RESEARCH ARTICLE

Molecular cytogenetic characterization of a new wheat *Secale africanum* 2R^a(2D) substitution line for resistance to stripe rust

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Abstract

A stable, highly fertile wheat *Secale africanum* substitution line LF24, derived from the F_7 generation of a cross between Mianyang11 (MY11) and *Triticum durum*, *S. africanum* amphiploid (YF) was identified through molecular cytogenetic analysis. Application of C-banding, *in situ* hybridization and molecular markers analysis showed that LF24 was a wheat *S. africanum* 2R^a(2D) substitution line. When inoculated with stripe rust isolates, *T. durum* and MY11 were highly susceptible, while *S. africanum*, YF and LF24 were immune. It is confirmed through molecular cytogenetic analysis that the stripe rust resistance of LF24 was derived from *S. africanum* chromosome 2R^a. We compared the banding patterns and disease resistance of reported chromosomes 2R from different *S. cereale* introduced into wheat background, and found that there was new stripe rust resistance gene(s) on *S. africanum* 2R^a. LF24 is a new substitution line which can be used as stripe rust resistant source in wheat improvement.

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Introduction

Stripe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* (Pst), is a devastating fungal disease that afflicts wheat in many regions of the world. Losses of 40% can be common and in some fields the crop is totally destroyed (Roelfs *et al.* 1992). Growing resistant cultivars is the most economical and ecological method of controlling the disease (Chen 2005). Rye (*Secale cereale* L.), a species closely related to wheat, has been extensively used as a valuable source of genes for disease resistance, yield improvement and environment adaptation for wheat breeding (Friebe *et al.* 1996). Resistance gene *Yr9* originating from Petkus rye (*Secale cereale* L.), first introduced into wheat through a wheat–rye 1BL.1RS translocation chromosome (Zeller 1973), broke down after the 1990s (Rabinovich 1998). In addition to *Yr9*, other stripe rust resistance genes from different origins of cultivated rye and wild *Secale* species are also worthy of study (Luo *et al.* 2008).

Secale africanum (genome R^a), a wild species of genus *Secale*, has excellent resistance to wheat stripe rust, leaf rust and powdery mildew (Gill *et al.* 1991). The first step to transfer wheat diseases resistance from *S. africamum* to wheat is to develop wheat *S. africamum* amphiploid. Since wheat *S. africanum* amphiploids have already been developed (Jiang *et al.* 1992), a programme for introducing wheat diseases resistance genes from *S. africamum* to common wheat was initiated by crossing the amphiploids with cultivated wheat (Yang *et al.* 2001). So far, we have bred a large number of wheat *S. africamum* introgression lines, of which, one wheat *S. africanum* 1BL.1R^{a}S translocation line with high resistance to stripe rust was obtained (Yang *et al.* 2009), still there are unidentified resistance genes in *S. africanum*, which allowed us to transfer novel resistance genes by developing addition, substitution or translocation lines and undertake their identification through molecular cytogenetic analysis.

In the present study, we identified a wheat *S. africanum* 2R^a(2D) substitution line LF24, which can further be used as resources for transferring stripe rust resistance gene(s) from *S. africanum* into wheat.

Materials and methods

Plants materials

Secale africanum was obtained from Missouri Botanical Garden, St Louis, USA. *T. durum S. africanum* amphiploid

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(YF) was developed through colchicine treatment of the F_1 hybrids (Jiang *et al.* 1992). The line LF24 was selected from the F_7 generation of the cross between wheat cultivar Mianyang11 (MY11) and YF. Nulli-tetrasomic stocks, nullisomic-2A tetrasomic-2D (N2AT2D), nullisomic-2B tetrasomic-2A (N2BT2A) and nullisomic-2D tetrasomic-2B (N2DT2B) of common wheat Chinese Spring (CS) were provided by Wheat Genetic and Genomic Resources Center, Kansas State University, USA. *T. durum* and wheat cultivar MY11 are maintained at the Key Laboratory of Plant Genetics and Breeding, Sichuan Agricultural University, China.

C-banding and in situ hybridization

Chromosome identification was executed by following genomic *in situ* hybridization (GISH), sequential C-banding and fluorescence *in situ* hybridization (FISH) by Mukai *et al.* (1992). Wheat D-chromosome specific probe pAs1 (Raybun and Gill 1986) and rye genome repetitive sequence pSc119.2 (McIntyre *et al.* 1990) were obtained from Kansas State University, Manhattan, USA.

Molecular marker analysis

PCR-based landmark unique gene (PLUG) primers TNAC1178, TNAC1233 and TNAC1102 were synthesized according to Ishikawa *et al.* (2009). EST-derived 2RL-specific primers NSFT03P2_Contig4445 and NSFT03P2_Contig 17755 were synthesized according to Lee *et al.* (2009). PCR amplification of all sequences was performed in 25 μL reactions containing: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM of each dNTP, 10 μM of each primer, 0.5 U Taq polymerase and 50 ng template DNA; using the following programme: 3 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 53–63°C (depending on different primers), 2 min at 72°C; termination by 10 min of final extension at 72°C.

Reaction to stripe rust

Lines LF24, CS, MY11, *T. durum*, *S. africanum* and YF were grown in local plots that have favourable environments for stripe rust development. The stripe rust race CYR32 was provided by the Crop Protection Institute, Sichuan Academy of Agricultural Sciences, China. For disease evaluation, infection types (IT) were scored upon fully development of pustules (Bariana and McIntosh 1993).

Results

Cytogenetic identification

Chromosome counts were done at meiotic metaphase-I for 10 cells each of 20 plants in genotype LF24. Chromosome counts showed that the chromosome number of all the plants was 42. Meiotic observations showed 21 bivalents in all the cells indicating that line LF24 is cytogenetically stable.

GISH using rye genomic DNA as probe showed that LF24 carried a pair of rye chromosomes (figure 1a). Subsequently, FISH was performed on the same cell using wheat D chromosome specific sequences pAs1 as a probe. The result showed that 12 D-chromosomes gave rise to hybridization signals, only wheat chromosome 2D was absent (figure 1b). Sequential C-banding and FISH indicated a pair of *S. africanum* chromosomes in LF24 showed strong telomeric C-band at the short arms, weak centromere band and no telomeric C-band at the long arms which was similar to Chinese rye cultivar, *S. cereale* cv. Jingzhouheimai (Zhuang *et al.* 2010). The FISH using rye genome repetitive DNA sequence pSc119.2 as probe on the same mitotic metaphase showed that the hybridization signal pattern (figure 1, c&d) of this pair of *S. africanum* chromosomes was similar to rye chromosome 2R of *S. cereale* (Schwarzacher *et al.* 1989), which further verified that this pair of *S. africanum* chromosomes was $2R^a$ chromosome. Based on the above results, we concluded that LF24 was a new wheat *S. africanum* $2R^a$(2D) substitution line (figure 2).

Molecular analysis

PLUG primer TNAC1233 could amplify three bands from CS which originated from wheat 2A, 2B and 2D chromosomes, respectively (Ishikawa *et al.* 2009). In the present study, PCR using TNAC1233 also gives rise to three bands in wheat CS and MY11, while LF24, *T. durum* and YF only amplified two bands from chromosomes 2A and 2B (figure 3a). Other PLUG primers, TNAC1178 and TNAC1102, specific to wheat homoeologous group 2 also generated the similar amplification (data not shown). These results suggested that chromosome 2D was absent in LF24, which was similar to the results obtained through FISH (pAs1) analysis.

The 2R-specific primers NSFT03P2_Contig4445 and NSFT03P2_Contig17755a (Lee *et al.* 2009) were used to amplify genomic DNA from CS, MY11, *T. durum*, *S. africanum*, YF and LF24. The PCR products of 289 bp and 439 bp in length were amplified in *S. africanum*, YF and LF24, respectively, while wheat lines CS, MY11 and *T. durum* have no amplification at the target band sites (figure 3, b&c). These PCR results supported that the *S. africanum* chromosomes in LF24 were $2R^a$ chromosomes.

Reactions of stripe rust

After inoculation with the currently prevalent isolate CYR32 of *P. striiformis* f. sp. *tritici* in Sichuan Province (figure 3), lines LF24, *S. africanum* and YF were immune (IT 0) to CYR32. In contrast, MY11 and *T. durum* were highly susceptible (IT 4), indicating the stripe rust resistance of LF24 derived from *S. africanum* $2R^a$ chromosome.

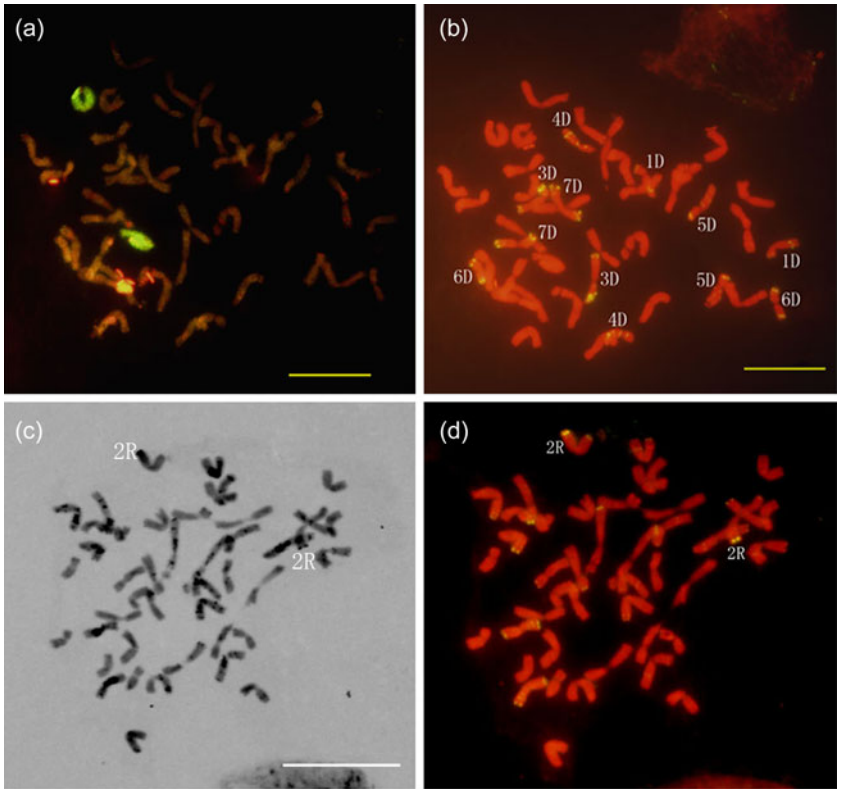

Figure 1. Cytogenetic analysis of LF24 by (a) GISH, (b & d) FISH and (c) C-banding.GISH using the genomic DNA of *S. africanum* as probe (a), FISH using the probes of pAs1 (b) and pSc119.2 (d), respectively (scale bar = 20 μm).

Discussion

GISH is a powerful technique to visualize alien chromatin in wheat-alien hybrids (Schwarzacher *et al.* 1989). In the present study, GISH using *S. africanum* genomic DNA as probe showed that a pair of *S. africanum* chromosomes were hybridized in LF24. FISH can localize the repetitive DNA sequences to specific chromosome sites and discriminate genome constitution (Alkhimova *et al.* 1999). Here we hybridized wheat D-chromosome specific sequence pAs1 to LF24 and found that wheat chromosome 2D was absent. With the development of functional molecular markers, particularly, the markers based on comparative mapping from different grass species (Ishikawa *et al.* 2009) and the EST based PCR markers (Ma and Gustafson 2008), it is effective and accurate for determining the homologous relationships of alien chromosomes in wheat background. In this research, PCR-based rye 2R molecular markers and PLUG markers clearly revealed that the *S. africanum* chromosomes in LF24 belonged to the $2R^a$ and the 2D chromosomes were absent. Thus combining GISH, FISH and molecular data, it was concluded that LF24 is a wheat *S. africanum* $2R^a$(2D) substitution.

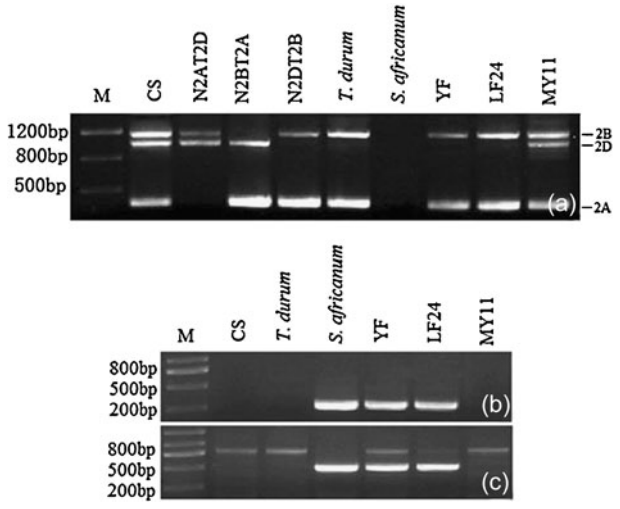

Figure 2. Amplification pattern of marker (a) TNAC1233, (b) NSFT03P2_Contig4445 and (c) NSFT03P2_Contig17755.

Large heterochromatic blocks, staining as C-bands, are a major structural characteristic of the rye genome and are present near the telomere of most chromosome arms (Richards *et al.* 1993; Alkhimova *et al.* 1999). However, the wild *Secale*, *S. africanum* and *S. silvestre*, displayed the least telomeric heterochromatin among the genus *Secale* (Bennett *et al.* 1977). Recently, we also observed the C-banding patterns of amphiploids between tetraploid wheat and *S. africanum*, and found that the *S. africanum*

Figure 3. Adult plants reaction to stripe rust when inoculated with isolate CYR 32 of *P. striiformis*.

chromosomes contained less heterochromatin in comparison with *S. cereale* (Yang *et al.* 2009). In reported wheat *S. cereale* substitution lines, the chromosome 2R from *S. cereale* cv. Imperial (Mukai *et al.* 1992), Chaupon (Friebe *et al.* 1990) and Onokhoiskaya (Silkova *et al.* 2006) displayed strong telomeric C-bands in both arms, and a small C-band adjacent to the centromere in the long arm (figure 4). In the present study, the added chromosome pair of *S. africanum* chromosome $2R^a$ in LF24 can be easily distinguished from the chromosomes of wheat by the presence of large telomeric C-bands in short arms and a faint C-band in centromere (figures 2c & 4a). Moreover, studies also revealed that the tandem repetitive sequences pSc200 and pSc250 hybridized on at least short arms of *S. cereale* chromosome 2R (Alkhimova *et al.* 1999; Zhou *et al.* 2010). Nevertheless, no FISH signal of pSc200 and pSc250 was detected in *S. africanum* chromosomes $2R^a$ (Yang *et al.* 2009). It is thus to conclude that the chromosome $2R^a$ in LF24 was different from those of *S. cereale* chromosome 2R on the constitution of heterochromatin.

Wheat–rye addition or substitution lines with different origin of rye may possess different disease resistance genes or alleles (Villareal *et al.* 1994; Ren *et al.* 2009). Rye chromosome 2R has been reported previously to contain a powdery mildew resistance gene *Pm7* (Heun and Friebe 1990). A powdery mildew resistance gene in chromosome 2R was identified from a German white rye cultivar, which may differ from *Pm7* (An *et al.* 2006). Recently, another gene for powdery mildew resistance, designated *PmJZHM2RL*, was also located on rye chromosome 2RL from Chinese rye Jingzhouheimai (Zhuang *et al.* 2010). Till now, only powdery mildew resistance was introduced from different origin rye 2R chromosome into wheat. In the present study, after inoculating with isolate of *P. striiformis*, we found that the $2R^a$(2D) substitution line LF24 is highly resistant to stripe rust at both seedling and adult stages. It is likely that the *S. africanum* chromosome $2R^a$ may possess new stripe rust resistance gene(s) and the wheat *S. africanum* substitution line LF24 might be useful resource in wheat breeding programme for stripe rust resistance.

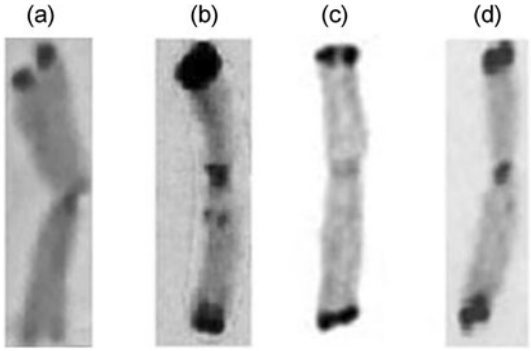

Figure 4. Comparison of C-banding pattern of chromosome $2R^a$ of (a) *S. africanum* and (b) the other chromosomes 2R of *S. cereale* cv. Imperial (Mukai *et al.* 1992), (c) cv. Onokhoiskaya (Silkova *et al.* 2006), (d) cv. Chaupon (Friebe *et al.* 1990).

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