Development of New PCR-Based Markers Specific for Chromosome Arms of Rye (*Secale cereale* L.)

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Development of New PCR-Based Markers Specific for Chromosome Arms of Rye

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Abstract

PCR-based rye (*Secale cereale* L.) chromosome-specific markers can contribute to the effective utilization of elite genes of rye in wheat (*Triticum aestivum* L.) breeding program. In the present study, 578 new PCR-based rye-specific markers have been developed by using Specific Length Amplified Fragment Sequencing (SLAF-seq) technology and 76 markers displayed different polymorphism among rye Kustro, Imperial and King II. A total of 427 and 387 markers were respectively located on individual chromosomes and chromosome arms of Kustro by using a set of wheat-rye monosomic addition lines and 13 monotelosomic addition lines, which were derived from *T. aestivum* L. Mianyang11 $\times$ *S. cereale* L. cv. Kustro. In addition, two sets of wheat-rye disomic addition lines, which were derived from *T. aestivum* L. var. Chinese Spring $\times$ *S. cereale* L. var. Imperial and *T. aestivum* L. cv. Holdfast $\times$ *S. cereale* L. var. King II, were used to test the chromosomal specificity of the 427 markers. The chromosomal locations of 281 markers were consistent among the three sets of wheat-rye addition lines. The markers developed in this study can be used to identify a given segment of rye chromosomes in wheat background and accelerate the utilization of elite genes on rye chromosomes in wheat breeding program.

Key words: Wheat; Rye; Chromosome-specific marker; PCR-based

Introduction

Rye (*Secale cereale* L.) ($2n=2x=14$), a relative of common bread wheat (*Triticum aestivum* L.) ($2n=6x=42$), has played an important role in wheat breeding program as a valuable reservoir of genes. Elite genes of rye have been introduced into wheat backgrounds through different types of wheat-rye hybrid derivatives such as substitution, addition and translocation lines (Jiang et al. 1994; Masoudi-Nejad et al. 2002; Khlestkina 2014). Fast and accurate identification and characterization of rye chromatin in wheat backgrounds can improve the application efficiency of elite rye genes in wheat breeding. DNA-based markers are convenient and efficient for identifying rye chromatin in wheat backgrounds. Some genetic linkage maps of rye based on restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR) and Diversity Arrays Technology (DArT) have been constructed (Devos et al. 1993; Philipp et al. 1994; Senft and Wricke 1996; Korzun et al. 1998; Saal and Wricke 1999; Masojć et al. 2001; Bednarek et al. 2003; Camacho et al. 2005; Milczarski et al. 2007; Milczarski et al. 2011). Additionally, by using wheat-rye addition lines, some rye chromosome-specific markers have also been developed (Fu et al. 2010; Tomita and Seno 2012; Xu et al. 2012; Li et al. 2013). Among these reported DNA-based markers, PCR-based markers, especially agarose gel electrophoresis-based markers, are much easier to perform than RFLP, AFLP and DArT markers. Although genomic resources of rye have been developed and opportunities for developing large numbers of SNP-based markers for

the study of rye genome and related research topics are now available (Martis et al. 2013), the practical application of PCR-based markers to rye chromosome mapping is limited because of the small number of this type of marker. Therefore, more rye chromosome-specific PCR-based markers, especially those suited to agarose gel electrophoresis, are needed to be developed for the production of a high density map of rye chromosomes.

In the present study, new PCR-based and rye chromosome arm-specific markers have been developed by using Specific Length Amplified Fragment Sequencing (SLAF-seq) technology.

Materials and methods

Plant materials

The octoploid triticales line MK was obtained by crosses between *T. aestivum* L. cv. Mianyang11 (MY11) and *S. cereale* L. cv. Kustro. Some progeny were obtained by controlled backcrossing of MK with MY11 followed by self-fertilization. From these progeny, seven wheat-rye monosomic addition lines (MA1R^{Ku}-MA7R^{Ku}), 13 monotelosomic addition lines (MTA1RS^{Ku}-MTA7RS^{Ku}, MTA1RL^{Ku}-MTA2RL^{Ku} and MTA4RL^{Ku}-MTA7RL^{Ku}) were detected and identified by FISH. These materials were developed by our laboratory. The 3RL^{Ku} monotelosomic addition line (MTA3RL^{Ku}) was not obtained. Two sets of wheat-rye disomic addition lines (DA1R^I-DA7R^I) and (DA1R^{Ki}-DA7R^{Ki}), which were respectively derived from *T. aestivum* L. var. Chinese Spring (CS) × *S. cereale* L. var. Imperial and *T. aestivum* L. var. Holdfast × *S. cereale* L. var. King II, were supplied by Dr. J. Perry Gustafson, USDA-ARS, University of

Missouri, Columbia, Missouri, USA. Additionally, Holdfast, Imperial and King II were also supplied by Dr. J. Perry Gustafson. Wheat CS is maintained in our laboratory. These materials were used to identify rye-specific markers, to screen polymorphisms of rye-specific markers and to test the chromosomal specificity of rye-specific markers.

Genomic DNA extraction

Genomic DNAs of materials used in this study were extracted from leaf tissue using cetyltrimethyl-ammonium bromide (CTAB) procedure according to the method described by Murray and Thompson (1980).

Development of molecular markers

Genomic DNA of *S. cereale* L. Kustro was sequenced by using the Specific Length Amplified Fragment Sequencing (SLAF-seq) method (Biomarker, Beijing, China). The sequencing procedure was performed as described by Chen et al. (2013) with some modifications. Briefly, genomic DNA of Kustro was digested with restriction enzyme *Hae*III. Subsequently, the sample was purified by using a Quick Spin column (Qiagen) and then run out on a 2% agarose gel to isolate the fragments between 450 to 500 bp by using a Gel Extraction Kit (Qiagen). These fragments were used in a PCR amplification described by Chen et al. (2013). Products with the size between 450 to 500 bp were excised and diluted for sequencing and the SLAFs were identified, filtered, clustered and corrected according to the methods described by Chen et al. (2013). The pair-end reads from Kustro were compared with the sequences of wheat A genome, D genome and *T. aestivum* L. Chinese Spring (supported by Biomarker,

Beijing, China) by using SOAP software (Li et al. 2009). The pair-end reads with low wheat homology were kept and rye-specific reads were obtained. Finally, primers were designed according to the rye-specific reads by using software Primer 3 (version 4.0).

PCR analysis

The PCR reaction mixture (20 μ l total) consisted of 80 ng of template DNA, 10 pmol of each primer, 0.2 mmol of dNTP, 1.0 unit of Taq polymerase, 20 nmol $MgCl_2$ and 1 $\times$ PCR buffer. PCRs were performed in a COYOTE BIO PCR System (COYOTE BIOSCIENCE), using a program that consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at annealing temperature, 2 min at 72°C, and final extension for 10 min at 72°C. The annealing temperature was 60°C. The amplified products were electrophoresed in 2% agarose gel in 1 $\times$ TAE buffer. For each pair of the primers, all the PCR reactions were repeated three times.

Physical location of markers on rye chromosome arm

The markers, whose products presented in rye cv. Kustro but absent in common wheat MY11, CS and Holdfast were regarded as rye-specific. The PCR analysis using the rye-specific markers was performed on the previously developed set of MY11-Kustro monosomic addition lines (MA1R^{Ku}-MA7R^{Ku}) and rye chromosome-specific markers were determined. Then the rye chromosome-specific markers were located onto rye chromosome arms using 13 MY11-Kustro monotelosomic addition lines (MTA1RS^{Ku}-MTA7RS^{Ku}, MTA1RL^{Ku}-MTA2RL^{Ku} and MTA4RL^{Ku}-MTA7RL^{Ku}). Subsequently, the polymorphisms of rye-specific markers

were tested by using Imperial and King II. Additionally, two sets of wheat-rye DA lines including DA1R^I-DA7R^I and DA1R^{Ki}-DA7R^{Ki} were used to test the chromosomal specificity of rye chromosome-specific markers.

Results

Development of rye chromosome-specific markers

A total of 1104 pair-end reads were randomly selected for designing primers. 578 of the 1104 pairs of primers amplified specific bands from rye cv. Kustro, but not from common wheat MY11, CS and Holdfast. Therefore, the 578 pairs of primers were regarded as rye-specific markers (Table S1). Among the 578 rye-specific markers developed by using Kustro, 51 did not amplify products from Imperial, two did not amplify products from King II, 12 did not amplify products from both Imperial and King II, and 11 amplified different rye-specific bands from Kustro, Imperial and King II (Table S1). Therefore, the 76 (51+2+12+11) markers displayed polymorphism among the three rye lines. For each of the remaining 502 markers, rye-specific bands with the same size were produced from Kustro, Imperial and King II (Table S1).

Subsequently, the complete set of MY11-Kustro monosomic addition lines (MA1R^{Ku}-MA7R^{Ku}) were used to locate the 578 markers onto individual chromosomes of rye Kustro. The assignment of the rye-specific markers on specific chromosomes of rye Kustro is presented in Table S2. Forty-nine of the 578 markers amplified rye-specific bands with all of the seven MA lines (MA1R^{Ku}-MA7R^{Ku}) (Fig. 1a, Table S2). Twenty-six markers produced rye-specific bands from more than one of the Kustro rye MA lines (Fig. 1b, Table S2). Seventy-six markers did not amplify

rye-specific bands from any of the MA lines (MA1R^{Ku}-MA7R^{Ku}) (Fig. 1c, Table S2). So, the 151(49+26+76) markers could not be definitely mapped to a specific chromosome of rye Kustro. The remainder of the 427 markers produced specific bands from particular MA lines and hence they were assigned to individual chromosomes of rye Kustro (Figs. 1d, 1e, 1f, 1g, 1h, 1i, 1j, Table S2). Therefore, 43, 50, 55, 101, 55, 72 and 51 markers were definitely located on 1R^{Ku}, 2R^{Ku}, 3R^{Ku}, 4R^{Ku}, 5R^{Ku}, 6R^{Ku} and 7R^{Ku} chromosomes, respectively (Table S2). It is notable that marker Ku.257 amplified a 2R^{Ku}-specific and also a 3R^{Ku}-specific band, however, only the 3R^{Ku}-specific band was located onto the 3RS^{Ku} arm (Table S2). Therefore, the marker Ku.257 was assigned to 3RS^{Ku} arm (Table S2).

Location of rye chromosome-specific markers on short and long arms

Subsequently, the 13 MY11-Kustro monotelosomic addition lines (MTA1RS^{Ku}-MTA7RS^{Ku}, MTA1RL^{Ku}-MTA2RL^{Ku} and MTA4RL^{Ku}-MTA7RL^{Ku}) were used to assign the 427 markers to specific rye chromosome arms (Fig. 2, Table S2). Among the 43 1R^{Ku}-specific markers, 11 and 31 markers were respectively assigned to 1RS^{Ku} and 1RL^{Ku} arms (Figs. 2a, 2b, Table S2). Marker Ku.532 did not amplify 1R^{Ku}-specific bands from either of the MTA1RS^{Ku} and MTA1RL^{Ku} lines. For the 2R^{Ku}-specific markers, 20 and 27 were mapped to 2RS^{Ku} and 2RL^{Ku} arms, respectively (Figs. 2c, 2d, Table S2). Three of the 2R^{Ku}-specific markers (Ku.92, Ku.501 and Ku.1001) didn't amplify products from either of the MTA2RS^{Ku} and MTA2RL^{Ku} lines. Among the 55 3R^{Ku}-specific markers, 30 were definitely mapped to 3RS^{Ku} arm (Fig. 2e, Table S2). However, the other 25 markers could not be confirmed

as located on 3RL^{Ku} arm because of the missing line MTA3RL^{Ku}. Totals of 31 and 68 of the 4R^{Ku}-specific markers were assigned to 4RS^{Ku} and 4RL^{Ku} arms, respectively (Figs. 2f, 2g, Table S2). No products were amplified from both MTA4RS^{Ku} and MTA4RL^{Ku} lines by Ku.717 and Ku.754, and consequently these two markers could not be assigned to specific chromosome arms (Table S2). For the 5R^{Ku}-specific markers, 19 and 33 markers were respectively mapped to 5RS^{Ku} and 5RL^{Ku} arms (Figs. 2h, 2i, Table S2). Three markers, Ku.575, Ku.598 and Ku.978, produced target amplicons from both MTA5RS^{Ku} and MTA5RL^{Ku} lines (Table S2). In all 20 and 51 of the 6R^{Ku}-specific markers were respectively assigned to 6RS^{Ku} and 6RL^{Ku} arms (Figs. 2j, 2k, Table S2). Marker Ku.491 did not amplify 6R^{Ku}-specific band from either MTA6RS^{Ku} or MTA6RL^{Ku} lines (Table S2). Among the 7R^{Ku}-specific markers, 27 were assigned to 7RS^{Ku} arm, 19 were assigned to 7RL^{Ku} arm, while one marker (Ku.441) amplified 7R^{Ku}-specific bands from either MTA7RS^{Ku} or MTA7RL^{Ku} lines, however, four didn't produce the target amplicons from both MTA7RS^{Ku} and MTA7RL^{Ku} lines (Figs. 2l, 2m, Table S2). Therefore, 387 rye chromosome-specific markers were certainly located on 13 chromosome arms of Kustro.

Testing of rye chromosome-specific markers

The 427 markers, which have been definitely located on individual chromosomes of rye Kustro, were tested against two long-established sets of wheat-rye disomic addition lines DA1R^I-DA7R^I and DA1R^{Ki}-DA7R^{Ki}. The results are listed in Table S3. Twelve of the 1R^{Ku}-specific markers amplified bands in both the DA1R lines (DA1R^I and DA1R^{Ki}) (Table S3). Ku.1043 amplified band only from DA1R^{Ki} and it was

located on chromosome 1R^{Ki} (Table S3). Six of the 1R^{Ku}-specific markers did not amplify bands from both DA1R^I and DA1R^{Ki}, and 27 of the 1R^{Ku}-specific markers could not be assigned to chromosomes 1R^I and 1R^{Ki} because they amplified bands from DA2R^I, DA2R^{Ki}, DA3R^I, DA3R^{Ki} or DA5R^I (Table S3).

Twelve of the 2R^{Ku}-specific markers did not amplify 2R^{Ku}-specific bands either from DA2R^I or DA2R^{Ki}, and 37 markers amplified bands exclusively from the DA2R lines (DA2R^I and DA2R^{Ki}) (Table S3). Ku.389 amplified bands from DA6R^I. Ku.941 produced bands from all of the seven CS-Imperial DA lines (DA1R^I-DA7R^I) (Table S3).

For the 3R^{Ku}-specific markers, 25 amplified bands only from the DA3R lines (DA3R^I and DA3R^{Ki}), 23 produced bands only from DA3R^I but not from DA3R^{Ki}, one amplified bands only from DA3R^{Ki} but not from DA3R^I and two did not amplify target bands from both DA3R^I and DA3R^{Ki} (Table S3). Ku.2, Ku.168 and Ku.461 amplified bands from DA1R^I, DA3R^I, DA5R^I, DA1R^{Ki} or DA3R^{Ki} lines, Ku.451 produced different bands from MA3R^{Ku} and DA3R^I, and Ku.257 amplified 500bp 3R-specific bands and 450bp 2R-specific bands (Table S3).

For the 4R^{Ku}-specific markers, 72 were mapped to only the 4R^I and 4R^{Ki} chromosomes, 14 were mapped to the 4R^I chromosome and not to 4R^{Ki}, five were assigned to 4R^{Ki} chromosome and not to 4R^I, and seven did not amplify bands from both DA4R^I and DA4R^{Ki} lines (Table S3). Three markers amplified bands from non-DA4R lines (Table S3). For example, Ku.754 amplified bands from DA3R^I and DA3R^{Ki} lines, Ku.771 amplified a band from DA2R^I, and Ku.972 amplified bands

from six of the seven CS-Imperial DA lines (Table S3).

Among the 55 5R^{Ku}-specific markers, 38 amplified bands only from DA5R lines (DA5R^I and DA5R^{Ki}), four did not amplify 5R-specific bands from both DA5R^I and DA5R^{Ki} lines, six produced bands only from DA5R^I line, four produced bands only from the DA5R^{Ki} line and four amplified 5R^{Ku}-specific bands from non-DA5R lines (Table S3).

For the 6R^{Ku}-specific markers, Ku.226, Ku.320, Ku.555 and Ku.857 produced different 6R-specific amplicons from MA6R^{Ku}, DA6R^I and DA6R^{Ki} lines (Table S3). Additionally, 58 amplified bands from exclusively DA6R lines (DA6R^I and DA6R^{Ki}), one amplified bands from DA4R^I, DA6R^I, DA7R^I, DA4R^{Ki} and DA7R^{Ki} lines, six amplified bands only from the DA6R^I line, four amplified 6R^{Ku}-specific bands only from the DA6R^{Ki} line and one did not amplify bands from both DA6R^I and DA6R^{Ki} lines (Table S3).

Among the 51 7R^{Ku}-specific markers, 39 were definitely located on chromosomes 7R^I and 7R^{Ki}, seven were located on chromosome 7R^I, two were assigned to chromosome 7R^{Ki} and three did not amplify bands from both DA7R^I and DA7R^{Ki} lines (Table S3). So, the chromosome location of 281 (12+37+25+72+38+58+39) markers was consistent among the three sets of wheat-rye addition lines.

Discussion

Why the need for more PCR-based and rye markers?

Some genetic maps of rye have been constructed by using isozyme, protein, RFLP,

DArT and SSR markers (Saal and Wricke 1999; Korzun et al. 2001; Ma et al. 2001; Milczarski et al. 2007; Gustafson et al. 2009; Milczarski et al. 2011). These markers can be used to investigate the genetic diversity of rye germplasm and to map useful genes in rye. However, the markers that can be used to detect rye chromatin in wheat backgrounds are rare. In addition, many of these previously reported markers are rye genome-specific but not rye chromosome-specific (Xu et al. 2012). To efficiently utilize the elite genes of rye in wheat breeding programs, it is necessary to precisely identify rye chromosome or chromosomal segments in wheat backgrounds. Some SCIM markers, EST-based markers, STS markers and PLUG markers have been assigned to individual rye chromosome or chromosome arm by using a set of wheat-rye disomic addition and monotelosomic addition lines that were derived from Chinese Spring $\times$ Imperial (Camacho et al. 2005; Tomita and Seno 2012; Xu et al. 2012; Li et al. 2013). These rye chromosome-specific markers not only can be used to construct molecular linkage maps of individual rye chromosomes, but also can be used to identify rye chromosomes and chromosomal segments in wheat backgrounds. However, the number of markers is far from enough for constructing well-saturated genetic linkage maps of rye chromosomes and for identifying small rye segments in wheat backgrounds. In the present study, 578 rye-specific markers have been developed. Among the 578 rye-specific markers, 76 displayed polymorphisms among rye Kustro, Imperial and King II, 427 were located on individual chromosomes of rye Kustro and 387 were mapped to individual chromosome arms of Kustro. These markers developed in this study have greatly enriched the available stocks of

rye-specific and rye chromosome-specific markers, which can be used to investigate the diversity of species in the *Secale* genus and to identify rye chromatin in wheat backgrounds. Furthermore, the markers developed in this study are convenient for their practical application because they are PCR-based and agarose gel electrophoresis-based markers.

In addition, the number of markers assigned to 4R and 6R chromosomes is more than that assigned to the other five rye chromosomes. About quarter of the 427 markers were located on the 4R chromosome. This case might be caused by the SLAF-seq technology that was used to develop markers in this study. Genomic DNA of Kustro was digested with restriction enzyme *Hae*III and fragments between 450 to 500 bp were isolated. Perhaps the use of restriction enzyme *Hae*III and selection of fragments between 450 to 500 bp led to the preferential isolation of fragments of 4R and 6R chromosomes. Until this present study, PCR-based and 4R-specific markers were rare. One 4RL-specific marker was developed by Camacho et al. (2005). Xu et al. (2012) reported six 4R-specific markers, Tomita and Seno (2012) developed two 4R-specific markers and Li et al. (2013) assigned eight markers to the 4R chromosome. In the present study, 101 markers were mapped to 4R chromosome of Kustro and this has enriched the specific markers for detecting 4R segment in wheat backgrounds and for investigating diversity of the 4R chromosome.

Variation of rye-specific markers

In this study, some rye-specific bands could not be assigned to any of the wheat-rye addition lines. Furthermore, some rye chromosome-specific bands could not be

mapped to the monotelosomic addition lines (Tables S2, S3). In most of the previous studies, only one set of wheat-rye addition lines were used to assign rye-specific markers to individual rye chromosome (Camacho et al. 2005; Fu et al. 2012; Tomita and Seno 2012; Xu et al. 2012; Li et al. 2013). In the present study, three sets of wheat-rye addition lines were used to map rye-specific markers to a single rye chromosome and it was found that the chromosomal specificity of some markers was inconsistent among the three sets of wheat-rye addition lines. For example, some $1R^{Ku}$ -specific markers amplified bands from non-DA1R lines and some $2R^{Ku}$ -specific markers amplified bands from non-DA2R lines (Table S3). Some $1R^{Ku}$ -specific and $2R^{Ku}$ -specific markers could not amplify 1R- and 2R-specific bands from $1R^I$ and $2R^{Ki}$ chromosomes, respectively (Table S3). That is, some $1R^{Ku}$ -specific and $2R^{Ku}$ -specific markers cannot definitely identify 1R and 2R chromosomes of rye Imperial and King II in wheat backgrounds. This discovery indicates that some rye chromosome-specific markers cannot be used to identify a given chromosome across all rye germplasm. The results obtained in this study revealed the variations of rye-specific and rye chromosome-specific markers. It has already been reported that some rye-specific PLUG markers could not be assigned to rye chromosomes due to the absence of rye-specific PCR products in all wheat-rye addition and substitution lines, and this case might be caused by the genetic polymorphism between rye line and wheat-rye addition lines (Li et al. 2013). Xu et al. (2012) also discovered that some 6R-specific amplicons could not be produced from both 6RS and 6RL telosomic addition lines, and it was deduced that this case might be due to the genomic

alterations after allopolyploidization. Rye chromosome variability and sequence rearrangements in wheat-rye addition lines have been reported (Alkhimova et al. 1999; Bento et al. 2010). Multiple evolutionary translocations in rye genome have also been reported (Devos et al. 1993; Li et al. 2013). When considered all these previous reports, two factors might contribute to the variations of rye-specific and rye chromosome-specific markers in this study. First, polymorphism may occur between the rye line and its corresponding wheat-rye addition lines that were used in this study. Second, the structures of rye chromosomes in wheat-rye addition lines have changed (eg. deletions, translocations) during the procedure of development of the alien chromosome addition lines.

In conclusion, the rye-specific and rye chromosome-specific markers developed in this study have enriched the specific markers available for detecting rye chromatin in wheat backgrounds. Furthermore, these chromosome arm-specific markers provide useful tools for introgression of elite genes on rye chromosome arms into wheat backgrounds.

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References

Alkhimova, A.G., Heslop-Harrison, J.S., Shchapova, A.I., and Vershinin, A.V. 1999.

Rye chromosome variability in wheat-rye addition and substitution lines.

- Chromosome Res. 7(3): 205-212. doi: 10.1023/A:1009299300018.
- Bednarek, P.T., Masojć, P., Lewandowska, R., and Myśków, B. 2003. Saturating rye genetic map with amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers. J. Appl. Genet. 44(1): 21-33.
- Bento, M., Gustafson, P., Viegas, W., and Silva, M. 2010. Genome merger: from sequence rearrangements in triticale to their elimination in wheat-rye addition lines. Theor. Appl. Genet. 121(3): 489-497. doi: 10.1007/s00122-010-1325-6.
- Camacho, M.V., Matos, M., Gonzales, C., Perez-Flores, V., Pernauta, B., Pinto-Carnida, O., and Benito, C. 2005. *Secale cereale* inter-microsatellites (SCIMs): chromosomal location and genetic inheritance. Genetica 123(3): 303-311. doi: 10.1007/s10709-004-5553-z.
- Chen, S.Q., Huang, Z.F., Dai, Y., Qin, Y.Y., Zhang, L.L., Gao, Y., and Chen, J.M. 2013. The development of 7E chromosome-specific molecular markers for *Thinopyrum elongatum* based on SLAF-seq technology. PLoS ONE 8(6): e65122. doi:10.1371/journal.pone.0065122.
- Devos, K.M., Atkinson, M.D., Chinoy, C.N., Francis, H.A., Harcourt, R.L., Koebner, R.M.D., Liu, C.J., Masojć, P., Xie, D.X., and Gale, M.D. 1993. Chromosomal rearrangements in the rye genome relative to that of wheat. Theor. Appl. Genet. 85(6): 673-680. doi: 10.1007/BF00225004.
- Fu, S.L., Tang, Z.X., Ren, Z.L., Zhang, H.Q., and Yan, B.J. 2010. Isolation of rye-specific DNA fragment and genetic diversity analysis of rye genus *Secale* L. using wheat SSR markers. J. Genet. 89(4): 489-492. doi:

10.1007/s12041-010-0070-6.

Gustafson, J.P., Ma, X.F., Korzum, V., and Snape, J.W. 2009. A consensus map of rye integrating mapping data from five mapping populations. *Theor. Appl. Genet.* 118(4): 793-800. doi: 10.1007/s00122-008-0939-4.

Jiang, J.M., Friebe, B., and Gill, B.S. 1994. Recent advances in alien gene transfer in wheat. *Euphytica* 73(3): 199-212. doi: 10.1007/BF00036700.

Khlestkina, E.K. 2014. Current applications of wheat and wheat-alien precise genetic stocks. *Mol. Breed.* 34(2): 273-281. doi: 10.1007/s11032-014-0049-8.

Korzun, V., Malyshev, S., Kartel, N., Westermann, T., Weber, W.E., and Börner, A. 1998. A genetic linkage map of rye (*Secale cereale* L.). *Theor. Appl. Genet.* 96(2): 203-208. doi: 10.1007/s001220050728.

Korzun, V., Malyshev, S., Voylov, A.V., and Börner, A. 2001. A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. *Theor. Appl. Genet.* 102(5): 709-717. doi: 10.1007/s001220051701.

Li, J.J., Endo, T.R., Saito, M., Ishikawa, G., Nakamura, T., and Nasuda, S. 2013. Homoeologous relationship of rye chromosome arms as detected with wheat PLUG markers. *Chromosoma* 122(6): 555-564. doi: 10.1007/s00412-013-0428-7.

Li, R., Yu, C., Li, Y., Lam, T.W., Yiu, S.M., Kristiansen, K., and Wang, J. 2009. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25(15): 1966-1967. doi: 10.1093/bioinformatics/btp336.

Ma, X.F., Wanous, M.K., Houchins, K., Rodriguez, Milla, M.A., Goicoechea, P.G., Wang, Z., Xie, M., and Gustafson, J.P. 2001. Molecular linkage mapping in rye

- (*Secale cereale* L.). Theor. Appl. Genet. 102(4): 517-523. doi: 10.1007/s001220051676.
- Martis, M.M., Zhou, R.N., Haseneyer, G., Schmutzer, T., Vrána, J., Kubaláková, M., König, S., Kugler, K.G., Scholz, U., Hackauf, B., Korzun, V., Schön, C.C., Dolež, J., Bauer, E., Mayer, K.F.X., and Stein, N. 2013. Reticulate evolution of the rye genome. Plant Cell 25(10): 3685-3698. doi: 10.1105/tpc.113.114553.
- Masojć, P., Myśków, B., and Milczarski, P. 2001. Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers. Theor. Appl. Genet. 102(8): 1273-1279. doi: 10.1007/s001220000512.
- Masoudi-Nejad, A., Nasuda, S., McIntosh, R.A., and Endo, T.R. 2002. Transfer of rye chromosome segments to wheat by a gametocidal system. Chromosome Res. 10(5): 349-357. doi: 10.1023/A:1016845200960.
- Milczarski, P., Banek-Tabor, A., Lebiecka, K., Stojałowski, S., Myśków, B., and Masojć, P. 2007. New genetic map of rye composed of PCR-based molecular markers and its alignment with the reference map of the DS2 × RXL10 intercross. J. Appl. Genet. 48(1): 11-24. doi: 10.1007/BF03194653.
- Milczarski, P., Bolibok-Bragoszewska, H., Myśków, B., Stojatowski, S., Heller-Uszyńska, K., Góralska, M., Brągoszewski, P., Uszyński, G., Kilian, A., and Rakoczy-Trojanowska, M. 2011. A high density consensus map of rye (*Secale cereale* L.) based on DArT markers. PloS ONE 6(12): e28495. doi: 10.1371/journal.pone.0028495.
- Murray, M.G., and Thompson, W.F. 1980. Rapid isolation of high molecular weight

- plant DNA. Nucleic Acids Res. 8(19): 4321-4326. doi: 10.1093/nar/8.19.4321.
- Philipp, U., Wehling, P., and Wricke, G. 1994. A linkage map of rye. Theor. Appl. Genet. 88(2): 243-248. doi: 10.1007/BF00225904.
- Saal, B., and Wricke, G. 1999. Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42(5): 964-972. doi: 10.1139/g99-052.
- Senft, P., and Wricke, G. 1996. An extended genetic map of rye (*Secale cereale* L.). Plant Breed. 115(6): 508-510. doi: 10.1111/j.1439-0523.
- Tomita, M., and Seno, A. 2012. Rye chromosome-specific polymerase chain reaction products developed by primers designed from the EcoO109I recognition site. Genome 55(5): 370-382. doi: 10.1139/g2012-024.
- Xu, H., Yin, D., Li, L., Wang, Q., Li, X., Yang, X. Liu, W., and An, D. 2012. Development and application of EST-based markers specific for chromosome arms of rye (*Secale cereale* L.). Cytogenet. Genome Res. 136(3): 220-228. doi: 10.1159/000336478.

Figure captions

Fig. 1. Location of rye-specific markers on individual chromosomes of Kustro. (a, b, c, d, e, f, g, h, i and j) Products amplified by markers Ku.384, Ku.1008, Ku.478, Ku.752, Ku.902, Ku.671, Ku.828, Ku.140, Ku.653 and Ku.401, respectively. Arrows indicate the target bands amplified by each of the rye-specific markers. M=DNA marker. CS=Chinese Spring. MY11=Mianyang 11.

Fig. 2. Location of rye-specific markers on individual chromosome arms of Kustro. (a, b, c, d, e, f, g, h, i, j, k, l and m) Products amplified by markers Ku.1071, Ku.341, Ku.197, Ku.503, Ku.342, Ku.973, Ku.447, Ku.47, Ku.640, Ku.791, Ku.24, Ku.419 and Ku.162, respectively. Arrows indicate the target bands amplified by each of the rye-specific markers. M=DNA marker.