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ORIGINAL RESEARCH

Identification and Physical Mapping of New PCR-Based Markers Specific for the Long Arm of Rye (*Secale cereale* L.) Chromosome 6

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ABSTRACT

To effectively use elite genes on the long arm of rye chromosome 6 (the 6RL arm) in wheat breeding programs, precise and fast identification of 6RL chromatin in wheat backgrounds is necessary. PCR-based 6RL-specific markers can facilitate the detection of elite genes on 6RL in wheat breeding. However, only a limited number of 6RL-specific markers have been developed. In the present study, 300 new PCR-based 6RL-specific markers were identified using specific length amplified fragment sequencing (SLAF-seq) technology, and were further physically mapped to four regions on the 6RL arm using 6R and 6RL deletion lines. Interestingly, 127 of the 300 markers were physically localized to a region from the site between 2.3 and 2.5 to the telomere, the same region where the powdery mildew resistance gene was mapped. In addition, 95 of the 300 markers exhibit polymorphisms, which can be used to investigate the diversity of rye 6RL arms. The markers developed in this study can be used to identify given segments of 6RL in wheat backgrounds and accelerate the utilization of elite genes on 6RL in wheat breeding.

KEYWORDS: Wheat; Rye; 6RL-specific marker; Physical mapping; SLAF-seq; Powdery mildew

INTRODUCTION

Rye (*Secale cereale* L.) is one of the most widely used sources in wheat breeding programs because it has great potential for expanding the genetic variability of cultivated common wheat (*Triticum aestivum* L.). The long arm of rye chromosome 6 (6RL) is notable due to the presence of some elite genes that can be exploited for wheat cultivar improvement. For instance, 6RL carries resistance genes *CreR* and *H25*, which are against the cereal cyst nematode *Heterodera avenae* Woll. and Hessian fly, respectively (Asiedu et al., 1990; Friebe et al., 1991; Mukai et al., 1993; Dundas et al., 2001). It also carries the powdery mildew resistance gene *Pm20* (Friebe et al., 1994), which provides a broad spectrum of resistance to *Blumeria graminis* f. sp. *Tritici* (*Bgt*) isolates (Zhao et al., 2013).

Development of 6RL-specific markers is conducive to the detection of 6RL chromatin in wheat backgrounds and to the utilization of the elite genes on 6RL. Some 6RL-specific markers have been developed, including representational difference analysis markers and isozyme markers (Benito et al., 1991; Delaney et al., 1995), as well as SSR, EST-SSR, and PCR-based landmark unique gene (PLUG) markers (Saal and Wricke, 1999; Wang et al., 2010; Xu et al., 2012; Li et al., 2013). Additionally, restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers for 6R have been reported (Wanous et al., 1995; Korzun et al., 1998; Saal and Wricke, 2002). A consensus map of 6R consisting of 104 molecular marker loci, and a high-density consensus map of 6R based on Diversity Arrays Technology (DArT) markers have been

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constructed (Stojałowski et al., 2009; Milczarski et al., 2011). Compared with the markers mentioned above, PCR-based markers, especially agarose gel electrophoresis-based markers, are much easier to use. However, the practical application of PCR-based markers to 6RL mapping is greatly limited due to their small number. Therefore, there is a genuine need for new PCR-based 6RL-specific markers that could be used to construct a high-density map and accelerate the transfer of important genes in wheat cultivars.

In this study, 300 new PCR-based 6RL-specific markers were developed by the specific length amplified fragment sequencing (SLAF-seq) technology. These new markers were further physically mapped into four regions (bins) on 6RL using deletion mutants of 6R and 6RL in a wheat background (6R and 6RL deletion lines).

RESULTS

Generation and identification of DEL6R^{Ku} and DEL6RL^{Ku}

The octoploid triticale line MK was derived from common wheat *T. aestivum* L. Mianyang11 (MY11) × rye *S. cereale* L. Kustro. From the progeny of MK × MY11, a wheat-rye 6R monosomic addition line (MA6R^{Ku}) was identified by simultaneous genomic *in situ* hybridization (GISH) and fluorescence *in situ* hybridization (FISH) analyses using the rye genomic DNA of Kustro (red), the *Aegilops tauschii* clone pAs1 (red), and the rye clone pSc119.2 (green) as probes (Fig. 1A) (Bedbrook et al., 1980; Rayburn and Gill, 1986). In line MA6R^{Ku}, the telomeric region of 6RS carried a single strong pSc119.2 signal; however, the 6RL arm carried four obvious pSc119.2 signal sites and three heterochromatic blocks reflected by rye genomic DNA (Fig. 1A). From the selfed progeny of line MA6R^{Ku}, a 6RL monotelosomic addition line (MTA6RL^{Ku}) and a 6RS monotelosomic addition line (MTA6RS^{Ku}) were cytologically identified, respectively (Fig. 1B and C). In lines MTA6RL^{Ku} and MA6R^{Ku}, the signal patterns of pSc119.2 and rye genomic DNA on 6RL were identical (Fig. 1A and B). The signal sites of pSc119.2 and the heterochromatic blocks on 6RL of Kustro were numbered (Fig. 1F) according to the standard *in situ* hybridization map constructed by Cuadrado et al. (1995).

From the selfed progeny of line MA6R^{Ku}, a 6R deletion line (DEL6R^{Ku}) was also identified, in which the 6R chromosome was broken near site 2.1, resulting in a deletion from the break point to the telomere (Fig. 1D and F). Furthermore, from the selfed progeny of line MTA6RL^{Ku}, a 6RL deletion line (DEL6RL^{Ku}) containing a broken 6RL was obtained, in which a break between sites 2.3 and 2.5 caused a deletion from this break point to the telomere (Fig. 1E and F).

From the progeny of MK × MY11, the other six monosomic addition lines (MA1R^{Ku}−5R^{Ku} and MA7R^{Ku}) were also detected (Fig. S1). In addition, two sets of disomic addition lines DA1R^{I}−7R^{I} and DA1R^{Ki}−7R^{Ki}, derived respectively from *T. aestivum* L. Chinese Spring (CS) × *S. cereale* L. Imperial and *T. aestivum* L. Holdfast × *S. cereale* L. King II, were cytologically confirmed (data not shown).

Identification and physical mapping of PCR-based 6RL-specific markers

To obtain PCR-based 6RL-specific markers, 1045 pairs of primers were designed based on 1045 sequences which were selected randomly from the 48,381 6RL-specific pair-end reads obtained using the SLAF-seq technology. Among the 1045 primer pairs, 300 amplified specific bands from Kustro, MA6R^{Ku}, and MTA6RL^{Ku} but not from MY11, MTA6RS^{Ku}, MA1R^{Ku}−5R^{Ku}, and MA7R^{Ku} (representative PCR results are shown in Fig. 2), indicating that these 300 primer pairs are 6RL-specific markers (Table S1). Among the 300 markers, 295 amplified one 6RL-specific band (Fig. 2A and B), and the other five amplified two 6RL-specific bands (Fig. 2C). The latter markers are KU-6RL.445, KU-6RL.712, KU-6RL.794, KU-6RL.904, and KU-6RL.915. Subsequently, the physical localization of these 300 6RL-specific markers on 6RL were detected using lines DEL6R^{Ku} and DEL6RL^{Ku}. Among the 300 markers, four amplified 6RL-specific bands from DEL6R^{Ku} but not DEL6RL^{Ku}, 89 amplified 6RL-specific bands from both lines, 80 amplified 6RL-specific bands from DEL6RL^{Ku} but not DEL6R^{Ku}, and 127 did not amplify 6RL-specific bands from either line (representative PCR results are shown in Fig. 3). For each of the five markers that amplified two 6RL-specific bands, both bands showed the same amplification profiles in these two lines and they were thus regarded as double-band markers. According to the amplification patterns, the 300 6RL-specific markers were physically mapped to four regions on 6RL arm of Kustro (Fig. 4). Four markers were located in region I (near the centromere), 89 markers were located in the region II (from the site between the centromere and 1.5 to the site between 2.1 and 2.3), 80 were located in region III (from the site between 2.1 and 2.3 to the site between 2.3 and 2.5), and 127 markers were located in the region IV (from the site between 2.3 and 2.5 to the telomere).

Polymorphism analysis of 6RL-specific markers

Holdfast, CS, Imperial, King II, and two sets of wheat-rye disomic addition lines (DA1R^{I}−7R^{I} and DA1R^{Ki}−7R^{Ki}) were used to further test the polymorphism of 6RL-specific markers by PCR. The polymorphism patterns were summarized in Table S2. Among the 300 6RL-specific markers, 192 markers, including three double-band markers KU-6RL.445, KU-6RL.904 and KU-6RL.915, amplified 6RL-specific bands from Imperial, King II, DA6R^{I}, and DA6R^{Ki} but not from Holdfast, CS, DA1R^{I}−5R^{I}, DA7R^{I}, DA1R^{Ki}−5R^{Ki}, and DA7R^{Ki}, indicating these 192 markers have no size polymorphisms. Another 31 markers did not amplify 6RL-specific amplicons from Holdfast, CS, Imperial, King II, and the two complete sets of disomic addition lines (DA1R^{I}−7R^{I} and DA1R^{Ki}−7R^{Ki}), 29 markers including the double-band maker KU-6RL.794 amplified 6RL-specific bands only from King II and DA6R^{Ki}, and 35 markers including the double-band maker