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RESEARCH ARTICLE

Molecular mapping of leaf rust resistance genes in the wheat line Yu 356-9

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Abstract

The Chinese wheat line Yu 356-9 exhibits a high level of resistance to leaf rust. In order to decipher the genetic base of resistance in Yu 356-9, gene postulation, inheritance analyses, and chromosome linkage mapping were carried out. Gene postulation completed using 15 leaf rust pathotypes and 36 isogenic lines indicated that Yu 356-9 was resistant to all pathotypes tested. F_1 and F_2 plants from the cross Yu 356-9 (resistant)/Zhengzhou 5389 (susceptible) were tested with leaf rust pathotype "FHNQ" in the greenhouse. Results indicated a 3:1 segregation ratio, indicative of the presence of a single dominant leaf rust resistance gene in Yu 356-9 which was temporarily designated as *LrYu*. Bulk segregant analysis and molecular marker assays were used to map *LrYu*. Five simple sequence repeat (SSR) markers on chromosome 2BS were found closely linked to *LrYu*. Among these markers, *Xwmc770* is the most closely linked, with a genetic distance of 5.7 cM.

Keywords: wheat, leaf rust, resistance gene, inheritance analyses, molecular mapping

1. Introduction

Wheat is cultivated as a food crop throughout the world, and the wheat planting area in China is only slightly smaller than that of rice (Peng and He 2009). Among three rusts of wheat, leaf or brown rust caused by *Puccinia triticina* is the most widely distributed and can inflict 15% production losses and severe infection can result in losses up to 40% (Knott 1989; Kolmer 1996). Severe losses in wheat production were recorded due to leaf rust outbreaks during 1969, 1973, 1975 and 1979 in the Southwest China and some regions of the Yangtze River Basin, China (Dong 2001). During a recent leaf rust epidemic in 2012, large wheat acreage was affected in the Gansu Province of China. Development and deployment of resistant varieties has long been a preferred approach for preventing and controlling wheat leaf rust. More than 100 leaf rust resistance genes and alleles have been identified in wheat and 71 of these are officially named (Singh *et al.* 2013). However, a very limited number of these genes confer effective resistance to leaf rust in China. Therefore, it is essential to undertake regular screening for identifying novel rust resistance genes to breed and develop resistant wheat varieties.

Various methods can be employed for the genetic analysis of wheat leaf rust resistance including gene postula-

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tion, and chromosome linkage mapping. The locations of several leaf rust resistance genes have been successfully determined using simple sequence repeat (SSR) markers including genes *Lr13* (Messmer *et al.* 2000), *Lr34* (Suenaga *et al.* 2001), *Lr39* (Raupp *et al.* 2001), *Lr46* (Suenaga *et al.* 2001), *Lr50* (Brown *et al.* 2003), *Lr37* (Blaszczyk *et al.* 2004), *Lr19* (Li *et al.* 2005; Zhang *et al.* 2005), *Lr52* (Hiebert *et al.* 2005), *Lr45* (Zhang *et al.* 2007), *Lr58* (Kuraparthy *et al.* 2007), and *Lr61* (Herrera-Foessel *et al.* 2008).

Two novel leaf rust resistance genes were identified and reported by our laboratory using SSR markers *LrBi16* (in wheat variety Bimai16) and *LrNJ97* (in Neijiang 977671) located on chromosomes 7B and 2B, respectively (Zhang *et al.* 2011; Zhou *et al.* 2013). The wheat genotype Yu 356-9 displays high resistance to leaf rust over sites and seasons but the genetics of resistance has not been reported. In the present study, the genetics of leaf rust resistance of Yu 356-9 was investigated and reported. The objectives of this study are to map the leaf rust resistance gene in Yu 356-9 using SSR markers.

2. Results

2.1. Gene postulation

Results of leaf rust seedling screening are presented in Table 1. Yu 356-9 displayed resistance to pathotype PHJS, whereas leaf rust resistance genes including *Lr1*, *Lr2C*, *Lr3*, *Lr16*, *Lr26*, *Lr11*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr13*, *Lr14b*, *Lr20*, *Lr21*, *Lr23*, *Lr33*, *Lr36*, and *Lr45* were susceptible to this pathotype. Thus, it was inferred that there is resistance in Yu 356-9 that is unique from these 19 known genes. Yu356-9 was also found resistant to pathotype TGTT which was virulent to *Lr2a*, *Lr3ka*, *Lr30*, *Lr18*, *Lr2b*, and *Lr15*. It was proposed that Yu 356-9 possesses resistance that is unique from these six known genes. Resistance in Yu 356-9 was also found to be different from *Lr29* and *Lr44* as Yu 356-9 was resistant to pathotype FHDS, whereas *Lr29* and *Lr44* were found susceptible. Leaf rust resistance genes including *Lr9*, *Lr24*, *Lr19*, *Lr28*, *Lr39*, *Lr42*, *Lr47*, *Lr51*, and *Lr53* were found resistant to all tested pathotypes. Yu 356-9 displayed a reactive level of 2+ toward pathotype THJL; in contrast, the plants harboring *Lr9*, *Lr28*, and *Lr47* had reactive levels of 0, and those with *Lr9*, *Lr24*, *Lr39*, *Lr42*, *Lr51*, and *Lr53* exhibited reactive levels of 1. These infection types were lower than those observed for Yu 356-9, indicating that Yu 356-9 may possibly contain gene(s) different from the previously mentioned known genes or a widely effective gene combination.

2.2. Seedling resistance identification

The resistant parent Yu 356-9, the susceptible parent Zhengzhou 5389, and their F_1 and F_2 progeny seedling reactions following inoculation with *P. triticina* pathotype FHNQ are presented in Table 2. All 20 seedlings of the resistant parent Yu 356-9 were found resistant to pathotype FHNQ, while 20 seedlings of the susceptible parent displayed susceptible reactions to pathotype FHNQ. In the F_2 generation, out of the tested 159 seedlings, 110 were found resistant while 49 were susceptible, which fit a single-locus segregation ratio (P=0.09) (Table 2). Thus, it was hypothesized that the leaf rust resistance of Yu 356-9 in response to pathotype FHNQ at the seedling stage is controlled by a single dominant gene.

2.3. Molecular marker screening and gene location

A total of 1 082 SSR primers distributed across 21 chromosomes were screened between the two parents and the resistant and susceptible DNA pools. Five markers that were polymorphic between the parents and pools were selected (*Xwmc25*, *Xbarc55*, *Xgwm148*, *Xgwm410*, and *Xwmc770*); all five of these markers were distributed on chromosome 2BS. Thus, we preliminarily positioned the gene on chromosome 2BS and temporarily named it as *LrYu*.

2.4. Linkage analysis and molecular mapping

Five pairs of primers that revealed polymorphisms between the parents and bulks were used for PCR amplification and electrophoresis detection of the same 159 F_2 plants used in the seedling screening. Linkage with *LrYu* was evident, with genetic distances ranging from 5.7 to 28 cM; the most closely linked marker was *Xwmc770*, with a genetic distance of 5.7 cM (Fig. 1). A polyacrylamide gel image of *Xwmc770* is shown in Fig. 2.

2.5. Temperature sensitivity test

Genetic analysis and linkage mapping suggested that *LrYu* is positioned on chromosome 2BS near the *Lr13* locus, which displays temperature sensitivity (Pretorius *et al.* 1984). Table 3 shows that *LrYu* also exhibited temperature-sensitive resistance and was resistant to all three strains. Yu 356-9 was most resistant at 18°C, whereas the plants harboring *Lr13* were susceptible to all three of the pathotypes, suggesting that *LrYu* differs from *Lr13*.

3. Discussion

3.1. Preliminary positioning of *LrYu*

The F_2 progeny from crosses of Yu 356-9 and Zhengzhou 5389 were inoculated and studied in the greenhouse. The