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# Quantitative trait loci for resistance to Sharp Eyespot (*Rhizoctonia cerealis*) in recombinant inbred wheat lines from the cross Niavt 14 × Xuzhou 25

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**Abstract**

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This paper studies QTL (quantitative trait locus) resistance to Sharp Eyespot in wheat using a genetic population of the recombinant inbred line (RIL) hybridized from Niavt 14 and Xuzhou 25. Based on three-year phenotypic data and a resultant RIL genetic map, three QTLs in total associated with Sharp Eyespot resistance were detected. Two out of the three loci were detected on chromosome 2B, namely *Qses.jaas-2b1* and *Qses.jaas-2b2*, of which *Qses.jaas-2b1* accounted for 5.46 and 8.56% of the phenotypic variation in the field after inoculation in two successive years and *Qses.jaas-2b2* accounted for 6.04, 8.10 and 12.92% after field inoculation for three successive years. A further QTL for resistance gene named *Qses.jaas-7d* was detected on chromosome 7D in addition to the two on 2B and this exceeding 11.25% of the phenotypic variation. These results indicate that QTLs associated with Sharp Eyespot possibly exist on the linkage groups of chromosomes 2B and 7D in wheat and lay the foundation for further research of QTL resistances to Sharp Eyespot in wheat.

**Keywords**: genetic map; molecular marker; QTL; *Rhizoctonia cerealis*; Sharp Eyespot; wheat

In recent years, the wheat Sharp Eyespot disease has worsened in the wake of changes to crop systems and advances in cultivation. Wheat Sharp Eyespot is particularly associated with temperate wheat-growing regions such as in China (Wang *et al*. 1994; McBeath & McBeath 2010), Egypt (Hammouda 2003), England and Wales (Clarkson & Cook 1983; Polley & Thomas 1991), New Zealand (Cromey *et al*. 2006), Poland (Lemańczyk 2010; Lemańczyk & Kwaśna 2013), and the USA. (Lipps & Herr 1982; Mazzola *et al*. 1996). Severe Sharp Eyespot can considerably decrease wheat grain yield (Clarkson & Cook 1983; Lemańczyk & Kwaśna 2013). In terms of wheat acreage affected by Sharp Eyespot, China is the largest epidemic region in the world, as exemplified by 8.1 million hectares of winter wheat affected in 2005 (McBeath & McBeath 2010).

Wheat Sharp Eyespot is caused by *Rhizoctonia cerealis* and *Rhizoctonia solani* and has covered a vast area of winter wheat in the regions of the Yangtze-Huai river basin and the middle and lower reaches of the Yellow River in China. Every year, about one-fifth of the wheat planting area has been affected by the Sharp Eyespot disease, and billions of dollars in economic losses have been recorded (Cai *et al*. 2006; McBeath & McBeath. 2010; Lemańczyk & Kwaśna 2013). Cultivating disease-resistant varieties is the most effective way to reduce damage caused by the wheat Sharp Eyespot disease. Resistance to wheat Sharp Eyespot is a typical quantitative trait (Huo 2002; Zhang *et al*. 2005; Cai *et al*. 2006; Ren *et al*. 2010; Chen *et al*. 2013), and is dependent on the environment during initial infection and development. Consequently, its genetic improvement is likely to be difficult.

Wheat Sharp Eyespot resistance has been genetically mapped by molecular markers and the molecular marker approach of detection of linkage to quantitative trait loci (QTL) should be relevant to breeding for resistance to wheat Sharp Eyespot. Over years, many studies on the mapping of QTL resistance to wheat Sharp Eyespot have been conducted using molecular markers, and main-effect QTLs (Kosambi 1944; Sambrook *et al.* 1992; Ren *et al.* 2004) for resistance to wheat Sharp Eyespot have been found on chromosomes 7D and 1A. However, different sources of resistance may expose different resistant genes, and by using different genetic populations for QTL mapping and molecular mapping, different QTL resistances and relevant linked molecular markers can be obtained. This paper uses an $F_{6:8}$-generation recombinant inbred line (RIL) population of Niavt 14 × Xuzhou 25 for mapping QTL resistance to wheat Sharp Eyespot, and provides theoretical and material reference for the effective use of the resistance source Niavt 14 and molecular breeding for resistance to wheat Sharp Eyespot.

## MATERIAL AND METHODS

**Experimental materials**. The wheat RIL population used in this experiment is a hybridized combination of resistant parent Niavt 14 (from France) and susceptible parent Xuzhou 25. It was developed by a single-seed descent method from the $F_2$ generation, having 215 lines. R0301, an isolate of *Rhizoctonia cerealis* CAG 1, was used as the pathogen material, being kindly provided by Professor Huaigu Chen at the Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China. R0301 has been reported to be highly virulent on wheat (Ren *et al.* 2010).

**Sharp Eyespot resistance assessment**. Toothpick inoculation method: first commercially available toothpicks were soaked for 24 h and later arranged at the bottom of an intact aluminium box after having been folded in half. The toothpicks were soaked by one-fourth in PDA culture medium sterilized beforehand by conventional high-pressure steam. The R0301 strain of *Rhizoctonia cerealis* bred in a culture dish was transplanted into the sterilized solid medium with toothpicks aseptically after cooling, then cultured in an incubator at 25°C; and not used until hyphae grew all over the toothpicks. Inoculation took place at the jointing stage of wheat (when the temperature rose to more than 10 degrees in spring, the base of the wheat began to elongate and the internode was exposed to the ground about 1.5–2.0 cM after the tillering growth stage); 20 to 30 stems were inoculated for each strain, selecting sheaths close to the ground and carefully inserting short toothpicks covered with hyphae into locations between the sheaths and stems. The cultures were kept moist for one week after inoculation, then assessed for the disease level of Sharp Eyespot at the milk stage of wheat.

Sharp Eyespot resistance assessment: a disease level standard 0–5 scale is the standard scale used by Yuzhong Wang (Ren *et al.* 2007) but with some changes. Grade 0 indicates a disease-free state, which means no symptom of Sharp Eyespot; Grade 1 indicates that sheaths are infected but stems are not, which means one or more Sharp Eyespot lesions on sheaths but no symptoms on the stem; Grade 2 indicates that stems are infected and scabs occur on more than 1/4 of the area of the stems, which means one or more Sharp Eyespot lesions girdling in total less than or equal to 1/4; Grade 3 indicates that scabs occur on between 1/4 and 1/2 of the area of the stems; Grade 4 indicates that scabs occur on between 1/2 and 3/4 of the area of the stems, with the stem remaining unsoftened; Grade 5 indicates that scabs occur on more than 3/4 of the area of the stems or the stems are withered. The formula for a disease index of wheat Sharp Eyespot is provided as follows:

$$\text{Index (\%)} = \frac{(1 \times x_1 + 2 \times x_2 + 3 \times x_3 + 4 \times x_4 + 5 \times x_5)}{5 \times \sum_{i=0}^{5} X_i} \times 100$$

where:

$x_1, x_2, x_3, x_4, x_5$ – number of stems at Grade 1–5 of Sharp Eyespot

The disease index is converted through an anti-sine conversion in the process of data analysis.

**SSR analysis**. Genomic DNA of the two parents and 215 RIL lines of the $F_7$ generation were extracted from young leaves by the sodium dodecyl sulfate (SDS) method (Santos *et al.* 1993). According to the published genetic maps such as genetic and physical map (Somers *et al.* 2004), NW map (Sourdille *et al.* 2004) and consensus map (Tang *et al.* 2004), 503 pairs of SSR primers were synthesized. Resistant parents and susceptible parents were screened for polymorphism by means of the synthesized SSR primers to get polymorphic markers for a subsequent genetic analysis of the RIL population. Based on the GrainGenes database, expressed sequence tags (ESTs) located on chromosome 2B were used for developing sequence tagged site (STS) markers following the principle that each deletion bin is provided with 2 to 3 EST sequences. Primer design was obtained by the

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Table 1. Statistical analysis of disease index in Niavt14, Xuzhou25 and recombinant inbred lines (RILs) population

| Method | Year | Parents | | RIL population | | | |
|---|---|---|---|---|---|---|---|
| | | Niavt14 | Xuzhou25 | means | range | skewness | kurtosis |
| Toothpick inoculation method in field | 2009 | 32.08 | 47.92 | 39.68 | 28.32–53.51 | 0.44 | –0.25 |
| | 2010 | 36.11 | 41.07 | 40.03 | 30.57–51.07 | 0.22 | –0.29 |
| | 2011 | 28.98 | 47.08 | 35.45 | 26.57–59.22 | 1.25 | 1.64 |
| Toothpick inoculation method in greenhouse | 2011 | 37.54 | 56.48 | 46.18 | 27.64–70.44 | 0.37 | –0.12 |

MACVECTOR V10.0 software (Accelrys, UK). For PCR reaction and product analysis, refer to Santos *et al.* (1993) and Wang (1996).

**Data analysis**. Marker linkage was analysed using the mapping software JoinMap 3.0; LOD = 3.0, recombination rate is 0.4, and a genetic linkage map was drawn using the Kosambi mapping function (Xue *et al.* 2008). Mapping software MapChart 2.2 was used for drawing the linkage map.

## RESULTS

**Analysis of the RIL population resistance to Sharp Eyespot**. In field inoculation assessments over the three successive years from 2009 to 2011, the resistant parent Niavt 14 was highly resistant to Sharp Eyespot, and the disease index over three years was at 32.08, 36.11 and 28.98, respectively (Table 1). The susceptible parent Xuzhou 25 was very susceptible to Sharp Eyespot, and its index over three years was at 47.92, 41.07 and 47.08, respectively. The index of the population from three-year testing ranges from 28.32 to 53.51, 30.57 to 51.07 and 26.57 to 59.22, respectively. In the greenhouse toothpick inoculation assessments, the indexes of Niavt 14 and Xuzhou 25 were at 37.54 and 56.48, respectively. The index of the population ranges from 27.64 to 70.44. This shows that the index of the population resistant to Sharp Eyespot goes beyond the ranges of both parents in all tests, and transgressive segregation exists in the population resistance to Sharp Eyespot. Through normal SPSS detection, the indexes of five assessments show a continuous distribution, and skewed and curtailed data indexes are low and substantially accordant with normal distribution and mapping of QTL interval mapping. Moreover, the analysis of variance indicates that there exist significant differences between different lines in terms of resistance.

**Genetic linkage mapping**. 177 polymorphic loci were mapped genetically by the JoinMap 3.0 software and fitted to obtain 41 linkage groups composed of 148 SSR marker loci. A total of 884.4 cM was covered with the 41 linkage groups, with average distance being 6.0 cM, and the screened polymorphic loci covering all chromosomes except chromosome 6B.

**Analysis of QTL resistance to wheat Sharp Eyespot**. Three additive QTLs associated with wheat Sharp Eyespot resistance were detected in total and located on chromosomes 2B and 7D, respectively, two of which were detected on 2B and one was detected on 7D (Table 2).

The two QTLs detected on chromosome 2B are located between flanking markers *Xbarc101-2* and *Xbarc183* and between *Xbarc55* and *Xwmc149*, being

Table 2. Quantitative trait loci (QTLs) associated with Sharp Eyespot resistance detected by common information model (CIM)

| QTLs | Method | Chromosome | Flanking marker | LOD value | Contribution (%) |
|---|---|---|---|---|---|
| *Qses.jaas-2b1* | 2009, in field | 2B | Xbarc101-2~Xbarc183 | 3.12 | 5.46 |
| | 2010, in field | 2B | Xbarc101-2~Xbarc183 | 4.62 | 8.56 |
| *Qses.jaas-2b2* | 2009, in field | 2B | Xbarc55~Xwmc149 | 2.46 | 6.04 |
| | 2010, in field | 2B | Xbarc55~Xwmc149 | 3.60 | 8.10 |
| | 2011, in field | 2B | Xbarc55~Xwmc149 | 6.53 | 12.92 |
| *Qses.jaas-7d* | 2009, in field | 7D | Xbarc126~Xwmc702 | 5.26 | 11.25 |
| | 2011, in greenhouse | 7D | Xbarc126~Xwmc702 | 3.54 | 6.84 |

LOD – logarithm of the odds

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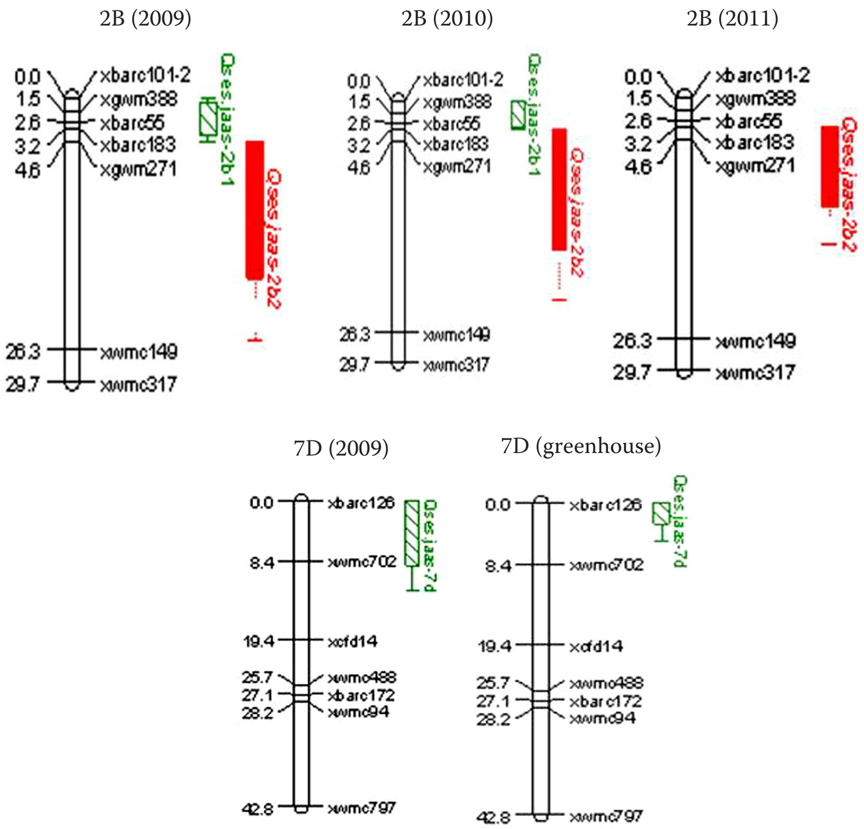

Figure 1. Graphic display of quantitative trait loci (QTLs) for wheat Sharp Eyespot on chromosomes 2B and 7D using composite interval mapping

respectively named *Qses.jaas-2b1* and *Qses.jaas-2b2* (Figure 1). *Qses.jaas-2b1* was detectable in field assessments in 2009 and 2010, of which LOD values are 3.12 and 4.62, which accounts for 5.46% and 8.56% of the phenotypic variation and exhibits a close linkage with marker *Xgwm388*. *Qses.jaas-2b2* was detectable in field assessments over the three successive years from 2009 to 2011, of which LOD values were 2.46, 3.60 and 6.53, accounting for 6.04, 8.10 and 12.92%, and being only 2.02 cM away from marker *Xgwom271*. The resistance of the two QTLs originates from the resistant parent Niavt 14. Although the LOD value of *Qses.jaas-2b2* detected in 2009 was low, LOD value and contribution were high during detection in 2010 and 2011. Therefore, *Qses.jaas-2b2* does exist, and these two QTLs are main-effect QTLs for resistance.

One QTL associated with wheat Sharp Eyespot resistance was detected on chromosome 7D and is located between markers *Xbarc126* and *Xwmc702*, being temporarily named *Qses.jaas-7d*. It was found in field inoculation assessments in 2009, and greenhouse inoculation assessments in 2011, respectively, and LOD values for the two years are 5.26 and 3.54, respectively. These account for 11.25% and 6.84% of phenotypic variation and are closely linked to marker *Xbarc126*. Resistance comes from Niavt 14. It can be inferred that this QTL may be a main-effect QTL for resistance (Figure1).

## CONCLUSION AND DISCUSSION

This experiment selected 503 pairs of SSR primers over the genome and detected QTLs within an RIL population through the use of composite interval mapping. Three QTLs associated with wheat Sharp Eyespot resistance were detected in total and located on chromosomes 2B and 7D, respectively. A single locus can account for 12.92% of phenotypic variation. They can be repeatedly detected, and their LOD values and phenotypic contribution are high. Moreover, the phenotypic effect of the QTLs is remarkable, meaning that it can be inferred that the three QTLs are main-effect QTLs.

In this research, two QTLs *Qses.jaas-2b1* and *Qses.jaas-2b2* associated with resistance are found on chromosome 2B and in close linkage to markers *Xgwm388* and *Xgwm271*. These two QTLs are very close to each other and whether or not they are associated with the same QTL remains to be demonstrated. Of the two QTLs, *Qses.jaas-2b1* is close to the flanking marker where the locus found on 2B by Lijuan Ren (Zhang *et al*. 2005) is located. One QTL for resistance *Qses.jaas-7d* detected on chromosome 7D is located between markers *Xbarc126* and *Xwmc702* and is closely linked to the marker *Xbarc126* and next to the QTL which was found on 7D by Cai *et al.* (1997). This indicates that loci resistant to Sharp Eyespot do exist on chromosome 7D.

Based on this research, we believe that the three QTLs on chromosomes 2B and 7D are main-effect loci. Genes can be precisely mapped by expanding the population and encrypting markers, and this can lay the foundation for assisted selection of gene markers and future gene cloning.

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