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Full Length Research Paper

The differential expression of *Rhopalosiphum padi* resistance in sibling wheat-rye amphiploids

Zongxiang Tang and Zhenglong Ren*

State Key Laboratory of Plant Genetics and Breeding, Sichuan Agriculture University, Ya'an, Sichuan 625014, China.

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Thirteen amphiploids were synthesized by chromosome doubling of an F_1 plant of a cross between *Triticum aestivum* Mianyang11 × *S. cereale* Kustro, which shows resistance to Bird cherry-oat aphid (*Rhopalosiphum padi)*. The amphiploids were confirmed by GISH analysis and tested for their reaction to *R. padi*. Eleven amphiploids exhibited resistance to *R. padi* and two of them exhibited high level of susceptibility. The PCR analysis using rye-specific markers provided evidence for the elimination of W-box elements from the susceptible amphiploids. The possible mechanism of loss resistance in some amphiploids was discussed. The resistant amphiploids can be exploited in wheat breeding programs to transfer *R. padi* resistance to susceptible wheat varieties.

Key words: *Rhopalosiphum padi*, amphiploid, wheat, rye.

INTRODUCTION

Aphid is an important insect pest of wheat. Aphid damage can severely reduce yield and grain quality of wheat. The Russian wheat aphid (RWA) has been reported to cause losses more than $800 million in the western USA from 1987 to 1993 (Morrison and Peairs, 1998). The most effective method of controling the aphid is to use resistant wheat cultivars. Thus far, 11 wheat aphid resistance genes have been reported that are effective against RWA. These RWA resistance genes are *Dn1*, *Dn2* (Du Toit, 1987), *Dn3* (Nkongolo et al., 1991a), *Dn4* (Nkongolo et al., 1991b), *Dn5* (Du Toit et al., 1995), *Dn6* (Harvey and Martin, 1990), *Dn7* (Marais et al., 1994), *Dn8*, *Dn9*, *Dnx* (Liu et al., 2001) and *DN2414* (Peng et al., 2007). Most of these resistance genes are dominant (Du Toit 1987; Harvey and Martin 1990; Nkongolo et al., 1991b; Marais et al., 1994; Du Toit et al., 1995; Liu et al., 2001; Peng et al., 2007). Among these genes, *Dn1*, *Dn2, Dn4, Dn5, Dn6, Dn8*, *Dn9* and *Dnx* were derived from wheat (Liu et al., 2005), *Dn3* from goatgrass [*Aegilops tauschii* (Coss.) Schmal] line SQ24 (Nkongolo et al., 1991a), and *Dn7* and *DN2414* from rye (*Secale cereale* L.) (Marais et al., 1994; Peng et al., 2007). It has been reported that *Dn* genes only conferred resistance to RWA but not to other aphid species such as *Rhopalosiphum padi* (L).

*Corresponding author. E-mail: rzhlong2010@sina.com.

In South-West regions of China, *R. padi* is the most prevalent species of the wheat-infecting aphids. However, sources of resistance against this aphid have not been reported. Therefore, identification and exploitation of resistance genes against *R. padi* should be a high priority area in wheat breeding programs. Wild relatives of common wheat are the rich sources of genes for resistance to diseases and pests (Jiang et al., 1994). Among these, rye (*Secale cereale* L.) is an important source of disease and pest resistance genes for improvement of cultivated wheat (Riley and Macer, 1966; Zeller and Hsam, 1983).

In this study, a cultivated rye (*Secale cereale* L.), Kustro, which displayed a high level of resistance to *R. padi*, was crossed to a common wheat genotype Mianyang11 (*Triticum aestivum* L.). A number of synthetic allopolyploids (amphiploids) were generated, characterrized and tested for their reaction to *R. padi.* The possible mechanism of loss resistance in some amphiploids was also discussed.

MATERIALS AND METHODS

Plant materials

Thirteen octoploid triticales (amphiploids) derived from *Triticum aestivum* Mianyang11 (AABBDD) × S. *cereale* Kustro (RR) cross were used in the study. The synthetic amphiploids were obtained

by treating young seedling of one F_1 hybrid with 0.05% colchicine. All the 13 amphiploids were derived from a same F_1 plant and were designated as MK09S_1-1 to MK09S_1-13. The parental rye, Kustro, was inbred for at least 8 generations, whereas parental wheat Mianyang 11 was selfed for at least 15 generations.

Genomic in situ hybridization (GISH)

The root-tip preparations of hybrids between wheat and rye were analyzed by GISH following Tang et al. (2008). The total genomic DNA from Kustro was labeled with digoxigenin-11-dUTP according to the manufacturer's instruction (Roche) for GISH analysis.

DNA extraction and PCR amplification

Genomic DNA of amphiploids and their parental plants was extracted according to the method described by Zhang et al. (1995). Two primer pairs specific to W-box motifs were designed from the two rye specific sequences, EF535876 and EF535871 deposited in GenBank by Tang et al. (2008). The two primer pairs named W1 (F: 5'TTC AAA GCC CAA GTT CGC C3'; R: 5'TTC TTC TAC GTC TGC ATG CCC3') and W2 (F: 5'CGA TTT ATT TGG GCC ACT GGA3';R: 5'TGC AGG TTT CGC TAC CGC TA3') flanking W-box were synthesized using the software DNAMAN (Version 4.0). The sequence EF535876 contains W-box (TTGACC) at 573-578 bp sites. The forward and reverse primers of W1 located at 236-253 bp and 680-699 bp sites of EF535876, respectively. The anticipatory fragment size of W1 was 464 bp. The sequence EF535871 contains W-box (TTGACC) at 298-303 bp sites. The forward and reverse primers of W2 located at 2-21 bp and 514-532 bp sites of EF535871, respectively. The anticipatory fragment size of W2 was 531 bp. The PCR amplification was carried out according to Tang et al. (2008). Wheat cultivar Chinese Spring (CS) DNA was used as a positive control in PCR.

Test of *Rhopalosiphum padi* resistance

The final test of *Rhopalosiphum padi* was performed according to Li et al. (2002) with some modifications. Plants were grown in individual pots in greenhouse. Seven aphids were placed directly on each plant at the seedling stage. After a four-week aphids exposure, the number of aphids on each plant was recorded for three times. Then the number ratio (the number of aphids on each plant/the number of aphid on all the plants) was used to evaluate the resistance to aphids. The scales of 0-0.01, 0.01-0.03, 0.03-0.05, 0.05-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.7 were rated as immune, highly resistant, moderately resistant, weakly resistant, weakly susceptible, moderately susceptible, highly susceptible, respectively.

RESULTS

The 13 seeds derived from one F_1 hybrid were first used for GISH analysis. The root-tip preparations in which rye chromatin was present were distinguishable by yellow-green fluorescing domains at interphase and metaphase. Chromosome counts indicated that the chromosome number of each seed was 56. Among the chromosomes of these seeds, 14 exhibited strong hybridization signals (Figure 1) and were identified as rye chromosomes. These results indicated that the 13 plants derived from the same F_1 plant were amphiploids (octoploid triticales).

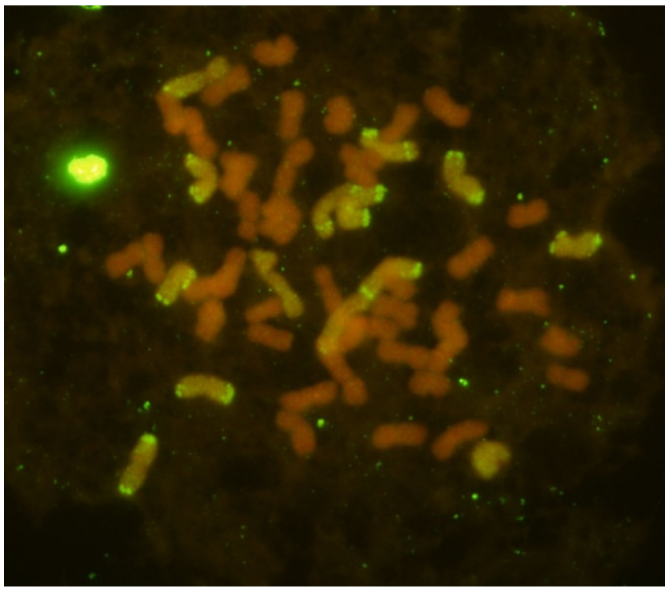

Figure 1. Amphiploid of Mianyang11×Kustro shown by GISH analysis to contain 56 chromosomes including 14 S. cereale chromosomes distinguishable by yellow-green fluorescing domains.

The 13 wheat-rye amphiploids displayed difference in their reaction to *R. padi* (Figure 2). Four amphiploids, MK09S_1-1, MK09S_1-11, MK09S_1-12 and MK09S_1-13 exhibited immune response, whereas five namely, MK09S_1-2, MK09S_1-5, MK09S_1-8, MK09S_1-9 and MK09S_1-10 exhibited high level of resistance to *R. padi*. MK09S_1-3 and MK09S_1-4 displayed moderate resistance and MK09S_1-6 and MK09S_1-7 were highly susceptible to the aphid.

Rye-specifc markers W1 and W2 did not amplify bands from wheat genomic DNA, but amplified nearly 460 and 530 bp sized products, respectively from the parental rye genomic DNA (Figure 3), thus suggesting their rye-specific origin. Both markers W1 amplified rye-specfic bands in all the amphiploids except MK09S_1-6 and MK09S_1-7 (Figure 3), which also displayed susceptibility to *R. padi*. The results suggest that the rye-specific W-box elements may have changed in the genomes of MK09S_1-6 and MK09S_1-7.

DISCUSSION

In this study, the resistance of *S. cereael* Kustro to *R. padi* has been introduced into wheat genome through synthesis of wheat-rye amphiploids. Although several RWA resistance genes derived from rye have been reported previously (Lapitan et al., 2007; Nkongolo et al., 1990; Lukaszewski et al., 2001; Marais et al., 19941), none of these is effective against *R. padi*. Recently, Hesler (2005) reported identification of three triticale accessions 8TA5L, H7089-52, and Stniism 3 exhibiting

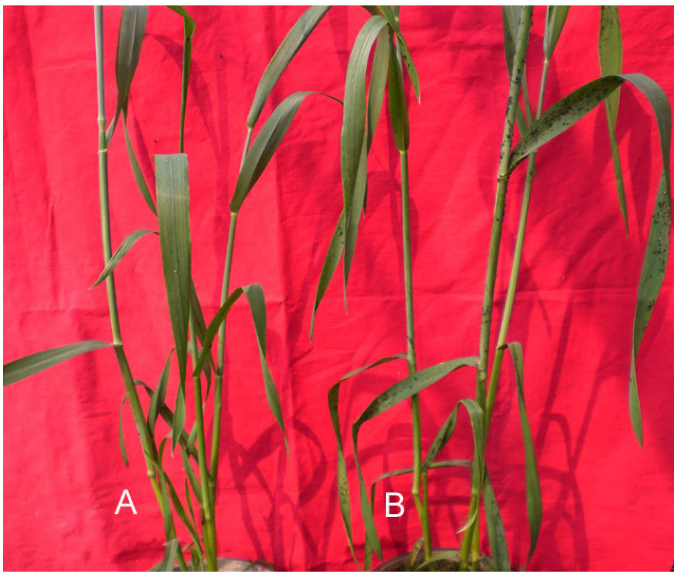

Figure 2. Resistance of wheat-rye amphiploids to *R. padi*. (A) MK09S1-2 displaying high level of resistance. (B) MK09S1-7 displaying high susceptibility.

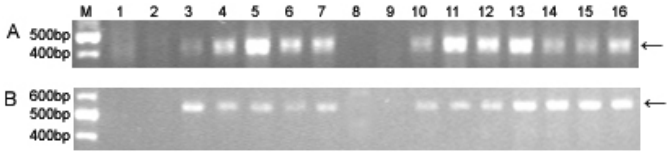

Figure 3. Amplification products obtained with W1 and W2 from genomic DNAs of Chinese Spring, Mianyang11, Kustro, and the 13 amphiploids. (A) Products amplified by W1. (B) Products amplified by W2. 1, Chinese Spring; 2, Mianyang11; 3, MK09S1-1; 4, MK09S1-2; 5, MK09S1-3; 6, MK09S1-4; 7, MK09S1-5; 8, MK09S1-6; 9, MK09S1-7; 10, MK09S1-8; 11, MK09S1-9; 12, MK09S1-10; 13, MK09S1-11; 14, MK09S1-12; 15, MK09S1-13; 16, Kustro. M, DNA marker.

resistance to *R. padi.* A comparative study of spectrum and mechanistic basis of resistance sources identified in *S. cereale* Kustro and those reported by Hesler et al. (2005) is required for their effective utilization in wheat breeding programs. The amphiploids with resistance to *R. padi* can serve as useful bridge for the further transmission of the aphid resistance gene(s) to common wheat.

Given that the wheat-rye amphiploids tested in this study are sibling lines derived from homozygous parents, its unusual to find variation in their reaction to *R. padi*. A series of studies on newly synthesized allopolyploid species of Triticeae have revealed rapid genomic and epigenomic changes involving sequence elimination and DNA methylation (Liu et al., 1998; Ozkna et al., 2001; Shaked et al., 2001; Han et al., 2003, 2004, 2005; Ma et al., 2004; Ma and Gustafson, 2006). Most of these studies have reported that the eliminated DNA sequences included low-copy, non-coding, coding and repetitive DNA sequences (Feldman et al., 1997; Liu et al., 1998; Ozkna et al., 2001; Shaked et al., 2001; Kaushkush et al., 2002; Ma et al., 2004; Han et al., 2005; Ma and Gustafson, 2006). Therefore, the observed variation in resistance phenotypes of amphiploids can be attributed to genomic alterations occurring during the hybridization and allopolyploidization process. Although we did not conduct thorough investigation on causes of variations in wheat-rye amphiploids, the deletion of W-box elements in $MK09S_1$-6 and $MK09S_1$-7 is suggestive of such genomic alterations. W-box (T)TGAC(C/T) is a cis-acting DNA element found frequently in the promoter of defense-related genes (Du and Chen, 2000; Yu et al., 2001). The susceptibility of $MK09S_1$-6 and $MK09S_1$-7 to *R. padi* might have resulted from the variation of W-box elements.

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