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Production of wheat–*Leymus racemosus* chromosome addition lines

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Abstract We produced ten wheat–*Leymus racemosus* chromosome addition lines. Eight chromosomes (A, C, F, H, I, J, k, and l) were recovered as disomic additions and two (E and n) as monosomic. Screening of the addition lines was done by fluorescence in situ hybridization using several repetitive sequences as probes, which allowed us to identify different *L. racemosus* chromosomes and find many aberrant *L. racemosus* chromosomes. RFLP analysis revealed partial conservation of homology between *L. racemosus* and wheat chromosomes, depending on the homologous groups. Chromosomes A and l belonged to group 2, chromosomes C and I to group 5, and chromosome k to group 6. Chromosomes H and J were a mixture of groups 1, 3, and 7, chromosome n of groups 3 and 7, and chromosomes E and F were of group 4 and others. Comparison of our addition lines with other addition lines showed large cytological differences.

Introduction

Leymus racemosus (2*n*=4*x*=28; genome NsNsXmXm) is evolutionarily distant to wheat and has many characteristics that do not appear in wheat, such as perennial growth habit and propagation by rhizomes. *L. racemosus* is distributed from central Asia to eastern Europe, forming colonies along seashores and inland dry areas. Besides its salt and drought tolerance (McGuire and Dvorak 1981), its habitat in harsh environmental conditions of coastlines suggests the potential of the species to tolerate adverse environmental conditions. The most attractive agronomic character of *L. racemosus* is its high level of resistance to wheat scab (also called *Fusarium* head blight), which is one of the serious wheat diseases. Scab outbreaks occur in moist regions such as Japan and China at several years' intervals (Ban 2002); and scab is predicted to become a worldwide epidemic through the effects of global warming. It can cause substantial damage. For instance, the estimated cost of damages attributed to it amounted to one billion dollars in the United States in 1993 (McMullen et al. 1997).

Qi et al. (1997) reported production of five wheat-*L. racemosus* disomic addition lines and several additional double-disomic addition, di-telosomic addition, and disomic substitution lines. However, these still do not cover the entire genomes of *L. racemosus*; and a high level of scab resistance has not yet been achieved. Here, we report the production of ten wheat–*L. racemosus* addition lines, using an accession of Bulgarian origin. We also characterize the homology of the *L. racemosus* chromosomes.

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Materials and methods

Plant materials

L. racemosus (Lam.) Tzvelev (2*n*=4*x*=28; genome NsNsXmXm) seeds were collected along the Black Sea coast of Balchik, Bulgaria (accession number HT15405). As recipient, common wheat (*Triticum aestivum* L. cv. Chinese Spring; 2*n*=6*x*=42, AABBDD, accession number KT20-3) was used. A hybrid plant between wheat and *L. racemosus* was obtained by treatment with gibberellic acid (100 mg/l) and 2,4-D (50 mg/l) 24 h after pollination, followed by embryo rescue on MS medium [MS salt (Sigma), 2% sucrose, 0.6% type IA agarose (Sigma), pH 5.8]. The F_1 plant was backcrossed with wheat and embryo-rescued. One BC_1F_1 plant was obtained and further backcrossed with wheat to obtain

generations from BC_3F_1 to BC_7F_1. Two lines of Qi et al. (1997), TA7643 and TA7646, were kindly provided by Wheat Genetics Resource Center (Kansas State University, United States) and used for comparison.

Identification of *L. racemosus* chromosomes

Chromosomes were analyzed by fluorescence in situ hybridization (FISH) and genomic (G)ISH, following Mukai (1996) with a modification by Kishii et al. (1999). Five kinds of probes were used for FISH: pLrTaiI-1 (TaiI family sequence in *L. racemosus*; Kishii et al. 1999), pLrPstI-1 (350-bp family sequence in *L. racemosus*; Kishii et al. 1999), pLrAfa1 (Afa family sequence in *L. racemosus*; Nagaki et al. 1999), pTa71 (45S rDNA of wheat; Gerlach and Bedbrook 1979), and 5S rDNA sequences amplified by PCR using *L. racemosus* genomic DNA as a template (Kishii and Tsujimoto 2002). These clones were labeled with digoxigenin-11-dUTP or biotin-16-dUTP by PCR, or in the case of 45S rDNA, were labeled by nick translation with the BioNick labeling system (Gibco BRL).

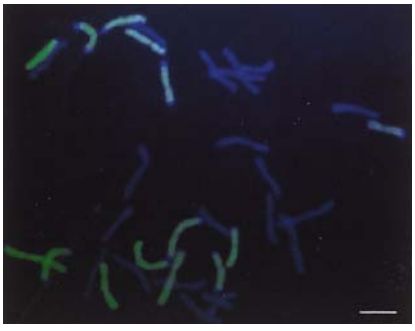

Fig. 1 *Leymus racemosus* chromosomes in the F_1 hybrid. The probe was the total genomic DNA of *L. racemosus*. *Bar* 10 μm

For GISH, genomic DNA of *L. racemosus* was labeled with biotin-16-dUTP using the BioNick labeling system.

Determination of homology

Plant DNA was isolated by the CTAB method (Murray and Thompson 1980) and 15-μg aliquots of the DNA were digested with *Eco*RI, *Dra*I, *Hin*dIII, or *Xba*I. Southern hybridization was conducted as described by Kishii et al. (2001). A total of 42 RFLP markers (ABC, BCD, CDO, KSU, PSR libraries) that exhibited *L. racemosus*-specific bands were used to identify the homology of *L. racemosus* chromosomes in the addition lines and plants.

Results

Production of wheat-*L. racemosus* chromosome addition lines

One embryo was obtained using the embryo-rescue technique and grown to a mature plant. This plant was sterile and showed a perennial growth habit but did not propagate rhizomes. GISH analysis confirmed the presence of 14 *L. racemosus* chromosomes in the F_1 hybrid (Fig. 1). The parental *L. racemosus* chromosomes were assigned by Kishii et al. (1999) as A–J and k–r. The chromosomes indicated by a lowercase letter were those that could not be identified as homologous pairs (Kishii et al. 1999). In the F_1 hybrid, we found the presence of chromosomes k, l, n, and q in addition to the A–J chromosomes, indicating that those were homologous to one of the remaining chromosomes (m, o, p, r).

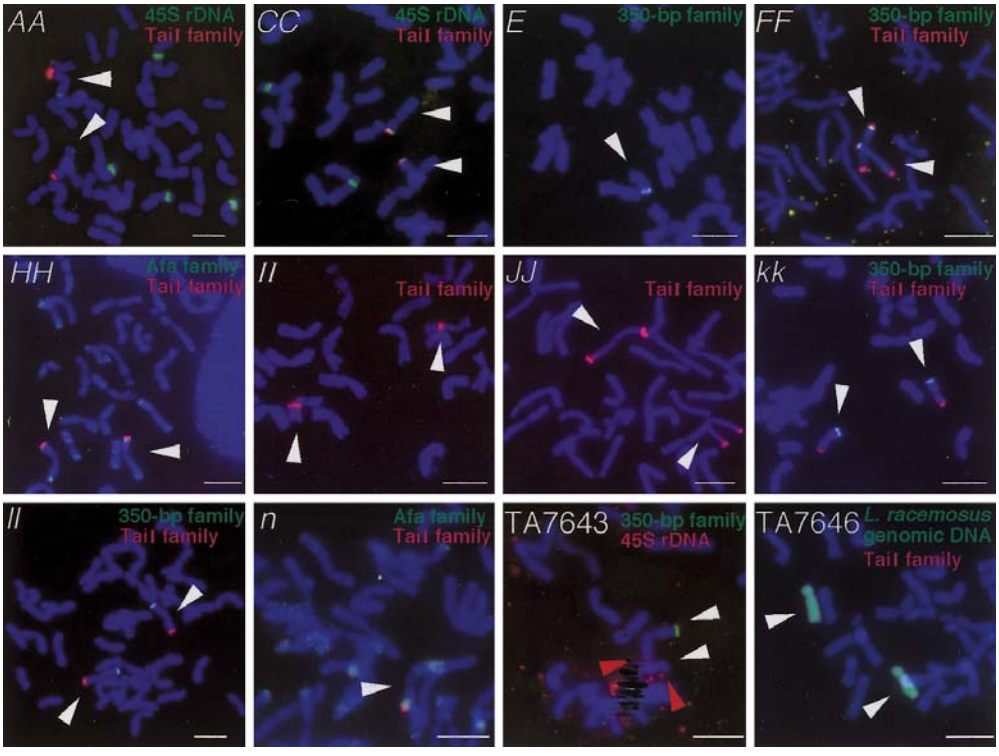

Fig. 2 *L. racemosus* chromosomes in monosomic or disomic addition lines. *Double* or *single letters* indicate disomic or monosomic addition lines, respectively. The probe combinations are indicated at the *upper right* in colors corresponding to the signals. *White arrowheads* indicate *L. racemosus* chromosomes. *Red arrowheads* in line TA7643 point to 45S rDNA sites. *Bars* 10 μm

To obtain amphiploids, we treated the hybrid with colchicine. However, no fertile plants were obtained. Thus, we backcrossed wheat pollen directly to the F_1 plant and obtained one plant after embryo culture. This BC_1F_1 plant showed male sterility and an annual growth habit. We selected monosomic addition plants in the generations from BC_3F_1 to BC_7F_1 and disomic addition lines in their self-pollinated populations. Finally, eight disomic addition lines for chromosomes A, C, F, H, I, J, k, and l and two monosomic addition plants for chromosomes E and n were obtained (Fig. 2). Each chromosome in the addition lines was assigned from their hybridization pattern of several tandem repetitive sequences.

Chromosomes A and C can be identified from their 45S rDNA and TaiI family signals. Other chromosomes can be determined from the number of 350-bp and TaiI family signals: chromosome J has three TaiI family sites (two chromosome ends, one interstitial), chromosome F has two TaiI family and one 350-bp family signals, chromosomes k and l have TaiI family signals in one chromosomal end and one 350-bp family signal in the other end, chromosomes H and n have one TaiI family signal in one chromosomal end, and chromosome E has one 350-bp family signal. Chromosomes k and l can be distinguished by arm ratio and chromosomes H and n are determined by Afa family signals, where chromosome *n* has more signals than chromosome H.

Spikes of the addition lines with these chromosomes are shown in Fig. 3. Chromosomes A and l produced awns; and chromosome E also had awns but they were short. The internodes of chromosomes C and I were elongated, but they were shortened in chromosome F. Spikes of chromosome H became taper-shaped.

Several rearranged chromosomes were observed during the selection of addition lines. Some were derived from a simple rearrangement, like a Robertsonian translocation or misdivision, whereas others originated from more complex rearrangements. However, in many case, these aberrant chromosomes could be distinguished from normal ones based on their morphology and the FISH patterns of chromosomes present in the F1 plant (data not shown).

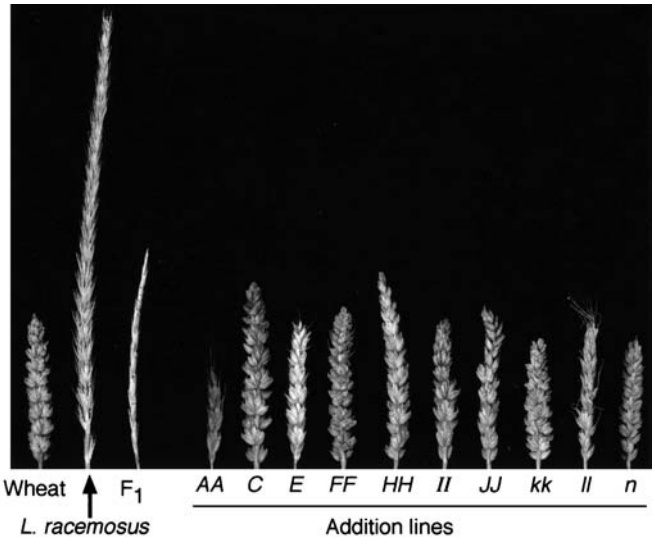

Fig. 3 Spikes of common wheat (cv. Chinese Spring), *L. racemosus*, their F_1 hybrid, and the addition lines. *Double* or *single letters* indicate disomic or monosomic lines, respectively. Spike of chromosome C addition line is of the monosomic plant because of the unavailability of the spike of disomic lines

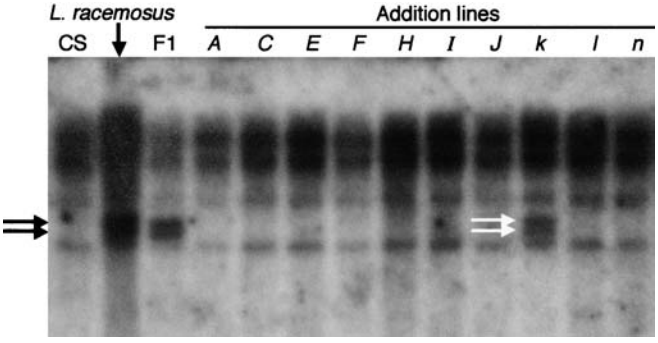

Fig. 4 An example of RFLP analysis for homologous group identification of *L. racemosus* chromosomes added to wheat. Genomic DNAs digested by *Dra*I were probed by PSR142. *CS Triticum aestivum* cv. Chinese Spring, *F1* F_1 hybrid between CS and *L. racemosus*. *Letters* indicate the different monosomic or disomic addition lines. Polymorphic bands of *L. racemosus* are seen in the F_1 hybrid and in the k chromosome addition line

Homology of *L. racemosus* chromosomes in addition lines

We determined the homology of *L. racemosus* chromosomes present in the addition lines, using 42 RFLP markers that we selected because they were entirely distributed within the seven homologous chromosomes (Fig. 4, Table 1).

Group 2 chromosomes

The addition lines carrying chromosomes A and l showed polymorphic bands with markers of homologous group 2. Since *L. racemosus* is tetraploid, chromosome A and l must be in a homologous relationship in group 2. This result was further supported by the awn formation in spikes that is a morphological diagnostic character for group 2 chromosome addition (Fig. 3). However, because it was impossible to distinguish the Ns and Xm genomes by GISH (data not shown), we could not designate them as 2Ns and 2Xm.

Group 5 chromosomes

The addition lines carrying chromosomes C and I showed polymorphism with markers of group 5 only. Thus, these are homologous group 5 chromosomes. However, the conservation of homology was largely different between these two chromosomes. Chromosome I was structurally

Table 1 RFLP profiles of *Leymus racemosus* chromosomes added to wheat. The order of markers reflects the physical order in the wheat consensus map. + Presence of polymorphic bands of *L. racemosus*

Marker	Group	Chromosome											
		AA	C	E	FF	HH	II	JJ	kk	ll	n	TA7643	TA7646
BCD98	1S	−	−	−	−	+	−	+	−	−	−	−	−
PSR596a		−	−	−	−	−	−	−	−	−	−	−	−
BCD371		−	−	−	−	−	−	−	−	−	−	−	−
BCD207	1L	−	−	−	−	−	−	−	−	−	−	−	−
BCD808		−	−	−	+	+	−	−	−	−	−	−	−
KSUG34		−	−	−	−	−	−	−	−	−	−	−	−
PSR666	2S	+	−	−	−	−	−	−	−	+	−	+	−
BCD855		−	−	−	−	−	−	−	−	+	−	−	−
PSR107		+	−	−	−	−	−	−	−	−	−	+	−
PSR126		+	−	−	−	−	−	−	−	+	−	+	−
PSR112	2L	+	−	−	−	−	−	−	−	+	−	+	−
PSR388		+	−	−	−	−	−	−	−	−	−	+	−
KSUF15		+	−	−	−	−	−	−	−	+	−	+	−
KSUH16		−	−	−	−	−	−	−	−	+	−	+	−
PSR598	3S	−	−	−	−	−	−	−	−	−	+	−	−
PSR902		−	−	−	−	−	−	−	−	−	+	−	−
PSR116	3L	+	−	−	−	+	−	+	−	−	−	−	−
PSR394		−	−	−	−	+	−	+	−	−	−	−	−
PSR931		−	−	−	−	+	−	+	−	−	−	−	−
PSR920	4S	+	−	−	+	−	−	−	−	−	−	−	−
KSUF8		−	−	−	+	−	−	−	−	−	−	−	−
CDO1387	4L	−	−	+	+	−	−	−	−	−	−	−	−
PSR164		−	−	−	−	−	−	−	−	−	−	−	−
PSR628	5S	−	−	−	−	−	+	−	−	−	+	−	+
PSR326		−	+	−	−	−	+	−	−	−	−	−	+
KSUD16	5L	−	+	−	−	−	+	−	−	−	−	−	+
PSR360		−	−	−	−	−	+	−	−	−	−	−	+
PSR929		−	−	−	−	−	+	−	−	−	−	−	+
PSR637		−	+	−	−	−	+	+	−	−	−	−	+
PSR370		+	−	−	−	−	−	−	−	−	−	+	−
PSR580		−	−	−	+	−	+	−	−	−	−	−	+
KSU128	6S	−	−	−	−	−	−	−	+	−	−	−	−
CDO534		−	−	−	−	−	−	−	+	−	−	−	−
KSUD17	6L	−	−	−	−	−	−	−	+	−	−	−	−
PSR142		−	−	−	−	−	−	−	+	−	−	+	−
PSR371		−	−	−	−	−	−	−	+	−	−	−	−
KSUF37		−	−	−	−	−	−	−	+	+	−	−	+
PSR160	7S	−	−	−	+	−	−	+	−	−	−	−	−
ABC151		−	−	−	−	+	−	−	−	−	+	−	−
PSR311	7L	−	−	−	−	−	−	+	−	−	−	−	−
PSR129		−	−	−	−	−	−	+	−	−	−	−	−
KSUD2		−	−	−	−	−	−	+	−	−	−	−	−

more similarly conservative to that of wheat than chromosome C. Chromosome C lacked markers in the distal regions of both arms (PSR628, PSR370, PSR580). These markers were located in chromosomes A (PSR370) or chromosome F (PSR580).

Group 6 chromosome

Chromosome k showed polymorphism with all markers of homologous group 6, indicating the structural similarity of this chromosome with wheat one. No other group 6 chromosome could be isolated as an addition line.

Group 1, 3, 4, and 7 chromosomes

Other chromosomes were less conserved and were complicated. Both chromosomes H and J exhibited polymorphisms matching the markers of group 1 (short arm), group 3 (long arm), and group 7 (short arm). Chromosome J also had the markers of group 7 (long arm). Likewise, both chromosomes E and F had the common marker of group 4 (CDO1387), but only chromosome F carried the short arm markers. Chromosome n was also complicated, carrying markers of groups 3, 5, and 7.

Estimated roughly from Table 1, both of the two group 1 chromosomes, one group 6 chromosome, one of the group 3 short arms, and one of the group 7 long arms were missing from the population of the present addition lines. The remaining four chromosomes not obtained here must carry these missing markers.

Comparison of present lines with other lines

We compared the present addition lines with the lines produced by Qi et al. (1997; Fig. 2, Table 1). The RFLP profile of their TA7643 line was similar to our chromosome A line. In addition, with both chromosomes carrying a 45S rDNA locus in the distal region of the long arm (Fig. 2). Thus, these two chromosomes should be homologous. However, the distribution of the repetitive sequences in the terminal region of the short arm was different in each: chromosome A carried TaiI family sequences, whereas the chromosomes in line TA7643 carried 350-bp family sequences.

Likewise, the RFLP profile of TA7646 was identical to those of our chromosome l line, except for the presence of KSUF37 in line TA7646. However, the chromosome of line TA7646 did not carry a notable TaiI family signal in the interstitial region (Fig. 2).

Discussion

Homology of *L. racemosus* chromosomes

The conservation of homology between *L. racemosus* chromosomes and those of wheat varied, depending on the homologous group (Table 1). Chromosomes A and l (group 2), C and I (group 5), and k (group 6) relatively conserved their homology, whereas the other chromosomes consisted of different homologous groups. For instance, H and J carried RFLP markers belonging to three homologous groups of wheat. This result indicates the occurrence of chromosome rearrangements during the evolution of the genomes of *Leymus* and/or wheat from their common ancestor. For example, in group 3, the long-arm markers and the short-arm markers were located on different chromosomes of *L. racemosus*. This must be attributed to a separation within the group by a translocation during the evolution of *L. racemosus*, or to the fusion of two arms originally belonging to chromosomes within different groups during the evolution of wheat.

Although the genomes of the Triticeae have been classified mainly on the chromosome pairing of their hybrids, the phylogenic relationship among the genomes has been inconsistent. In this study, we discuss the evolutional events by allocation of RFLP markers to the alien chromosomes. To use addition lines for this purpose, however, it is important to distinguish the intact chromosomes from rearranged ones. Otherwise, we would mistake the rearrangements occurring during the breeding process of the addition lines as events which occurred during chromosomal evolution. During the study, we carefully selected plants with intact chromosomes present in the original *L. racemosus*, using five chromosome markers as probes for FISH. Actually, two chromosomes of *L. racemosus* in homologous relation, such as A and l, C and I, and H and J, consisted of a similar combination of RFLP markers, indicating that the inconsistency of homologous group seen in the addition lines is attributable to evolutional events.

To name the chromosomes in the manner adopted for wheat chromosome nomenclature, we have to know information about the genome to which the chromosomes belong. Although we tried to distinguish the Ns and Xm genomes of *L. racemosus* using GISH probes from diploid *Psathyrostachys* species carrying the Ns genome, the two genomes could not be discriminated. Thus, it was impossible to rename the *L. racemosus* chromosomes following the wheat manner.

Chromosomal variation of *L. racemosus*

The RFLP profiles of *L. racemosus* chromosomes in the present lines were well consistent with those of the chromosomes in the lines of Qi et al. (Table 1), e.g., our chromosome A corresponds to the chromosome in their line TA7643 and our chromosome I to that in their line TA7646. In addition, the sizes of restriction fragments were mostly the same between both the accessions of *L. racemosus*. However, the distribution of TaiI family and 350-bp family sequences in their chromosomes were greatly different from each other (Fig. 2). The accession used here originated from Bulgaria and was rich in TaiI family sequences (Fig. 2; Kishii et al. 1999). In contrast, the accession used by Qi et al. (1997) was rich in 350-bp family sequences (data not shown). According to Wang et al. (1986), that accession derived from the gene bank of Utah State University and, according to Dr. R.C. Wang (Utah State University), it was collected in Russia. These sorts of repetitive sequence would evolve very rapidly. Indeed, we previously showed the polymorphic nature of the distribution of these repetitive sequences in the chromosomes of *L. racemosus* (Kishii et al. 1999).

Use of wheat-*L. racemosus* addition lines for practical breeding

The present wheat–*L. racemosus* addition lines could be used in practical wheat breeding. Preliminary studies showed the following characters: the addition line with chromosome J showed resistance to leaf rust (courtesy of Dr. R.A. McIntosh, University of Sydney, Australia) and an increase in SDS sedimentation value (courtesy of Mr. H. Tanaka, Tottori University, Japan). This chromosome also transmits preferentially from the male side. The addition line with chromosome H showed early heading; and that with chromosome I showed large seeds. The present addition lines are now open for the evaluation of practical breeding.

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