.001	14	0.63
		GY13	69.78	1.17E-13	0.001	36	0.6	28.22	5.17E-07	0.002	12	0.59
3064366	1BL	CYR32	126.46	8.72E-21	0.001	50	0.71	39.96	4.42E-09	0.001	15	0.64
		GY13	81.77	1.97E-15	0.001	39	0.62	31.72	1.13E-07	0.001	12	0.57
4439854	3B	CYR32	95.05	4.79E-17	0.001	43	-0.66	21.17	1.05E-05	0.041	9	-0.54
4408062	4BL	GY14	-	-	-	-	-	24.55	2.28E-06	0.015	13	-0.39

Note: (1) Marker-F is F value from the F test on marker; (2) Marker-p is the corresponding P value from the F test on marker; (3) The perm-p is a permutation test derived using a step-down MinP procedure and controls the family-wise error rate (FWER); (4) marker R-square is the percentage of total variation explained by the marker in the model; (5) effect means the function of allele 1 relative to allele 0 for a silico DArT marker, which is expressed by increasing or decreasing the IT by several standard deviation units. Only markers with perm-p values of 0.05 or less are accepted as significant.

Table 5.5 List of significant markers associated with stripe rust resistance detected using DS data in Q model

Marker	Chromo.	Marker-F	Marker-p	Perm-p	Marker R^2 (%)	Estimate	Tests
1093943	6AS	29.07	3.56E-07	0.006	17	-0.28	GY14
3948356	6AS	25.56	1.54E-06	0.029	14	-0.35	GY14
3956745	6AS	25.56	1.54E-06	0.029	14	-0.35	GY14
1127223	6AS	21.83	7.81E-06	0.037	12	-0.25	GY13
3064366	1BL	24.54	2.37E-06	0.014	12	0.29	GY13
4394514	3B	22.52	5.64E-06	0.03	13	-0.25	MY14

Note: GY13, GY14 and MY14 indicate that the markers were detected to be in association with stripe rust resistance at the field tests in Guiyang in 2013, 2014 and in Mianyang in 2014, respectively.

To investigate the silico DArT's precise position on wheat chromosomes or their probable function related to stripe rust resistance, a BLAST search with the corresponding nucleotide sequences were used to search similar sequence with the silico DArTs, which were associated with stripe rust resistance in a Q model AM. There were two markers (1093943 and 4394514) with significant alignments. Marker 1093943 on 6AS was identical to the wheat genomic survey sequence (GSS) CL901863, which is expressed in young shoot tissue and originated from 1639HC library, 06G18 clone. Marker 4394514 on 3B was identical to the wheat EST CA733384, which is expressed in lemma and palea and originated from wlp1c library, pk008.m6 clone. The latter was located at a position of 517073450-517073515 bp on the genomic scaffold of chromosome 3B by alignment. But the function of the two sequences are still unknown.

5.5 Discussion

5.5.1 Impact of LD on 6A and 1B, population structure on AM

Because of the limitations of QTL mapping in biparental populations, association mapping has gained wide acceptance as an efficient method for mapping QTLs in plant populations. This has been facilitated by the availability of increasing numbers of markers and advances in computational tools. In most crops, reference germplasm collections of diverse accessions have been or are being actively assembled for association study. But the pre-characterization of the accessions is very critical because a common feature of germplasm collection is the presence of non-random, background co-ancestry among accessions that in some case can reach notable levels (Flint-Garcia et al., 2003). The presence of such population structure greatly increases the Type Ⅰ errors. The LD extent, averaged for each chromosome or whole genome, is the second main feature of a germplasm collection used for association mapping. It relates to the resolution or detection capacity of the AM and marker density in AM.

In this study, we used infection type data for identifying the QTLs or genes for stripe rust resistance in elite wheat germplasm using both SFA model and Q model. Genome-wide analyses using a SFA model identified 595 loci associated with stripe rust resistance in wheat germplasm that map to all chromosomes. But the majority of loci converged on the chromosomes 6A and 1B, which accounted for 43.5% and 27.9%, respectively. But before the AM, the LD analysis was carried out chromosome by chromosome for all marker pairs. It showed that LD extent was far higher on 6A and 1B than other wheat chromosomes, which were obtained by statistic ratio of marker pairs in significant LD and average allele frequency correlations (r^2) for each chromosome. Due to higher LD extent or lower LD decay rate, a small number of

markers would suffice for AM. However, silico DArTs were also not evenly distributed with higher proportion on 1B, 2B, 3B, 5B and 6A. The higher LD extent and large amount of markers led to results that lots of markers in LD on 6A or 1B were also detected to be associated with stripe rust resistance. Population structure analysis in chapter Ⅳ also proved that 2 subpopulations were divided into mainly according to 6VS/6AL translocation lines. Zhang et al. (2011) also reported that there was population stratification in Chinese wheat according to 1BL/1RS translocation lines. However, the Q model taking the population structure into account showed contrasting results. Only 4 markers were detected to be associated with stripe rust resistance with IT data. Among them, two markers were on the 1BL but none were on 6A. Therefore, Q model can efficiently exclude the false positive association in AM. Wang et al. (2008b) also reported that single factor analysis of variance (SFA) and mixed linear model (MLM) analyses were both used to discover marker/trait associations. The MLM analyses, which include population structure, kinship or both factors, reduced the number of markers significantly associated with IDC by 50% compared with SFA. Zhao et al. (2007) used association mapping to investigate the genetic basis of variation with *Brassica rapa*. Marker-trait associations were investigated both with and without taking population structure into account. One hundred and seventy markers were found to be associated with the observed traits without correction for population structure. But correction for population structure led to the identification of 27 markers. Therefore, markers that show association after correction for substructure in our study may more reliably be linked to stripe rust resistance QTLs.

5.5.2 Principal components analysis corrects for stratification in genome-wide association

Principal component analysis (PCA) has long been used in genetic diversity analysis. It was recently proposed as a fast and effective way to diagnose population structure (Patterson et al., 2006). The PCA summarizes variation observed across all markers into a smaller number of underlying component variables. These principle components (PCs) could be interpreted as relating to separate, unobserved subpopulations from which the individuals (or their ancestors) originated (Zhu et al., 2008). Therefore, principal components analysis can correct for stratification in genome-wide association studies as Q matrix. Replacing Q with PC in the mixed model shows some promise (Weber et al., 2008; Zhao et al., 2007). How many top PCs (K) should be included in an AM have been studied. Generally, the rigorous approach is to set K equal to the number of statistically significant PC vectors (Patterson et al., 2006). But it is not always the case. Zhao et al. (2011) used top 4 PCs explaining nearly half of genetic variance and SNPs as fixed effect in AM. In this study, the top 2, 5 and 10 PCs were tried in AM to exclude the confounding of the population structure, respectively. The results showed that 98 marker-trait associations were detected with 2 PCs. Among them, 30 and 42 were converged on 2A and 2B, which departed quite far from the expected distribution (unpublished data). With 5 or 10 PCs considered in AM, fewer marker-trait associations were detected (6 and 10), but the 4 most significant marker-trait associations were identical (unpublished data). It indicated that AM with 2 PCs could not minimize the confounding and still created false positive associations. However, the top 5 PCs, which could explain nearly half of genetic variance, were enough to capture the true population structure effect. Price et al. (2006) also noted

that using more PCs than necessary would in theory lead to a loss in power; however, for K＜＜N (sample number) the effect will be minimal. Therefore, it is important to confirm how many PCs should be involved before the AM.

5.5.3 Comparisons with previous mapping results for stripe rust resistance genes or QTLs

In this study, 4 loci and 6 loci were confirmed to be associated with stripe rust resistance using Q model association mapping with an infection type data (scored at both seedling stage and adult plant stage) and disease severity data (scored at adult plant stage), respectively. The two markers (1395486 and 3064366) on 1BL were detected in two test (CYR32 test and field test in Guiyang in 2013) with infection type data. Besides, the marker 3064366 was also detected in the field test of Guiyang in 2013 with disease severity data. Therefore, their association with stripe rust resistance are very stable. LD of these two markers was highly significant, with r^2 of 0.79. Therefore, there are high probability that they were linked to the same resistance gene. At present, there are two ASR genes on 1BL that have been reported (*Yr*Exp1 and *Yr*26) (Lin and Chen, 2008; Wang et al., 2008a). We also analyzed the LD between the *Yr*26 linked marker we173 (1.4 cM) and 3064366, which was also highly significant. Therefore, we may deduce that the two markers on 1BL are tightly linked with *Yr*26, which can be used for development of more tightly linked and user-friendly marker of *Yr*26. One and three markers on 6AS were also confirmed to be in association with stripe rust resistance in field test of Guiyang in 2013 and 2014, respectively. All of 4 markers were also in highly significant LD. Among the 4 markers, 1093943 was in consistent with the wheat genomic survey sequence (GSS) CL901863. But its position was still unknown. QTLs of stripe rust resistance mapped on 6AS have also been

reported (Hao et al., 2011; Rosewarne et al., 2012). But the QTL in both reports could interpret phenotypic variation with 6% to 7%, which are lower than R^2 in our research (12% to 17%). Two markers on 3B were also confirmed to be associated with stripe rust resistance, but one (4439854) is detected in the test of CYR32 and the other (4394514) is detected in field test with disease severity data. Therefore, 4439854 may be linked to an ASR gene, while, 4394514 may be linked with a QTL. There is only one ASR gene (*Yr*4) mapped on 3B, but *Yr*4 is susceptible to CYR32 (Wan et al., 2004). Therefore, we deduce that 4439854 may relate to a new ASR gene. There are a lot of QTLs that were mapped on both arms of 3B (Maccaferri et al., 2015). Marker 4394514 have been mapped on the genomic scaffold of chromosome 3B at a position of 517073450-517073515 bp by alignment. Because it is the unique QTL detected in Mianyang site in 2014, which is very meaningful to wheat resistant breeding in Southwestern China. It should be paid more attention in future study. Marker 4408062 on 4BL was associated with stripe rust resistance in Guiyang site in 2014, which contributed phenotype variation of 13%. On the 4BL, *Yr*50, *Yr*62 and some other QTLs were mapped (Liu et al., 2013; Lu et al., 2014; Maccaferri et al., 2015). We could not found similar sequence with Marker 4408062 by BLAST. But in future, consensus map with silico DArT markers will be available along with widely application of DArT-seq marker in wheat. Map position of each significant marker will be known and we can compare map position with other researches to confirm whether they are the same genes or QTLs. In the next step, we can also check the plus effect alleles in the resistant cultivars, and cross them with a susceptible cultivar to construct a segregation population for fine mapping. These will be favorable for wheat resistant breeding in China.

5.6 Reference

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CHAPTER VI

CONCLUSION

In this study, commercial cultivars and breeding lines from Southwestern China were evaluated for stripe rust resistance at seedling stage and at adult plant stage. Six stripe resistance genes (*Yr*9, *Yr*10, *Yr*15, *Yr*18, *Yr*26 and *Yr*29) were also identified in these cultivars (lines) by molecular detection. Whole genome were profiled with DArT-seq technique and genetic diversity of Southwestern China wheat were also analyzed. Finally, association mapping was conducted for stripe resistance. Results are as following:

1. Different ratio of cultivars were resistant to stripe rust at different sites for two years. Forty nine cultivars (lines) were resistant at all sites and years. Durable resistance of Guizhou cultivars was better than Sichuan cultivars.

2. There were 52.2% cultivars that were resistant to CYR32. Guizhou cultivars had the highest frequency of resistant cultivars but other regional cultivars had the lowest frequency of resistant cultivars to CYR32.

3. Virulence of *Pst* races appeared to be higher in Sichuan (Mianyang site) than in Guizhou province (Guiyang and Hezhang site).

4. Six stripe rust resistance genes were not evenly distributed in Southwestern China wheat. *Yr*26 was the most abundant, *Yr*9 in the second place, but *Yr*10, *Yr*15, *Yr*18

and *Yr*29 were more scarce.

5. *Yr*26 is overused in Southwestern China wheat. It is still effective in Guizhou but begins to lose its resistance to the new *Pst* race (V26) at Mianyang site according to correlation analysis.

6. Gene combination analysis showed that the 6 *Yr* genes were not highly pyramided in Southwestern China wheat. It also showed the trend that the more *Yr* genes were pyramided together, the better resistance the cultivar would possess.

7. Precise molecular detecting of *Yr* genes should be combined with specific *Pst* race test or pedigree analysis.

8. Two groups of wheat varieties (Group I and Group II) were identified using principal-coordinates analysis (PCoA) of the whole-genome level and the chromosome 6AS level. Group I and Group II were divided based on T6VS/6AL and 6AS/6AL.

9. Dendrogram with bootstrap values revealed that population stratification of Southwestern China wheat was not obvious, but accessions were clustered together based on their pedigree or origin.

10. Genetic similarity (GS) coefficient between pairs of genotypes ranged from 0.60 to 0.99 with a mean GS of 0.72, which suggest that the collection of Southwestern China wheat were not diverse.

11. Linkage disequilibrium (LD) analysis showed that larger extent of LD were estimated on chromosome 6A and 1B.

12. In association mapping (AM) for stripe rust resistance using infection type (IT) data with single factor analysis (SFA) model, there were 595 markers in total that were detected on 21 wheat chromosomes in significant association and the main were

distributed on 6A and 1B. But in AM with Q model, there were only 4 markers that were detected on 1BL, 3B and 4BL in significant association.

13. In AM for stripe rust resistance using disease severity (DS) data with Q model, there were 6 markers in total that were detected on 6AS, 1BL and 3B in significant association, which suggest the presence of QTLs for stripe rust resistance.

APPENDICES

Table 2.1 Stripe rust resistance genes with an official designation (Dong and Xu, 2009)

Gene	Chromo.	Type	Source	Representative cultivar	Marker type/ marker/ genetic distance (cM)
Yr1	2AL	ASR	*T.aestivum*	Chinese spring	SSR/gwm372
Yr2	7B	ASR	*T.aestivum*	Heines Ⅶ	SSR/WMC 364/5.6
Yr3a	1B	ASR	*T.aestivum*	Vilmorin23	
Yr3b	1B	ASR	*T.aestivum*	Hybird 46	
Yr3c	1B	ASR	*T.aestivum*	Minister	
Yr4a	6B	ASR	*T.aestivum*	Cappelle-Desprez	
Yr4b	6B	ASR	*T.aestivum*	Hybird 46	
Yr5	2BL	ASR	*T.spela album*	*T.spelta album*	RGAP/Xwgp21/0.5; RGAP/Xwgp27/0.9
Yr6	7BS	ASR	*T.aestivum*	Heines Kolben	SSR/Xwmc76; SSR/Xwmc276
Yr7	2BL	ASR	*T.durum*	Lee	SSR/Xgwm526/5.3
Yr8	2A/2D	ASR	*Ae.comosua*	Compare	RAPD/OP-D11
Yr9	1B/1R	ASR	*S.cereala*	Lovrin 13	SSR/Xgwm582/3.7
Yr10	1BS	ASR	*T.aestivum*	Moro	SCAR/SC200/0.5; SSR/Xpsp3000/1.2
Yr11	/	APR	*T.aestivum*	Joss cambier	
Yr12	/	APR	*T.aestivum*	Mega	
Yr13	/	APR	*T.aestivum*	Maris Huntsman	
Yr14	/	APR	*T.aestivum*	Hobit	
Yr15	1BS	ASR	*T.dicoccoides*	*T.dicoccoides*-G25	RGA/Yr15-R2/2; SSR/Xgwm33/5
Yr16	2DL	APR	*T.aestivum*	Cappelle-Desprez	
Yr17	2AS	ASR	*Ae.ventricosa*	VPM1	SCAR/Sc-Y15/0.8±0.7
Yr18	7DS	APR	*T.aestivum*	Jypateco 73R	STS/csLV34/0.4
Yr19	5B	ASR	*T.aestivum*	Compair	
Yr20	6D	ASR	*T.aestivum*	Fieldeer	
Yr21	1B	ASR	*T.aestivum*	Lemhi	
Yr22	4D	ASR	*T.aestivum*	Lee	
Yr23	6D	ASR	*T.aestivum*	Lee	
Yr24 (YrCh 42)	1BS	ASR	*T.turgidum*	K733	SSR/Xgwm273/6.1; SSR/Xgwm11/7.1
Yr25	1D	ASR	*T.aestivum*	Tp1295	
Yr26	1BL	ASR	*T.turgidum*	T.turgidum	SSR/Xgwm11/1.9; SSR/Xgwm18/1.9

Table 2.1 (Continued) Stripe rust resistance genes with an official designation (Dong and Xu, 2009)

Gene	**Chromo.**	**Type**	***Source***	**Representative cultivar**	**Marker type/ marker/ genetic distance (cM)**
Yr27	2BS	ASR	*T.aestivum*	Selkirk	
Yr28	4DS	ASR	*Ae.tauschii*	*T.tauschii* W-219	
Yr29	1BL	APR	*T.aestivum*	Pavon76	AFLP/(P84/M78)/ 5.8
Yr30	3BS	APR	*T.aestivum*	Opata85	
Yr31	2BS	ASR	*T.aestivum*	Pastor	
Yr32 （*YrCV*）	2AL	ASR	*T.aestivum*	Senat	SSR/Xwmc198/2
Yr33	7DL	ASR	*T.aestivum*	Batavia	SSR/Xgwm437/linkage
Yr34	5AL	ASR	*T.aestivum*	WAWHT2046	SSR/B1/12.2
Yr35	6BS	ASR	*T.dicoccoides*	*T.dicoccoides* 479	
Yr36	6BS	APR	*T.dicoccoides*	Glupro	Xbarc101/co-segregated
Yr37	2DL	ASR	*Ae.kotschyi*	Line 8078	
Yr38 （*YrS12*）	6A	ASR	*Ae.sharonensis*	Line 8028	
Yr39	7BL	APR	*T.aestivum*	Alpowa	RGAP/Xwgp36/close linkage
Yr40	5DS	ASR	*Ae.geniculata*	WL711、TA6675	

Table 2.2 Information of the formally named stripe rust resistance genes (McIntosh et al., 2010, 2011, 2012; McIntosh et al., 2014)

Genes	Chromo.	Type	Representative cultivar	Marker/ genetic distance (cM)
Yr41 (*YrCN19*)	2BS	ASR	Chuannong 19	Xgwm410/0.3 cM
Yr42/Lr62	6AS	ASR	*Ae. neglecta*	
Yr43	2BL	ASR	IDO377s	Xwgp110/4.4 cM; Xwgp103/5.5cM
Yr44	2BL	ASR	Zak	Yr5/12.7 cM; Xwgp100/3.9 cM
Yr45	3DL	ASR	PI181434; PI 660056	Xwp118/4.8 cM; Xwp115/5.8 cM
Yr46/Lr67	4DL	APR	RL6077; PI 250413	Xbarc98/4.4cM
Yr47/Lr52	5BS	ASR	AUS 28183	Lr52 /4.1cM; Xcfb309/9.6cM
Yr48	5AL	APR	PI 610750	Xwmc727/4.4cM; Xwms291/0.3cM
Yr49	3DS	APR	Chuanmai 18; AUS 91433	Xgwm161/1cM; Xgpw7321/co-segregated
Yr50	4BL	ASR	*Th. intermedium*.; CH233	Xbarc1096/6.9cM; Xbarc-4B/7.2cM
Yr51	4AL	ASR	5515; AUS 91456	wPt4487/9.8cM; wPt0763/4.4cM
Yr52	7BL	APR	PI 183527	Xbarc182/1.2 cM; Xwgp5258/1.1cM
Yr53	2BL	ASR	PI 480148	Xwmc441/5.6 cM;
Yr54	2DL	APR	GID6032209	Xgwm301/0.4cM
Yr55	2DL		AUS 38882	Xmag4089/11.4cM; Xmag3385/8.4cM
Yr56	2AS	ASR	AUS 91575	Xsun167/5.7cM; Xsun168/7.6cM
Yr57	3BS	ASR	AUS 91463	Xgwm389/2.0cM
Yr58	3BL		Sonora W195; AUS19292	123392/4.6cM; 1121669/3.9cM
Yr59	7BL	APR	PI 660061; PI 178759	Xwgp5175/2.1cM; Xbarc32/1.1cM
Yr60	4AL	ASR	Lal Bahadur	Xwmc776/ 0.51cM
Yr61	7AS	ASR	Pindong 34	Xwgp5765b/3.9cM; Xwp5467/1.9cM
Yr62	4BL	APR	PI 192252; PI 660060	Xgwm251/3.3cM; Xgwm192/2.0cM
Yr63	7BS		AUS 27955	IWB33120/0.9cM; IWB52844/1.5cM
Yr64	1BS	ASR	PI 660064; PI 331260	Xgwm413/ 3.5cM; Xgdm33/2.0cM
Yr65	1BS	ASR	PI 480016	Xgwm18/1.2cM; Xgwm11/2.1cM
Yr66	3DS		VL892	IWB47165/3.1cM; IWB18087/2.9cM
Yr67	7BL		C306; C591	IWB37096 /1.1cM; IWB71995/0.6cM
YrAlp	1BS	ASR	Alpowa	Xgwm18/15.2 cM

Table 2.3 Temporarily designated genes for resistance to stripe rust (Maccaferri et al., 2015)

Genes	Chromo.	Type	Representative cultivar	Marker/ genetic distance (cM)
YrAS2388	4DS	ASR	*Ae. tauschii*	Xwmc617/34.6 cM; Xwmc285/1.7 cM
YrAvS		ASR	Avocet S	
YrC142	1BS	ASR	Synthetic CI142	
YrC51	2B	ASR	C51	C51STS-4/1.4cM
YrC591	7BL	ASR	C591; Zhongzhi 1	Xcfa20-40/8.0cM; SC-P35M48/11.7cM
YrCK	2DS	ASR	Cook; Sunco	
YrCN17	1BL.1RS	ASR	(*S. cereale*) CN17 ;	
YrExp1	1BL	ASR	Express	Xwgp78/4.2cM; Xwmc631/3.4cM
YrExp2	5BL	ASR	Express	Xwgp8/1cM; Xwgp82/0.7cM
YrH52	1B	ASR	Hermon H52 (*Triticum dicoccoides*)	Xgwm413/1.3cM; Xgwm273/2.7cM
YrH9014	2B, 2BS	ASR	H9014-14-4-6-1	Xbarc13/1.4cM; Xbarc55/3.6cM
YrH9020	2DS	ASR	(*Psathyrostachys huashanica*)	Xgwm455/5.8cM; Xgwm261/4.4cM
YrKK	2B	APR	Kenya Kuku	Xgwm148/3.2cM; Xwmc474/1.8cM
YrLM168	6A	APR	LM168-1	R1/2.4cM; R2/2.4cM
Yrns-B1	3B	APR	Lgst. 79-74	3cM interval between Xgwm493 and Xgwm1329
YrP81	2BS	ASR	P81; Xu29	
YrR212	1BL.1RS	ASR	(S. cereale.) R185; R205; R212	Xgwm42/1.8cM; Xwmc770/4.1cM
YrR61	2AS	APR	Pioneer 26R61	
Yrs2199	2BL	ASR	S2199	Xgwm120/11.0cM ;Xdp269/0.7cM
YrSp	2B, 2BL	ASR	Spaldings Prolific	Allelic with Yr5/Yr7
YrV23	2B	ASR	Vilmorin 23	Xwmc356/9.4cM
Yrxy1	7AS	HTAP	Xiaoyan 54	Xbarc49/15.8cM
Yrxy2	2A	HTAP	Xiaoyan 54	Xwmc794/4.0cM; Xbarc5/6.4cM
YrZH84	7BL	ASR	Zhou 8425B	Xbarc32/4.8cM

Table 2.4 Previously reported Stripe rust QTLs (Maccaferri et al., 2015)

Loci	Donor parents	QTL Interval (%)	Loci	Donor parents	QTL interval (%)
QYr.ufs-2A (Yr16)	Cappelle-Desprez	2.1~7.6	*QYr.cau-5AS*	AQ24788-53	22.3~23.9
QYr.ufs-2DS	Cappelle-Desprez	34.2~36.9	*QYr.caas-4DL*	Bainong64	45.4~53.7
QYr.ufs-4B	Palmiet	34.6~51.9	*QYr.caas-6BS*	Bainong64	12.6~16.4
QYr.ufs-5B	Cappelle-Desprez	37~41.2	*QYr.caas-7A*	Jingshuan16	12.5~22.8
QYr.ufs-6D	Cappelle-Desprez	42.4~56.1	*QYr.caas-1AL*	Naxos	77.8~80.3
QYr.sun-1B	Wollaroi	84.2~96.3	*QYr.caas-1BL.1RS*	SHA3/CBRD	28.2~34.6
QYr.sun-2A	Wollaroi	2.3~2.5	*QYr.caas-1DS*	Naxos	0.8~8.5
QYr.sun-3B	Wollaroi	68.6~71.9	*QYr.caas-2BL*	Naxos	60.1~66.6
QYr.sun-5B	Wollaroi	84.4~97.1	*QYr.caas-2DL*	Naxos	44.1~55.8
QYr.sun-1A	Janz	24.5~46.8	*QYr.caas-5AL.2*	SHA3/CBRD	81.2~84.6
QYr.sun-1B	Kukri	80~96.7	*QYr.caas-5BL.3*	SHA3/CBRD	65.4~67.4
QYr.sun-2AS	Kukri	37.9~41.4	*QYr.caas-6BS.2*	Naxos	10.3~16.4
QYr.sun-3B	Kukri	28.3~37.6	*QYr.caas-7B.1*	SHA3/CBRD	33.8~56.3
QYr.sun-5B	Janz	63.3~72.9	*QYr.caas-7BL.2*	SHA3/CBRD	71.8~73.6
QYr.sun-6B	Janz	17.1~34.8	*QYr-2B*	Avocet	89.2~90.8
QYr.sun-7B	Kukri	43.8~58.7	*QYr-2B (Yr27)*	Attila	18.6~21.3
QYr.tam-2D	Quaiu	68.4~79.5	*QYr-7B*	Attila	82.6~88.4
QYr.tam-1A (YrA)	Avocet-	8.1~10.2	*QYr.cim-1AL*	Pastor	81.9~83
QYr.tam-1B	Quaiu	90.2~98.7	*QYr.cim-1BL (Lr46/Yr29)*	Pastor	93.6~100
QYr.tam-3B	Quaiu	2.1~13.7	*QYr.cim-1BS*	Pastor	41.2~54.5
QYr.tam-3D	Quaiu	4.6~24.1	*QYr.cim-3A*	Avocet	69.7~75.5
QYr.tam-1AL	TAM112	75.7~100	*QYr.cim-3B*	Pastor	42.1~60.7
QYr.tam-1AS	TAM111	21.7~31.8	*QYr.cim-4DS*	Pastor	18.4~34
QYr.tam-2AS	TAM111	0~6.1	*QYr.cim-5AL*	Pastor	66.8~79.9
QYr.tam-2BL	TAM111	36.6~47.9	*QYr.cim-6A*	Avocet	7.6~17.8
QYr.tam-6BS	TAM111	9.4~19.7	*QYr.cim-6BL*	Pastor	70~79.9
QYr.inra_2AL.2	Camp Remy	79.1~100	*QYr.cim-7AS*	Avocet	0~6.1
QYr-2B	Opata85	35.4~44.3	*QYr.cim-7BL*	Avocet	74.4~85.5
QYr-5A	Opata85	66.8~79.2	*QYr.cim-7BL*	Pastor	73.5~87.6
QYr-6D	W-7984	71.6~77.8	*QYr.wgp-6B.1*	Stephens	24.1~34.8

Table 2.4 (Continued) Previously reported Stripe rust QTLs

Loci	Donor parents	QTL interval (%)	*Loci*	Donor parents	QTL interval (%)
QYrlo.wpg-2BS	Louise	29.6~44.5	*QYr.wgp-6BS.2*	Stephens	15.9~21.4
QYr.vt-4BL	VA00W-38	58.1~60.4	*QYr-3B*	Opata85	0~6.2
QYrpi.vt-3BL	VA00W-38	51.2~65.1	*QYr-1B*	Sachem	61.3~67.2
QYrva.vt-2AS	VA00W-38	4~15	*QYr-4A*	Sachem	74.2~79.1
QYr.inra-2AS.1	Recital	2.5~7.6	*QYr-4B*	Sachem	32.9~40.7
QYr.inra-2BS	Renan	10~15.5	*QYr-7BL*	Strongfield	80.5~86.2
QYr.inra-3Bcentr	Renan	50.5~60.9	*QYr-3B*	Oligoculm	2.3~7
QYr.inra-3BS	Renan	2.8~7.9	*QYr-4B*	Oligoculm	65.8~73.3
QYr.inra-6B	Renan	42.3~50.2	*QYr-4D*	Oligoculm	54~62.3
QYr.inra-7A	Recital	6.5~10.1	*QYr-5B*	Oligoculm	47.6~52.3
QYr.tem-5B.1	Flinor	37.5~38.9	*QYr-6B*	Oligoculm	23.1~28.6
QYr.tem-5B.2	Flinor	70.3~75.7	*QYr-7B*	Oligoculm	21~27.7
QYraq.cau-2BL	Aquileja	62.1~73.6	*QYr.orr-4AL*	Stephens	74.2~81.2
QYrlu.cau-2BS1	Luke	19.9~30.4	*QYr.orr-6AL*	Stephens	71.3~74.7
QYrlu.cau-2BS2	Luke	30.6~32.3	*QYr.orr-7A*	Stephens	69.9~77.1
QYr.uga-2AS	26R61	1.9~16.8	*QYr.orr-7BS*	Stephens	41.8~45.9
QYr.uga-3BS.1	AGS2000	6.7~11.6	*QYrst.orr-1DS*	Stephens	1.4~10.1
QYr.uga-5B	AGS2000	11.4~16.7	*QYrst.orr-2AS*	Stephens	9.9~13.1
QYr.uga-6AS	26R61	0~7.1	*QYrst.orr-2B.1*	Stephens	10.6~11.4
QYr-7B	Tiritea	68.8~75.2	*QYrst.orr-2BS.2*	Stephens	30.4~36.4
QYr.jic-2D	Briagdier	72.1~81.5	*QYrst.orr-3AL*	Stephens	12.8~22.4
QYr.jic-4B	Alcedo	54.8~62.1	*QYr-3B.1*	Pavon76	0~5.3
QYr.cim-1BL	Francolin	90.2~98.7	*QYr-4B*	Avocet	39.4~51.9
QYr.cim-2BS	Francolin	44.3~47.9	*QYr-6A*	Avocet	63.9~73.1
QYr.cim-3BS.2	Frankolin	2.3~14.9	*QYr-6B*	Pavon76	42.3~50.2
QYr.cim-5AL	Francolin	35.7~42.3	*QYr.cim-2BS (Yr31)*	Chapio	29.1~32.2
QYr.cim-6AL	Francolin	49.7~68	*QYr.cim-3BS (Yr30)*	Chapio	0~6.7
QYr-1B	Saar	90.3~96.3	*QYr.cim-5BL*	Chapio	20.8~37
QYr-1B	Saar	5~9.2	*QYr-3B*	Alturas	2.3~5.7
QYr-6A	Saar	62.7~74.6	*QYr.sun-1B*	CPI133872	80~90.2

Table 2.4 (Continued) Previously reported Stripe rust QTLs

Loci	Donor parents	QTL interval (%)	*Loci*	Donor parents	QTL interval (%)
QYr.wgp-6AS	Express	3.8~7.1	*QYr.sun-1D*	CPI133872	5.1~8.5
QYrex.wgp-1BL	Express	75.3~100	*QYr.sun-4B*	Janz	36.3~64
QYrex.wgp-3BL	Express	88.2~99.5	*QYr.sun-7A*	CPI133872	12.5~40.2
QYr.ucw-2A	PI610750	6.7~14.1	*QYr.ui-4A*	IDO444	78.8~85.7
QYr.ucw-2B	UC1110	39.8~49.1	*QYr.ui-4B*	Rio Blanco	49.7~53.8
QYr.ucw-3BS	UC1110	4.4~6.7	*QYr.ui-5B*	IDO444	84.8~100
QYr.ucw-5AL	PI610750	84.5~100	*QYrid.ui-1A*	Rio Blanco	0~5.7
QYr.caas-2DS	Libellula	0~4.8	*QYrid.ui-2B.1*	IDO444	25.1~27.4
QYr.caas-4BL	Libellula	49.2~52.4	*QYrid.ui-2B.2*	IDO444	30.4~50.2
QYr.caas-5BL.1	Libellula	45.2~47.6	*QYrid.ui-3B*	Rio Blanco	85.6~98.1
QYr.caas-5BL.2	Libellula	70.3~73.2	*QYr.sgi-2B.1*	Kariega	28.2~32
QYr.inra-2AL	Camp Remy	8.3~11.9	*QYr.sgi-4A.1*	Kariega	76.1~84.5
QYr.inra-2B.1	Camp Remy	42.1~50.5	*QYr.sgi-4A.2*	Kariega	74.4~80.2
QYr.inra-2B.2	Camp Remy	59.8~60	*QYr.caas-2BS*	Pingyuan 50	32.3~49.1
QYr.inra-2DS	Camp Remy	31.4~55.8	*QYr.caas-5AL*	Pingyuan 50	81.5~88.3
QYr.inra-5B.1	Camp Remy	42.1~46.5	*QYr.caas-6BS*	Pingyuan 50	27.4~42.3
QYr.inra-5BL.2	Camp Remy	70.3~74.8	*QHtap.wsu-7BL*	Alpowa	41.6~47.8
QYr.jic-1B	Guardian	90.1~94.8	*QYr.ucw-1B*	IWA3892	87.9~89.7
QYr.jic-2D	Guardian	55.6~61.3	*QYr.ucw-1D*	IWA980	24~25.6
QYr.jic-4B	Guardian	58.1~62.1	*QYr.ucw-2A.2*	IWA422	2.9~4
QYr.sgi-1A.1	Kariega	16.5~20.1	*QYr.ucw-2A.3*	IWA424	26.3~27.4
QYr.sgi-2B.1	Kariega	27.4~30	*QYr.ucw-3B.2*	IWA5202	6.5~8
QYr.sgi-4A.1/4A2	Kariega	80.1~94.8	*QYr.ucw-4A*	IWA1034	84.3~85.8
QYr.sgi-7A	Kariega	74.6~100	*QYr.ucw-4D*	IWA5375	47.2~49.1
QYr.ufs-6A	Kariega	61.6~66.8	*QYr.ucw-5A.1*	IWA6988	86.7~88.1
QYr.ufs-6B	Kariega	6.9~12.3	*QYr.ucw-6B*	IWA7257	56.7~58.3
QYr.cau-1BS	AQ24788-53	5.1~28	*QYr.ucw-6D*	IWA167	47.9~49.8
QYr.cau-3AL	AQ24788-53	37.4~42			

Table 3.1 Information of wheat cultivars and lines in this study.

Name	Abb.	Origin	Pedigrees	Types
0308	0308	Guizhou	selection from powdery mildew resistance population	Line
9712	9712	Guizhou	n.a.	Line
Guimai12	GM12	Guizhou	n.a.	Line
Guiyu17	GY17	Guizhou	n.a.	Line
Qianmai12	QM12	Guizhou	Zongkang'ai2/Abandanza // Triticale///*Av. sativa*	Cultivar
Qianmai14	QM14	Guizhou	n.a.	Cultivar
Qianmai15	QM15	Guizhou	Xing'aikang1 /C39	Cultivar
Qianmai16	QM16	Guizhou	QR3/QR1	Cultivar
Qianmai17	QM17	Guizhou	P36/1726	Cultivar
Qianmai18	QM18	Guizhou	selection from Powdery mildew resistance population	Cultivar
Qianmai19	QM19	Guizhou	9665F8//Guinong21/ Abandanza	Cultivar
Qianxiafan28	QXF28	Guizhou	Guinong21/ Abandanza	Line
Qian0117-1	Q0117-1	Guizhou	Xiaoyan503/976F4	Line
QianAT6-5	QAT6-5	Guizhou	Aibai//Guinong21/Abandanza///Mohei25	Line
Qian9939-5	Q9939-5	Guizhou	Guinong775//Guinong21/Abandanza	Line
Qian9988-46	Q9988-46	Guizhou	Guinong775/98318	Line
Qian2032-8	Q2032-8	Guizhou	Chaoxuan/9639	Line
Qian 0240-2	Q0240-2	Guizhou	Guinong001/Xiafan61	Line
Qian0209-4	Q0209-4	Guizhou	Jieyan970012/9611F6	Line
Guinong18	GN18	Guizhou	Laizhou137/Guinong775	Cultivar
Guinong19	GN19	Guizhou	Zhongyan96-3/Guinong21	Cultivar
Guinong25	GN25	Guizhou	Hanyou2/Guinong775	Cultivar
Guinong26	GN26	Guizhou	Guinong97012/Guinong21	Cultivar
Guinong28	GN28	Guizhou	Guinong13 /Guinong775	Cultivar
Guinong19-4	GN19-4	Guizhou	Triticale / *Av. fatua* L. // *T. aestivum* L.	Line
Guinong21	GN21	Guizhou	*H. villosa* / *T. turgidum* var. *durum* // *T. aestivum* L.	Line
Guinong21-1	GN21-1	Guizhou	*H. villosa* / *T. turgidum* var. *durum* // *T. aestivum* L.	Line
Guinong22	GN22	Guizhou	*H. villosa* / *T. turgidum* var. *durum* // *T. aestivum* L.	Line
Guinong775	GN775	Guizhou	*Ae. squarrosa* / *Av. fatua* L // *Ae. ventricosa* Tausch / *T.durum*	Line
Baimian3	BM3	Guizhou	Systematic selection from Ailiduo	Line

Table 3.1 (Continued) Information of wheat cultivars and lines in this study.

Name	**Abb.**	**Origin**	***Pedigrees***	**Types**
JYP1	JYP1	Guizhou	*Ae. squarrosa* / *Av. fatua* L. // *Ae. ventricosa* Tausch / *T. turgidum* var. *durum*	Line
JYP2	JYP2	Guizhou	*Ae. squarrosa* / *Av. fatua* L. // *Ae. ventricosa* Tausch /*T. turgidum* var. *durum*	Line
JYP3	JYP3	Guizhou	*Ae. squarrosa* / *Av. fatua* L. // *Ae. ventricosa* Tausch / *T. turgidum* var. *durum*	Line
TG	TG	Guizhou	*T. timopheevi* Zhuk. / *Av. fatua* L.	Line
TP2	TP2	Guizhou	*T. timopheevi* Zhuk. / *Av. fatua* L.	Line
TP3	TP3	Guizhou	*T. timopheevi* Zhuk. / *Av. fatua* L.	Line
YP1	YP1	Guizhou	*T. turgidum* var. *durum* / *Av. fatua* L.var. Portugal	Line
YP2	YP2	Guizhou	*T. turgidum* var. *durum* / *Av. fatua* L.var. Portugal	Line
YLP8-1	YLP8-1	Guizhou	*T. monococcum* L. / *Av. fatua* L. var .Portugal	Line
YLP8-40	YLP8-40	Guizhou	*T. monococcum* L. / *Av. fatua* L. var .Portugal	Line
P1-2	P1-2	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P3M-8	P3M-8	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P5-1	P5-1	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P7-9	P7-9	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P7-26	P7-26	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
Yiguang-1	YG-1	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Yiguang-3	YG-3	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Yiguang-4	YG-4	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Yitexuan1	YTX1	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Yitexuan2	YTX2	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Guyou1	GY1	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Guixie1	GX1	Guizhou	*T. timopheevi* Zhuk. /*Av. fatua* L. var. Portugal	Line
Guixie3	GX3	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Guixie3-1	GX3-1	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Guixie4	GX4	Guizhou	*T. turgidum* var. *dicoccoides* / *Av. fatua* L.	Line
Guixie5	GX5	Guizhou	*T. turgidum* var. *dicoccoides*/ *Av. fatua* L.	Line
P13-1	P13-1	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P13-3	P13-3	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P13-4	P13-4	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line
P13-5	P13-5	Guizhou	Selection from mutation of *T. timopheevi* Zhuk.	Line

Table 3.1 (Continued) Information of wheat cultivars and lines in this study.

Name	Abb.	Origin	Pedigrees	Types
Anmai7	AM7	Guizhou	Sanshumai/8625	Cultivar
Bi2007-1	B2007-1	Guizhou	n.a.	Line
Bi2007-7	B2007-7	Guizhou	n.a.	Line
Bimai18	BM18	Guizhou	Bi89-2/Guinong93-4//Guinong93-4	Cultivar
Fengyou1	FY1	Guizhou	n.a.	Cultivar
Fengyou2	FY2	Guizhou	00435///S124//Sumai3/Wumang1	Cultivar
Fengyou3	FY3	Guizhou	8619-3-1/8524-1-6	Cultivar
Fengyou6	FY6	Guizhou	9106-1-8/9303-9-6	Cultivar
Fengyou7	FY7	Guizhou	87111/90225	Cultivar
Fengyou8	FY8	Guizhou	Xinong1376/Tedasui	Cultivar
Fengyou9	FY9	Guizhou	8619-3-1/8729-6-2	Cultivar
Fengyou10	FY10	Guizhou	Yun35334-3/Fengyou3	Cultivar
Fengyou92212	FY92212	Guizhou	n. a.	Line
Xingyu7	XY7	Guizhou	n. a.	Cultivar
Xingyu823	XY823	Guizhou	n. a.	Line
Mianmai1403	MM1403	Sichuan	Mianyang04854/Guinong21-1	Cultivar
Mianmai185	MM185	Sichuan	Mianyang96-5/Liaochun10	Cultivar
Mianmai228	MM228	Sichuan	1275-1/Nei2938//99-1522	Cultivar
Mianmai367	MM367	Sichuan	1275-1/99-1522	Cultivar
Mianmai37	MM37	Sichuan	96EW37/Mianyang90-100	Cultivar
Mianmai39	MM39	Sichuan	Mianyang96-78/Guinong21-1	Cultivar
Mianmai43	MM43	Sichuan	Miangyang92-8/88-304//Guinong19-4	Cultivar
Mianmai45	MM45	Sichuan	07146-12-1/Guinong19-4	Cultivar
Mianmai46	MM46	Sichuan	07242-3-1-1/Guinong21	Cultivar
Mianmai47	MM47	Sichuan	Mianyang96-5/Guinong19-4	Cultivar
Mianmai48	MM48	Sichuan	Mianyang01821/Guinong19-4	Cultivar
Miannong4	MN4	Sichuan	(75-21-4/76-19)F4//(Mianyang11/Alondras)F3	Cultivar
MY2002-5	MY2002-5	Sichuan	Mianyang01821/Guinong19-4	Line
Mianyang26	MY26	Sichuan	Mianyang81-5/Chuanyu81-24	Cultivar
Mianmai38	MM38	Sichuan	07146-12-1/Guinong19-4	Cultivar
Mianmai40	MM40	Sichuan	Mianyang01821/Guinong19-4	Cultivar

Table 3.1 (Continued) Information of wheat cultivars and lines in this study.

Name	Abb.	Origin	Pedigrees	Types
Mianmai42	MM42	Sichuan	Mianyang96-5/Guinong21-1	Cultivar
Guohaomai15	GHM15	Sichuan	Mianyang96-5/Guinong19-4//NE	Cultivar
Xikemai2	XKM2	Sichuan	Chuanyu11/Mo444xuan	Cultivar
Xikemai3	XKM3	Sichuan	Guinong21/5575	Cultivar
Xikemai5	XKM5	Sichuan	Guinong21/96 II -39	Cultivar
Chuanmai107	CM107	Sichuan	2469/80－28－7	Cultivar
Chuanmai16	CM16	Sichuan	n. a.	Cultivar
Chuanmai30	CM30	Sichuan	77/YAA//ALD'S'/3/YSZ//ST2022/983	Cultivar
Chuanmai39	CM39	Sichuan	Mo444/90-7	Cultivar
Chuanmai42	CM42	Sichuan	91T4135×88Fan8	Cultivar
Chuanmai44	CM44	Sichuan	96Xia440/Guinong21	Cultivar
Chuanmai50	CM50	Sichuan	Guinong21/3295	Cultivar
Chuanmai60	CM60	Sichuan	98-1231//Guinong21/Shenghe3295	Cultivar
Chuanmai45	CM45	Sichuan	GH430/SW1862	Cultivar
Chuanmai51	CM51	Sichuan	174/183//99-1572	Cultivar
Chuannong10	CAU10	Sichuan	78-5038/85-D.H.5015	Cultivar
Chuannong17	CAU17	Sichuan	91S-23/A302	Cultivar
Chuannong18	CAU18	Sichuan	Chuanyu12/87-429	Cultivar
Chuannong19	CAU19	Sichuan	91S-23/A302	Cultivar
Chuannong23	CAU23	Sichuan	R1685/MY26	Cultivar
Chuannong27	CAU27	Sichuan	Chuannong19/R3301	Cultivar
CN04-1	CN04-1	Sichuan	N1491/N1071	Line
CN04-2	CN04-2	Sichuan	N1491/N1071	Line
SY95-71	SY96-71	Sichuan	Eronga83/Fan6//Fan6	Line
Huaimai18	HM18	Jiangsu	Yumai13/Lumai14	Cultivar
92R137	92R137	Jiangsu	6AL.6VS translocation lines /Yangmai5	Line
NAU24	NAU24	Jiangsu	selection from T6AL.6VS lines	Line
NAU04y10	NAU04y10	Jiangsu	selection from T6AL.6VS lines	Line
NAU06y603	NAU06y603	Jiangsu	selection from T6AL.6VS lines	Line
Ermai15	EM15	Hubei	882-852//Een1 /Nppp-2///Guinong11	Cultivar
Xiangmai27	XM27	Hubei	84C-432/Guinong31-1//Guinong20-3///Fengyou5 ////Zhefeng2	Line

Table 3.1 (Continued) Information of wheat cultivars and lines in this study.

Name	Abb.	Origin	Pedigrees	Types
Xiangmai55	XM55	Hubei	8811/Guinong24-7//Emai19	Cultivar
Xiangmai83	XM83	Hubei	Guinong20-3/6038//Zhongyou9803	Line
Zheng9023	Z9023	Henan	Xinong881/Shan213	Cultivar
Yanyu898	YY898	Henan	Guinong25-8/Yumai18	Cultivar
05Zhong38	05Z38	Henan	n. a.	Line
Zhong91-13	Z91-13	Beijing	n. a.	Line
Zhongyou9507	ZY9507	Beijing	Selection from Zhongyou8	Cultivar
Zhongmai415	ZM415	Beijing	Guinong11/Jing411//Jing411	Cultivar
CA9722	CA9722	Beijing	Jing411/Guinong11//Jing411	Cultivar
Mingxian169	MX169	Shanxi	n. a.	Cultivar
Xianyan54	XY54	Shaanxi	Selection from Xiaoyan6	Cultivar
Linmai33	LM33	Gansu	92Yuan-11/Guinong20	Cultivar
Linmai34	LM34	Gansu	94Xuan4149/Guinong20//82316-1/Linmai26	Cultivar
Longjian9343	LJ9343	Gansu	Guinong21/77-69	Cultivar
Tianxuan43	TX43	Gansu	8845-1-1-1-1/Guinong22	Cultivar
Zhongzhi2	ZZ2	Gansu	Shan167, Guinong22 and *T. Spelta album* multiple cross	Cultivar

Table 3.2 Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars/ lines	Guiyang (2013)	Hezhang (2013)	Guiyang (2014)	Mianyang (2014)	*Yr9*	*Yr10*	*Yr18*	*Yr26*	*Yr29*
0308	0;	0	0;	0;				+	
9712	0;	4	0;	3				+	
Guimai12	3	2	1	2	+		+		
Guiyu17	0;	0	0;	3				+	
Qianmai12	0;	0	0;	3				+	
Qianmai14	4	4	4	4	+				
Qianmai15	0;	2	0;	2	+		+		
Qianmai16	4	4	1	2					
Qianmai17	3	4	3	3	+				
Qianmai18	0;	4	0;	1				+	
Qianmai19	0;	0	0;	2				+	
Xiafan28	0;	0	0;	3				+	
Qian0117-1	3	0	0;	3		+		+	
QianAT6-5	0;	0	0;	1				+	
Qian9939-5	0;	0	0;	2					
Qian9988-46	0;	0	0;	2				+	
Qian2032-8	4	4	4	4	+				
Qian2040-2	0;	0	0;	1				+	
Qian0209-4	0;	0	0;	2				+	
Guinong18	0;	0	0;	0				+	
Guinong19	0;	0	0;	0;			Na	+	
Guinong25	0;	0	0;	1				+	
Guinong26	0;	0	1	1	+			+	
Guinong28	0;	0	0;	0;				+	
Guinong19-4	0;	Na	0;	1	+				
Guinong21	1	1	0;	2	+				
Guinong21-1	4	2	2	1	+			+	
Guinong22	0;	0	0;	2				+	
Guinong775	0;	0	0;	2				+	

Table 3.2 (Continued) Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars/ lines	Guiyang (2013)	Hezhang (2013)	Guiyang (2014)	Mianyang (2014)	*Yr9*	*Yr10*	*Yr18*	*Yr26*	*Yr29*
Baimian3	4	3	3	3					
JYP1	4	3	4	4	+				
JYP2	4	3	4	4	+				
JYP 3	0;	0	0;	1				+	
TG	0;	0	0;	1				+	
TP2	0;	0	0;	1				+	
TP3	0;	0	0;	0;	+			+	
YP1	4	3	4	3	+				
YP2	3	3	3	3	+				
YLP8-1	3	3	1	2	+				
YLP8-40	4	3	0;	4	+				
P1-2	0;	0	0;	2				+	
P3M-8	0;	0	0;	1			+	+	
P5-1	0;	0	1	3				+	+
P7-9	0;	0	0;	1				+	+
P7-26	0;	0	0;	2				+	+
Yiguang-1	4	0	2	4	+	+			+
Yiguang-3	0;	0	1	3			+	+	+
Yiguang-4	0;	0	0;	4	+	+		+	+
YTX1	0;	0	0;	1				+	+
YTX2	0;	0	1	2				+	+
Guyou1	0;	0	0;	1				+	+
Guixie1	0;	0	0;	0;				+	+
Guixie3	0;	0	0;	0;		+			
Guixie3-1	0;	3	3	3					
Guixie4	0;	0	0;	1				+	
Guixie5	0;	0	0;	2			Na	+	
P13-1	0;	0	0;	1				+	

Table 3.2 (Continued) Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars / lines	Guiyang (2013)	Hezhang (2013)	Guiyang (2014)	Mianyang (2014)	*Yr9*	*Yr10*	*Yr18*	*Yr26*	*Yr29*
P13-3	0;	0	0;	2					
P13-4	0;	0	0;	1		+		+	
P13-5	0;	0	0;	1				+	
Anmai7	2	2	0;	0;					
Bi2007-1	3	3	0;	2	+				
Bi2007-7	3	4	1	2					+
Bimai18	2	3	0;	2	+				
Fengyou1	0;	1	0;	0;	+				
Fengyou2	4	4	3	2	+				
Fengyou3	4	4	3	3	+				
Fengyou6	0;	3	0;	2	+				
Fengyou7	2	2	0;	1					
Fengyou8	3	3	0;	4				+	
Fengyou9	3	3	2	3					+
Fengyou10	0;	0	0;	4				+	
Fengyou92212	4	4	4	4	+				
Xingyu7	3	4	4	3	+				+
Xingyu823	4	4	0;	3				+	
Mianmai1403	2	2	1	0;	+				
Mianmai185	0;	0	1	4				+	
Mianmai228	0;	0	0;	3					
Mianmai367	0;	0	2	1				+	
Mianmai37	0;	0	1	4				+	
Mianmai39	0;	0	2	4				+	
Mianmai41	3	3	0;	3	+				
Mianmai43	0;	0	1	0;	+				
Mianmai45	3	0	2	1	+				
Mianmai46	0;	0	0;	4					

Table 3.2 (Continued) Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars/ lines	**Guiyang (2013)**	**Hezhang (2013)**	**Guiyang (2014)**	**Mianyang (2014)**	***Yr9***	***Yr10***	***Yr18***	***Yr26***	***Yr29***
Mianmai47	3	2	2	2	+				
Mianmai48	3	0	0;	Na	+			+	
Miannong4	4	4	4	4					
MY2002-5	4	3	2	3					
Mianyang26	4	4	4	4					
Mianmai8	2	Na	1	3	+				
Mianmai40	3	Na	1	2					
Mianmai42	0;	Na	3	4	+				
GHM15	1	Na	2	2	+				
Xikemai2	4	Na	0;	1	+				+
Xikemai3	0;	Na	0;	4	+				
Xikemai5	0;	Na	0;	3				+	
Chuanmai107	3	2	2	1	+				
Chuanmai16	3	3	3	2	+				
Chuanmai30	4	4	3	2	+				
Chuanmai39	3	0	0;	3					
Chuanmai41	4	3	1	3					
Chuanmai44	0;	Na	1	4	+			+	
Chuanmai50	0;	Na	0;	4	+			+	
Chuanmai60	0;	Na	0;	3				+	+
Chuanmai45	2	0	2	1	+				
Chuanmai51	3	2	1	4					
Chuannong10	4	3	3	2	+				
Chuannong17	4	3	1	1					
Chuannong18	3	2	2	2	+				
Chuannong19	3	0	2	3					
Chuannong23	3	0	1	2			Na		
Chuannong27	3	2	0;	1					

Table 3.2 (Continued) Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars/ lines	Guiyang (2013)	Hezhang (2013)	Guiyang (2014)	Mianyang (2014)	*Yr*9	*Yr*10	*Yr* 18	*Yr*26	*Yr*29
CN04-1	1	2	0;	1	+				
CN04-2	3	3	3	4	+				
SY95-71	4	4	4	4					
Chinese Spring	1	0	0;	0;			+		
Huaimai18	3	4	2	3		+	+		
92R137	0;	0	0;	2				+	
NAU24	4	3	3	4	+				
NAU04Y10	3	3	1	2	+		+		
NAU06Y603	4	2	4	4					+
Emai15	4	Na	1	2	+				
Xiangmai27	4	Na	1	2		+			
Xiangmai55	3	Na	1	1					
Xiangmai83	4	Na	1	2		+			
Zheng9023	3	Na	2	2		+			
Yanyu898	4	Na	4	3					
05Zhong38	3	3	1	1	+				
Zhong91-13	3	1	1	3				+	
Zhongyou9507	3	4	4	2					
Zhongmai415	4	Na	4	3					
CA9722	4	Na	2	3					
Mingxian169	3	4	4	4			+		
Xiaoyan54	4	4	4	4		+			
Linmai33	0;	Na	0;	2	+			+	
Linmai34	0;	Na	2	4				+	+
Longjian9343	0;	Na	0;	3				+	

Table 3.2 (Continued) Stripe rust response of 140 wheat cultivars (lines) in 4 environments and screening results for resistance genes by molecular markers

Cultivars / lines	Guiyang (2013)	Hezhang (2013)	Guiyang (2014)	Mianyang (2014)	Yr9	Yr10	Yr18	Yr26	Yr29
Tianxuan43	0;	Na	0;	1			+	+	
Zhongzhi2	0;	Na	0;	3	+				

Note: Infection type to stripe rust for all cultivars were graded as 0, 0;, 1, 2, 3 and 4 with 0 for no visible uredia, 0; for small chlorotic flecks without sporulation, 1 for chlorosis and necrosis associated with extremely limited uredial development, 2 for chlorosis and necrosis with little intermediate sporulation, 3 for chlorosis with increased uredial development and 4 for abundant sporulation without chlorosis. Na and + mean missing data and positive alleles at the resistant loci, respectively.

Table 5.1 Seedling response of 140 wheat cultivar (lines) to the *Pst* races CYR32

Cultivar	IT	Cultivar	IT	Cultivar	IT	Cultivar	IT
AM7	3	XY823	0;	CM45	4	YTX2	1
B2007-1	1	CN04-1	3	CM51	0;	GY1	0;
B2007-7	3	CN04-2	4	05Z38	4	GX1	0;
BM18	3	HM18	4	Z91-13	0;	GX3	3
0308	0;	92R137	0;	ZY9507	4	GX3-1	0;
9712	1	NAU24	3	GN18	0;	GX4	0;
MX169	-	NAU04y10	4	GN19	0;	GX5	0;
GM12	-	NAU06y603	3	GN25	0;	P13-1	1
GY17	0;	XY54	3	GN26	0;	P13-3	1
QM12	0;	MM1403	3	GN28	0;	P13-4	1
QM14	-	MM185	0;	BM3	4	P13-5	0;
QM15	1	MM228	0	GN21	4	ZM415	4
SY95-71	-	MM367	0;	GN21-1	1	CA9722	4
QM16	3	MM37	0;	GN22	0;	YY898	3
QM17	1	MM39	0;	GN775	0;	EM15	3
QM18	1	MM41	0;	JYP1	4	XM27	4
QM19	0;	MM43	3	JYP2	3	XM55	4
QXF28	1	MM45	4	JYP3	0;	XM83	4
Q0117-1	1	MM46	0;	TG	0;	XKM2	4
QAT6-5	1	MM47	3	TP2	0;	XKM3	0;
Q9939-5	0;	MM48	0;	TP3	0;	Z9023	4
Q9988-46	0;	MN4	4	YP1	4	XKM5	0;
Q2032-8	3	MY2002-5	4	YP2	4	CM44	1
Q0240-2	1	MY26	4	YLP8-1	4	CM50	1
Q0209-4	0;	CAU10	4	YLP8-40	4	CM60	0;
FY1	3	CAU17	4	P1-2	0;	MM38	4
FY2	3	CAU18	4	P3M-8	0;	MM40	4
FY3	3	CAU19	4	P5-1	0;	MM42	1
FY6	3	CAU23	0;	P7-2	0;	GHM15	4
FY7	3	CAU27	4	P7-26	0;	LM33	0;

Table 5.1 (Continued) Seedling response of 140 wheat cultivar (lines) to the *Pst* races CYR32

Cultivar	IT	Cultivar	IT	Cultivar	IT	Cultivar	IT
FY8	1	CM107	3	YG-1	3	LM34	0;
FY9	3	CM16	4	YG-3	0;	LJ9343	0;
FY10	0;	CM30	4	YG-4	0;	TX43	0;
FY92212	4	CM39	4	CS	4	ZZ2	4
XY7	3	CM41	4	YTX1	1	GN19-4	4

Note: Infection type to stripe rust was graded as 0, 0;, 1, 2, 3 and 4 . The symbol '-' means missing data.

Table 5.2 Disease severity of 140 wheat cultivar (lines) to the stripe rust at 4 environments

Cultivars	DS (%)				Cultivars	DS (%)			
	GY13	HZ13	GY14	MY14		GY13	HZ13	GY14	MY14
AM7	4.9	3.8	0.0	2.3	CM45	6.7	0.0	5.0	5.0
B2007-1	7.9	5.0	0.0	10.1	CM51	14.2	5.0	0.0	80.0
B2007-7	26.7	37.7	3.7	31.6	05Z38	29.6	14.2	0.0	9.1
BM18	7.6	3.7	1.0	10.1	Z91-13	17.2	1.0	5.0	62.6
0308	0.0	0.0	0.0	0.0	ZY9507	63.5	12.8	27.6	14.8
9712	0.0	100.0	0.0	26.8	GN18	19.3	0.0	0.0	0.0
MX169	-	-	-	74.7	GN19	17.1	0.0	0.0	1.0
GM12	8.5	5.0	0.0	8.0	GN25	0.0	0.0	1.0	1.7
GY17	3.0	0.0	0.0	29.9	GN26	0.0	0.0	5.0	4.0
QM12	10.2	0.0	0.0	64.0	GN28	0.0	0.0	0.0	3.0
QM14	90.0	70.0	60.0	90.6	BM3	69.9	35.8	37.4	13.3
QM15	3.2	4.0	0.0	23.0	GN21	4.2	3.0	0.0	9.9
SY95-71	100.0	100.0	83.3	84.7	GN21-1	25.2	7.0	28.1	2.2
QM16	24.0	27.7	22.5	11.4	GN22	4.6	0.0	0.0	17.1
QM17	4.4	100.0	48.2	62.2	GN775	2.2	0.0	0.0	28.9
QM18	22.7	40.0	0.0	1.0	JYP1	58.0	35.2	83.1	76.7
QM19	0.0	0.0	0.0	11.4	JYP2	67.3	21.0	78.9	35.6
QXF28	12.7	0.0	0.0	36.1	JYP3	3.3	0.0	0.0	5.9
Q0117-1	21.9	0.0	0.0	42.7	TG	0.0	0.0	0.0	3.7
QAT6-5	0.0	0.0	0.0	1.4	TP2	0.0	0.0	0.0	2.3
Q9939-5	0.0	0.0	0.0	10.1	TP3	0.0	0.0	0.0	0.0
Q9988-46	7.9	0.0	0.0	8.8	YP1	78.7	16.0	26.6	80.9
Q2032-8	48.7	37.5	41.4	69.2	YP2	58.8	31.7	10.2	45.3
Q0240-2	0.0	0.0	0.0	5.4	YLP8-1	15.0	7.5	0.0	15.8
Q0209-4	0.0	0.0	0.0	14.0	YLP8-40	30.7	35.0	1.0	84.3
FY1	12.8	1.0	0.0	1.0	P1-2	0.0	0.0	0.0	10.3
FY2	80.7	44.2	42.8	19.3	P3M-8	0.0	0.0	0.0	3.7
FY3	73.5	36.7	43.4	25.9	P5-1	2.3	0.0	7.3	6.8

Table 5.2 (Continued) Disease severity of 140 wheat cultivar (lines) to the stripe rust at 4 environments

Cultivars	DS (%)				Cultivars	DS (%)			
	GY13	HZ13	GY14	MY14		GY13	HZ13	GY14	MY14
FY6	21.5	9.2	0.0	6.5	P7-2	0.0	0.0	0.0	2.4
FY7	4.3	1.0	0.0	2.1	P7-26	0.3	0.0	0.0	13.8
FY8	28.7	21.7	0.0	77.9	YG-1	46.7	0.0	34.7	87.6
FY9	20.0	45.0	1.0	24.3	YG-3	0.0	0.0	5.0	34.1
FY10	4.2	0.0	0.0	88.0	YG-4	0.0	0.0	0.0	89.3
FY92212	77.3	66.7	78.0	73.2	CS	2.0	0.0	0.0	1.0
XY7	56.5	53.3	54.3	18.2	YTX1	0.0	0.0	0.0	5.6
XY823	24.7	66.7	0.0	46.5	YTX2	0.0	0.0	1.0	5.1
CN04-1	28.3	5.3	0.0	6.4	GY1	0.0	0.0	0.0	5.8
CN04-2	8.2	11.0	26.0	31.3	GX1	0.0	0.0	0.0	1.3
HM18	54.0	21.7	7.1	35.8	GX3	0.0	0.0	0.0	0.0
92R137	18.0	0.0	0.0	9.9	GX3-1	27.3	19.2	45.8	88.3
NAU24	87.3	40.8	57.1	53.7	GX4	0.0	0.0	0.0	2.3
NAU04y10	38.8	26.7	5.5	6.5	GX5	0.0	0.0	0.0	7.8
NAU06y603	79.2	17.5	42.1	79.3	P13-1	0.0	0.0	0.0	5.7
XY54	51.7	13.3	82.2	50.5	P13-3	0.0	0.0	0.0	9.0
MM1403	20.5	7.0	1.0	0.0	P13-4	0.0	0.0	0.0	6.0
MM185	1.2	0.0	1.0	92.5	P13-5	0.0	0.0	0.0	10.1
MM228	0.0	0.0	0.0	83.3	ZM415	80.0	-	44.8	27.2
MM367	0.0	0.0	10.0	4.9	CA9722	55.3	-	7.0	40.4
MM37	0.0	0.0	1.0	90.4	YY898	82.0	-	68.9	62.1
MM39	0.0	0.0	5.0	87.3	EM15	39.3	-	4.4	16.1
MM41	7.7	17.5	1.0	26.4	XM27	79.7	-	9.0	12.0
MM43	1.4	0.0	5.0	1.0	XM55	44.2	-	3.7	5.3
MM45	6.9	0.0	1.0	3.4	XM83	52.7	-	10.1	15.1
MM46	2.6	0.0	0.0	92.2	XKM2	8.3	-	0.0	4.5
MM47	20.5	6.2	5.0	6.1	XKM3	0.0	-	20.0	87.6
MM48	19.8	0.0	0.0	0.0	Z9023	53.0	-	5.2	19.1

Table 5.2 (Continued) Disease severity of 140 wheat cultivar (lines) to the stripe rust at 4 environments

Cultivars	DS (%)				Cultivars	DS (%)			
	GY13	HZ13	GY14	MY14		GY13	HZ13	GY14	MY14
MN4	75.0	46.7	57.1	72.4	XKM5	0.0	-	0.0	56.9
MY2002-5	43.7	17.5	5.5	9.8	CM44	0.0	-	2.3	66.0
MY26	63.2	57.5	22.0	73.3	CM50	0.0	-	0.0	70.3
CAU10	51.7	29.2	23.2	51.2	CM60	0.0	-	0.0	36.6
CAU17	54.7	8.5	9.3	5.3	MM38	4.2	-	0.0	31.5
CAU18	18.5	5.3	10.0	7.6	MM40	11.0	-	5.0	5.5
CAU19	18.7	0.0	0.0	6.2	MM42	0.0	-	0.0	89.3
CAU23	14.1	0.0	0.0	7.0	GHM15	3.8	-	8.3	7.8
CAU27	5.6	10.0	0.0	4.7	LM33	6.7	-	0.0	17.1
CM107	16.9	10.0	5.5	6.6	LM34	11.1	-	0.0	72.2
CM16	18.7	32.5	10.8	19.3	LJ9343	0.0	-	0.0	30.7
CM30	55.8	38.3	45.4	15.2	TX43	0.0	-	0.0	1.8
CM39	25.4	0.0	0.0	32.2	ZZ2	6.7	-	0.0	20.1
CM41	9.5	7.5	2.3	35.8	GN19-4	7.8	-	0.0	7.1

Note: The symbol '-' means missing data. GY13, HZ13, GY14 and MY14 mean the field test at Guiyang and Hezhang site in the year 2013, at Guiyang and Mianyang site in the year 2014.

Table 5.3 Number of markers associated with stripe rust response in 5 tests by AM with SFA model and infection type data

Chromo.	CYR32	GY13	GY14	HZ13	MY14	SUM
1A	6	2		2		10
1B	156	82	3	34		275
1D	12	5		2		19
2A	17	10				27
2B	4	3				7
2D	1					1
3A	1	6				7
3B	11	18		4		33
3D	4	8				12
4A	7	13				20
4B	7	7		1		15
4D	3	4		2	1	10
5A	1	1	1			3
5B	1	2				3
5D	1	1	1			3
6A	252	245	1	135	1	634
6B	13	14		1		28
6D	14	13		2		29
7A	3	1				4
7B	6	6		2		14
7D		1				1
SUM	520	442	6	185	2	

Table 5.4 Number or ratio of markers simultaneously detected with association with stripe rust response in 1 to 4 tests by AM with SFA model and infection type data.

Chromo.	Number of markers (ratio)				
	1 test	**2 tests**	**3 tests**	**4 tests**	**all**
1A	4 (0.02)		2 (0.01)		6 (0.03)
2A	25 (0.07)	1 (0.00)			26 (0.07)
3A	5 (0.03)	1 (0.01)			6 (0.03)
4A	10 (0.05)	5 (0.02)			15 (0.07)
5A	3 (0.03)				3 (0.03)
6A	16 (0.03)	111 (0.24)	132 (0.29)		259 (0.56)
7A	4 (0.01)				4 (0.01)
1B	93 (0.26)	39 (0.11)	32 (0.09)	2 (0.01)	166 (0.46)
2B	7 (0.01)				7 (0.01)
3B	19 (0.05)	1 (0.00)	4 (0.01)		24 (0.06)
4B	4 (0.03)	4 (0.03)	1 (0.01)		9 (0.06)
5B	3 (0.01)				3 (0.01)
6B	2 (0.01)	13 (0.07)			15 (0.08)
7B	4 (0.02)	2 (0.01)	2 (0.01)		8 (0.03)
1D	7 (0.05)	3 (0.02)	2 (0.01)		12 (0.08)
2D	1 (0.00)				1 (0.00)
3D	4 (0.04)	4 (0.04)			8 (0.08)
4D	4 (0.05)		2 (0.02)		6 (0.07)
5D	1 (0.01)	1 (0.01)			2 (0.01)
6D	1 (0.01)	11 (0.06)	2 (0.01)		14 (0.07)
7D	1 (0.01)				1 (0.01)
Total	218	196	179	2	595

Table 5.5 The first five principal component (PC) used in AM to estimate the population structure

Cultivars	PC1	PC2	PC3	PC4	PC5	Cultivars	PC1	PC2	PC3	PC4	PC5
AM7	0.137	0.072	0.057	0.098	0.006	CM45	-0.152	-0.036	-0.127	-0.046	-0.031
B2007-1	-0.210	0.082	0.039	0.195	0.021	CM51	-0.105	-0.086	-0.094	-0.060	-0.004
B2007-7	-0.094	0.052	0.017	0.032	0.123	05Z38	-0.109	0.046	-0.015	-0.062	0.016
BM18	-0.187	0.180	0.058	0.141	-0.055	Z91-13	-0.090	-0.042	0.034	-0.088	0.047
0308	0.235	0.074	0.032	-0.052	-0.075	ZY9507	-0.089	0.057	0.021	-0.056	0.090
9712	0.193	-0.081	0.088	0.026	-0.046	GN18	0.191	-0.134	0.021	-0.018	-0.037
MX169	-0.099	0.000	-0.035	-0.070	0.091	GN19	0.121	0.039	-0.023	0.014	-0.013
GM12	-0.099	0.033	0.125	-0.014	-0.047	GN25	0.270	0.105	0.043	0.021	-0.054
GY17	0.135	-0.166	0.074	0.043	-0.040	GN26	0.244	0.084	-0.013	-0.002	-0.088
QM12	0.159	-0.101	0.114	0.046	-0.012	GN28	0.277	0.109	0.039	0.018	-0.052
QM14	-0.142	0.079	0.017	-0.046	-0.056	BM3	-0.065	0.095	0.019	-0.063	-0.009
QM15	-0.135	0.147	0.120	-0.021	-0.089	GN21	-0.221	0.137	0.017	0.259	0.054
SY95-71	-0.065	0.016	0.035	-0.110	0.026	GN21-1	-0.037	-0.035	0.005	-0.061	0.040
QM16	-0.091	0.032	0.121	-0.054	0.088	GN22	0.208	-0.170	0.089	0.031	-0.045
QM17	-0.097	0.014	0.079	-0.050	0.009	GN775	0.146	-0.098	0.140	0.172	0.084
QM18	0.239	0.080	0.025	-0.048	-0.076	JYP1	-0.086	0.050	0.081	-0.040	0.009
QM19	0.167	-0.139	0.106	0.063	0.070	JYP2	-0.104	0.081	0.126	-0.064	-0.047
QXF28	0.196	-0.124	0.102	0.009	0.017	JYP3	0.175	-0.102	0.137	0.146	0.088

Table 5.5 (Continued) The first five principal component (PC) used in AM to estimate the population structure

Cultivars	PC1	PC2	PC3	PC4	PC5	Cultivars	PC1	PC2	PC3	PC4	PC5
Q0117-1	0.120	-0.071	0.087	0.019	0.080	TG	0.324	0.103	0.003	0.001	-0.087
QAT6-5	0.185	0.020	0.093	-0.020	-0.041	TP2	0.308	0.128	-0.002	0.015	-0.068
Q9939-5	0.170	-0.146	0.112	0.053	0.069	TP3	0.268	0.075	-0.008	-0.012	-0.079
Q9988-46	0.163	-0.078	0.142	0.124	0.066	YP1	-0.099	0.074	0.056	-0.076	-0.121
Q2032-8	-0.119	-0.083	-0.054	-0.075	-0.125	YP2	-0.119	0.096	0.077	-0.045	-0.106
Q0240-2	0.216	-0.039	-0.004	0.014	-0.042	YLP8-1	-0.117	0.013	0.052	-0.042	0.002
Q0209-4	0.089	-0.041	0.129	0.102	0.069	YLP8-40	0.224	0.050	0.050	0.060	0.011
FY1	-0.177	0.134	0.135	0.072	-0.020	P1-2	0.246	0.130	-0.157	0.016	0.065
FY2	-0.113	0.052	-0.001	-0.070	-0.085	P3M-8	-0.060	-0.020	0.098	-0.022	0.078
FY3	-0.138	0.073	-0.003	-0.073	-0.125	P5-1	-0.061	-0.029	0.088	-0.029	0.071
FY6	-0.182	0.129	0.138	0.074	-0.019	P7-2	0.030	0.119	-0.106	-0.103	0.146
FY7	-0.118	0.035	-0.063	-0.096	0.025	YG-1	0.182	0.081	-0.135	-0.050	0.143
FY8	0.152	-0.073	0.087	-0.023	-0.006	YG-3	-0.089	-0.007	-0.043	-0.066	0.112
FY9	-0.090	0.042	0.102	-0.061	0.085	YG-4	0.246	0.129	-0.159	0.015	0.064
FY10	0.139	-0.083	0.102	-0.005	-0.033	CS	0.249	0.131	-0.159	0.013	0.063
FY92212	-0.124	0.151	-0.010	-0.001	-0.060	YTX1	0.278	0.154	-0.177	0.012	0.067
XY7	-0.127	-0.011	-0.070	-0.058	-0.066	YTX2	0.484	0.243	-0.007	0.051	-0.167
XY823	0.051	-0.081	0.093	-0.026	0.013	GY1	-0.110	0.023	0.016	-0.019	0.122

Table 5.5 (Continued) The first five principal component (PC) used in AM to estimate the population structure

Cultivars	PC1	PC2	PC3	PC4	PC5	Cultivars	PC1	PC2	PC3	PC4	PC5
CN04-1	-0.212	0.055	-0.025	0.089	-0.052	GX1	0.176	0.056	-0.042	0.025	0.095
CN04-2	-0.213	0.021	-0.032	0.021	-0.076	GX3	0.308	0.099	0.004	-0.001	-0.080
HM18	-0.079	-0.005	-0.017	-0.064	0.054	GX3-1	0.218	0.135	-0.157	-0.001	0.073
92R137	0.202	-0.168	0.090	0.028	-0.048	GX4	0.256	0.136	-0.165	0.012	0.062
NAU24	0.037	-0.073	-0.034	0.038	-0.067	GX5	0.260	0.136	-0.167	0.014	0.070
NAU04y10	-0.143	0.110	0.019	-0.066	-0.042	P13-1	-0.080	-0.005	-0.003	-0.069	0.004
NAU06y603	0.052	-0.127	0.067	-0.001	0.001	P13-3	0.257	0.136	-0.167	0.018	0.071
XY54	-0.083	-0.008	-0.027	-0.076	0.093	P13-4	-0.052	0.085	0.068	-0.125	0.026
MM1403	-0.249	0.013	-0.039	0.190	-0.024	P13-5	-0.055	0.068	0.039	-0.119	0.047
MM185	0.124	-0.184	-0.095	0.012	-0.031	ZM415	-0.100	-0.042	0.048	-0.012	0.055
MM228	0.084	-0.152	-0.144	0.011	-0.048	CA9722	-0.116	0.092	0.039	-0.039	-0.052
MM367	0.090	-0.201	-0.110	0.018	-0.016	YY898	-0.038	0.007	0.064	-0.144	0.131
MM37	0.125	-0.225	-0.034	0.017	-0.055	EM15	-0.062	-0.020	-0.007	-0.068	0.018
MM39	0.135	-0.122	-0.030	0.044	-0.057	XM27	-0.034	-0.004	0.069	-0.145	0.132
MM41	-0.129	-0.092	-0.112	0.117	-0.046	XM55	-0.127	0.007	-0.022	-0.055	-0.020
MM43	-0.145	-0.007	-0.028	0.147	-0.050	XM83	0.057	-0.218	-0.153	0.042	-0.043
MM45	-0.245	0.024	-0.044	0.194	-0.015	XKM2	-0.037	0.006	0.060	-0.144	0.128
MM46	-0.069	-0.115	-0.095	-0.064	-0.022	XKM3	0.066	-0.205	-0.126	0.055	-0.057

Table 5.5 (Continued) The first five principal component (PC) used in AM to estimate the population structure

Cultivars	PC1	PC2	PC3	PC4	PC5	Cultivars	PC1	PC2	PC3	PC4	PC5
MM47	-0.254	-0.003	-0.102	0.134	-0.006	Z9023	-0.055	-0.083	-0.065	-0.023	0.028
MM48	-0.071	-0.113	-0.130	-0.055	0.005	XKM5	-0.092	-0.124	-0.089	-0.050	0.010
MN4	-0.125	-0.118	-0.179	-0.043	-0.002	CM44	-0.103	-0.097	-0.036	-0.018	-0.035
MY2002-5	-0.206	0.000	-0.096	0.168	0.058	CM50	-0.255	0.003	-0.053	0.173	-0.008
MY26	-0.111	-0.132	-0.173	-0.043	0.012	CM60	-0.208	-0.013	-0.086	0.147	0.057
CAU10	-0.066	-0.118	-0.105	0.013	-0.074	MM38	0.210	-0.146	0.013	0.009	-0.052
CAU17	-0.175	0.036	-0.023	-0.067	-0.169	MM40	-0.251	-0.004	-0.102	0.137	-0.007
CAU18	-0.167	0.042	-0.025	-0.071	-0.182	MM42	-0.032	-0.005	0.044	-0.020	0.065
CAU19	-0.130	-0.050	-0.007	-0.021	0.076	GHM15	-0.046	-0.002	0.043	-0.072	0.010
CAU23	-0.061	-0.084	0.014	-0.066	-0.049	LM33	0.011	-0.042	0.112	-0.023	0.034
CAU27	-0.144	-0.044	-0.100	-0.060	0.066	LM34	-0.053	-0.018	0.041	-0.016	0.091
CM107	-0.170	0.039	-0.022	-0.067	-0.170	LJ9343	-0.042	-0.051	0.119	-0.010	-0.005
CM16	-0.167	-0.048	-0.019	-0.037	-0.090	TX43	-0.216	0.126	0.018	0.251	0.051
CM30	-0.105	-0.018	0.008	-0.116	-0.023	ZZ2	-0.142	0.019	0.039	0.069	0.055
CM39	-0.095	0.019	0.031	-0.107	0.010	GN19-4	0.043	0.042	0.062	0.031	-0.026

Table 5.6 LD squared correlation coefficients (R^2) between markers pairs on the same chromosome

		1BL		**3B**		**6AS**			
		1395486	**3064366**	**4394514**	**4439854**	**1093943**	**3948356**	**3956745**	**1127223**
1BL	1395486								
	3064366	0.79							
3B	4394514								
	4439854			0.04					
6AS	1093943								
	3948356					0.76			
	3956745					0.76	1.00		
	1127223					0.54	0.45	0.45	

BIOGRAPHY

Mr. Tianqing Chen was born on Feb 19 (Chinese calendar), 1982 in Feidong county, Hefei city, Anhui province, P. R. China. He received his Bachelor's degree in Biotechnology from Anhui Normal University (China) in 2003, and Master's degree in Crop Genetics and Breeding from Southwest University (China) in 2007. He started his career in Guizhou Institute of Upland Crops in 2007; his research field is wheat breeding and application. In 2009, he was accepted to the Ph.D. program under the supervision of Prof. Dr. Piyada Alisha Tantasawat at the School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Thailand.