Alien DNA introgression and wheat DNA rearrangements in a stable wheat line derived from the early generation of distant hybridization

ZHANG Lianquan, LIU Dengcai, YAN Zehong & ZHENG Youliang

Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan 611830, China
Correspondence should be addressed to Liu Dengcai (email: dcliu7@yahoo.com)

Received Augest 31, 2004; revised June 23, 2005

Abstract Polyploidy has been found to be common in plants. Bread or common wheat (*Triticum aestivum* L., 2*n* = 42) is a good example of allopolyploid made up of three diploid genomes A, B and D. In recent years, by the study of mimicking the origination of common wheat, it was found that changes of DNA sequence and gene expression occurred at the early stages of artificial allohexaploid between tetraploid wheat and *Aegilops tauschii*, which was probably favorable to genetic diploidization of new synthetic hexaploid wheat. Common wheat 99L2 is a new line stable in genetic, which was derived from the early self-pollinated generation of wide hybrids between common wheat and rye. In this study, it was found that at least two rye DNA segments had been introgressed into 99L2. This result suggested that a mechanism of alien DNA introgression may exist, which was different from the traditional mechanism of chromosome pairing and DNA recombination between wheat and alien species. Meanwhile, during the introgression process of alien rye DNA segments, the changes in DNA sequences of wheat itself occurred.

Keywords: wheat, rye, distant hybridization, early generation stability, genome evolution.

DOI: 10.1360/062004-45

In general, it requires five or more generations to develop a stable line by the traditional breeding method of cultivar hybrids in self-pollinated crops, such as wheat, barley, and rice. No doubt, the breeding period will be shortened and thus the breeding efficiency will be improved if the hybrids can stabilize in early generation. The method of haploid breeding, that is, obtaining stable pure lines (DH lines) of cultivar hybrids with the aid of anther culture and then chromosome doubling, can shorten breeding period[1]. Surprisingly, in rice, stable F_2 plants in genetic could be obtained from the cross between special diploid, triploid or tetraploid derived of diploid, and a normal diploid variety. These F_2 lines showed stability both in morphology and DNA level. They carried the DNA both maternal and paternal parents, with a bias of DNA bands to female or male. Some new DNA bands absent in parents appeared in these F_2 lines. Stability of F_2 generation can also be utilized in shortening rice breeding cycle[2—3].

Different from inter-cultivar cross, due to the large genetic differences between crop and alien species, progenies of distant hybridization segregate crazily, thus resulting in much more difficulty in stabilization. Sometimes, it is impossible to obtain stable progenies from distant hybridization. However, stable pure crop lines could be quickly obtained in early generation by

chromosome elimination of wide crosses followed by chromosome doubling[4,5]. There was no report about chromosome elimination in distant hybridization between common wheat and rye (*Secale cereale* L., $2n = 2x = 14$, RR). Unexpectedly, we obtained a wheat line 99L2[6], which was stabilized in early generation and produced by self-crossing of inter-generic hybrids between Shinchunaga, a common wheat landrace from Japan, and rye, *S. cereale* L. cv. Qinling from China. 99L2 was similar to normal common wheat in morphology and had the chromosome number of $2n = 42$. During the metaphase I of meiosis of pollen-mother-cells, these chromosomes showed normal pairing with 21 bivalents as common wheat did.

In the present work, at the cytological, biochemical and molecular level, we compared wheat 99L2 with its wheat parent "Shinchunaga" and rye parent Qinling. It was showed that not only were rye alien DNA segments introgressed into 99L2 but also wheat DNA sequences of 99L2 were rearranged.

1 Materials and methods

1.1 Plant materials

F_3-stabilized line 99L2 and its self-pollinated F_4 and F_5 generations, its common wheat parent "Shinchunaga" and rye parent Qinling were used in this study. All these materials were preserved in Triticeae Research Institute of Sichuan Agricultural University. Qinling rye was a Chinese rye cultivar, immune to powdery mildew and stripe rust disease. Shinchunaga was a common wheat landrace from Japan, with good resistance to wheat scab (head blight), provided by Dr. Shin Taketa, Barley Germplasm Center, Research Institute for Bioresources, Okayama University, Japan.

The production of 99L2 was as follows[6]. Three F_2 seeds were obtained by self-crossing of 15 F_1 hybrids between Shinchunaga and Qinling rye. These F_2 seeds were germinated in petri dishes and three F_2 plants were obtained, namely F_2-1, F_2-2, F_2-3. The three F_2 plants were planted into the field at the trifoliate stage, but F_2-1 and F_2-3 plants were destroyed by insect at the seedling stage and only F_2-2 plant remained. All the F_3 plants from self-pollinating F_2-2 showed the same agronomic phenotype and there was no segregation in morphological performance. This stable line was named 99L2. The F_2-1 and F_2-3 had no progenies due to injurious insect and thus there was no chance to compare F_2-2 with its sister plants in F_2 and F_3 generations. However, seed-setting and other agronomic performances were consistent between plants of F_2-2 and its progeny F_3, which indicated that 99L2 might have been stabilized in plant F_2-2.

1.2 Observations on mitotic chromosome of root-tip cells and meiosis of pollen mother cells (PMCs)

The root tips were stained with Feulgen and then were squashed with basic fuchsine. Anthers with PMCs from metaphase I to the tetrad stage were fixed in Carnoy solution I and slides were then made in basic fuchsine. Observations were made under a microscope Olympus BX-51.

1.3 Giemsa C-banding and Genomic *in situ* Hybridization (GISH) analysis

Giemsa C-banding were according to Gill et al.[7]. Chromosome identification of wheat and rye was respectively according to Gill et al.[7] and Lukaszewski and Gustafson[8]. GISH was performed according to the protocol published by Kansas State University, USA in http://www.ksu.edu/wgrc/Protocols.

1.4 Biochemical analysis

SDS-PAGE analysis referred to Yan et al.[9]; APAGE (pH3.2) analysis was according to the standard procedure published by ISTA in 1986.

1.5 RAPD analysis

Twelve RAPD markers (OPA-08, OPC-07, OPB-12, OPJ-13, OPJ-18, OPI-09, OPM-01, OPO-10, OPP-06, OPT-12, OPY-07, OPY-20) for rye chromosome arm 2RS were used and PCR reaction procedure was according to Brunell and Lukaszewski[10]. PCR products were fractionated in 0.8% agarose gels in 1× TAE buffer.

1.6 SSR analysis

58 SSR markers specific for different chromosome arms in wheat genome were selected for SSR analysis,

which were developed by Röder et al.[11]. They were chosen according to their map location in order to have at least one microsatellite on each chromosome arm. SSR markers amplification were according to Röder et al.[11]. PCR products were fractionated in 2.0% agarose gels at 5 V/cm in 1× TAE buffer. In this study, these SSR markers were numbered as follows. Number 1 — 4: X*gwm*164, X*gwm*550, X*gwm*192, X*gwm*233; Number 5—35: X*gwm*135-1AL, X*gwm*232-1DL, X*gwm*614-2AS, X*gwm*328-2AL, X*gwm*356-2AL, X*gwm*374-2BS, X*gwm*261-2DS, X*gwm*157-2DL, X*gwm*369-3AS, X*gwm*674-3AL, X*gwm*547-3BL, X*gwm*161-3DS, X*gwm*52-3DL, X*gwm*3-3DL, X*gwm*4-4AS, X*gwm*368-4BS, X*gwm*107-4BL, X*gwm*113-4BL, X*gwm*538-4BL, X*gwm*251-4BL, X*gwm*304-5AS, X*gwm*174-5DL, X*gwm*494-6AL, X*gwm*570-6AL, X*gwm*469-6DS, X*gwm*332-7AL, X*gwm*400-7BS, X*gwm*43-7BS, X*gwm*611-7BL, X*gwm*428-7DL, X*gwm*295-7DS; Number 36-58: X*gwm*136-1AS, X*gwm*11-1BS, X*gwm*140-1BL, X*gwm*106-1DS, X*gwm*337-1DS, X*gwm*512-2AS, X*gwm*429-2BS, X*gwm*148-2BS, X*gwm*526-2BL, X*gwm*120-2BL, X*gwm*162-3AL, X*gwm*389-3BS, X*gwm*160-4AL, X*gwm*6-4BL, X*gwm*149-4BL, X*gwm*194-4DL, X*gwm*609-4DL, X*gwm*186-5AL, X*gwm*234-5BS, X*gwm*408-5BL, X*gwm*459-6AS, X*gwm*132-6BS, X*gwm*219-6BL.

1.7 Rye-specific subtelomeric DNA sequence identification

For detecting rye-specific chromatin, PCR primers (5′-TGAGGGCCCAGACGGCCCTTTTTG-3′ and 5′-TTATCGCAATTACAACTCAAATTT-3′) designed for the approximately 350-bp rye sub-telomeric sequence pAW161 were used. PCR reaction system and amplification procedure were according to Bourdoncle and Ohm[12]. PCR products were fractionated in 2.0% agrose gels at 5 V/cm in 1× TAE buffer.

1.8 DNA cloning and sequencing of RAPD, SSR and rye-specific subtelomeric sequences

V-gene DNA Kit was used for extraction and purification of DNA fragment. The PCR products were resolved on 2% agarose gels. The repeatable RAPD, SSR or rye-specific sequences were respectively cloned into pMD18-T vector[9]. Clones were amplified with the original corresponding primers. After the positive clones were identified, the corresponding cloned DNA fragments were then sequenced by the commercial company TaKaRa Biotechnology (Dalian) Co., Ltd (http://www.takara.com.cn). Sequence analysis and similarity searching were performed with the BLASTN program, which was included in the URL: http://www.ncbi.nlm.nih.gov.

2 Results and analysis

2.1 Genetic stability of 99L2

By observations of several years, it was indicated that 99L2 did not segregate in morphologic characters and showed stable phenotypes, having no aberrant plants. During seedling stage, the morphological character of 99L2 was very like that of its wheat donor Shinchunaga and it was difficult to differentiate each other. After seedling stage, there had some differences in agronomic performances between 99L2 and Shinchunaga. 99L2 showed late sprouting and maturity, higher plant height, tough glumes, longer ears and good resistance to stripe rust, which may be derived from Qinling rye.

99L2 showed a stable cytology in F_3 generation[6]. In this study, the results of cytological and biochemical analysis on its F_4 and F_5 generations further proved the stability in genetics. 99L2 had the chromosome number with $2n = 42$, including two pairs of satellite chromosomes; PMCs of 99L2 regularly showed 21 bivalents in meiotic metaphase I; all the plants of 99L2 had consistent glutenin and gliadin patterns.

2.2 Alien genetic identificaton in 99L2

(i) PCR amplification and sequencing analysis of rye-specific subtelomeric DNA. Using primers designed for pAW161[12], a sharp amplification product of approximately 350-bp rye telomeric sequence was obtained for both introgression line 99L2 and Qinling rye parent, but no product could be observed for wheat parent Shinchunaga (Fig. 1), indicating that the band of 99L2 was probably from Qinling rye. The re-amplified PCR products were used for cloning. Three positive clones were respectively screened out

from 99L2 and Qinling rye. These positive clones were then sequenced. The results indicated that the sequence with a length of 348-bp (Fig. 2) derived from 99L2 (GenBank Accession No. AY952859) was totally matched to that of Qinling rye (GenBank Accession No. AY952860). This proved that the 348-bp fragment of 99L2 was derived from Qinling rye.

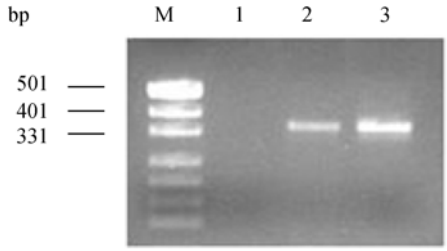

Fig. 1. Amplification of rye-specific subtelomeric sequence. M, Marker; 1, Shinchunaga; 2, introgression line 99L2; 3, Qinling rye.

Similarity searching in the GenBank database with the BLASTN program indicated that the 348-bp sequence showed highest homology with family pSc200 of rye subtelomeric repeat. The identities with the sequences from rye cultivar Onohoiskayia (GenBank Accession No. Z54189.1) and Imperial (GenBank Accession No. AY522380.1) were 97% (expect=e^{-169}) and 92% (expect=e^{-121}), respectively.

(ii) Giemsa C-banding and GISH analysis of 99L2. The C-banding analysis indicated that 99L2 had no rye terminal specific C-bands. There was no obvious difference between the C-bands of 99L2 and those of common wheat. 99L2 was then analyzed by GISH with the genomic DNA of Qinling rye used as a probe and Chinese Spring wheat genomic DNA as a block. However, no hybridization signals of rye chromosome segments were detected.

2.3 Comparison of biochemical markers between 99L2 and its donor parents

In comparison with its donor parents, 99L2 had the bands of *Sec-3* locus, which encodes high-molecular-weight (HMW) secalins located on 1RL and *Sec-2* locus, which encodes 75 K *γ-secalins* located on 2RS[13], indicating that the corresponding DNA fragments on 1RL and 2RS of rye were transferred into 99L2 (Fig. 3). At the same time, two HMW-GS bands in Shinchunaga, located on 1DL and 1BL, respectively, disappeared in 99L2. Gliadin analysis by APAGE suggested that some bands in α, β and ω regions of Shinchunaga disappeared in 99L2, while some new bands appeared in α, β, γ and ω regions of 99L2, indicating the changes of DNA sequences related to gliadin expression, which was located on the chromosome short arms of one or six homoeologous group in wheat (Fig. 4).

2.4 RAPD comparison between 99L2 and its donor parents

Twelve specific RAPD markers on 2RS[10] were used. The results showed that nine markers OPB-12, OPC-07, OPI-09, OPJ-13, OPT-12, OPJ-18, OPM-01, OPY-07 and OPY-20 respectively amplified same bands between 99L2 and Shinchunaga. Of these, seven markers respectively produced different bands between 99L2 and Qinling rye. Two markers respectively produced same bands among 99L2, Shinchunaga and Qinling rye. Marker OPO-10 amplified a sequence with similar size between 99L2 and Qinling

TGAGGGCCCA GACGGCCCTT TTTGGGCTCA GAATTTTCCG CGCACGTGAT CCTGTGTGTG
CACGTATGTA AAATCCTCCA GATCGTAAAA ACTAGAAGTC CTATCACCTC AATTCCCCAA
TAGCTTTCCA ACGCCTATGA AAACGACGAA ATCGGTGCTT GTTCTCACTT GCTTTGAGAG
TCTCGATCAA TTCGGACTCT AGGTTGATTT TTGTATTTTC TTTGATCACC GTTTCTTCGC
GAGAGTTGGC CTAACACCCT ATTGTATCCA TACATGGGTG GGGCGCCAAG GACCTGAACA
CCAAAGTGAT ATGCCGGCTC ATCAAAATTT GAGTTGTAAT TGCGATAA

Fig. 2. Qinling rye-specific subtelomeric sequence. Primer sequences are in border; tandem repeat sequences are in underlined boldface; different reverse repeat are respectively underlined in different styles.

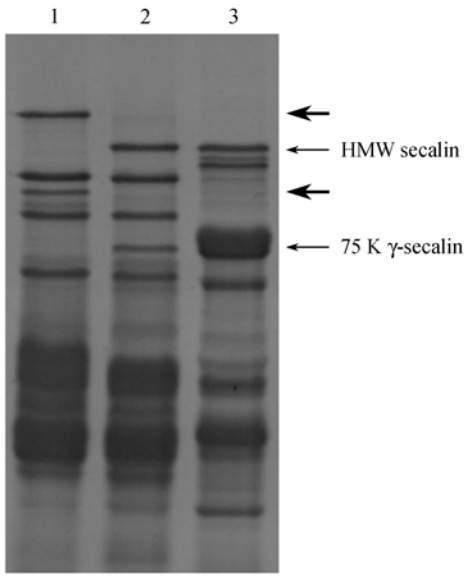

Fig. 3. Glutenin pattern of 99L2 and its donor parents. 1, Shinchunaga; 2, 99L2; 3, Qinling rye (Thin arrows indicated two rye secalins being introgressed into 99L2, thick arrows indicated disappearance of two subunits in 99L2).

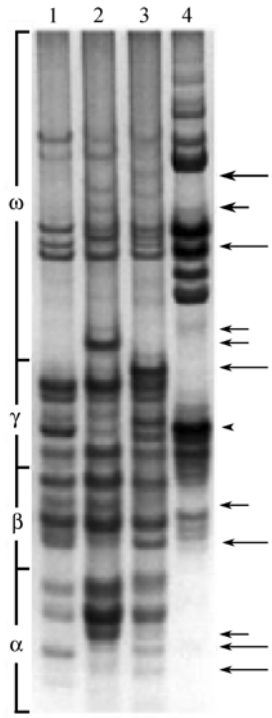

Fig. 4. Gliadin pattern of 99L2 and its donor parents. 1, Chinese spring; 2, Shinchunaga; 3, 99L2; 4, Qinling rye (Short arrows indicated disappearance of bands in 99L2, long arrows indicated appearance of new bands in 99L2, the triangular arrow indicated bands with varied molecular sizes).

rye (Fig. 5, band 1). However, the sequencing results showed that DNA sequences of the corresponding band in 99L2 were very different from that of Qinling rye (GenBank Accession No. AY954973, AY954974, identities=51%). This indicated that the RAPD band in 99L2 was not derived from Qinling rye.

RAPD markers OPA-08 and OPP-06 respectively amplified additional new bands in 99L2. OPO-10 amplified a sequence with smaller size in 99L2 than Shinchunaga (Fig. 5, band 2). These results indicated that DNA sequences in 99L2 were changed in comparison with that of Shinchunaga. The sequencing results showed that the smaller sequence in 99L2 (Fig. 5, band 2) amplified by OPO-10 had a size of 642 bp (GenBank Accession No. AY954977), which was a part of long terminal repeat of wheat retrotransposon Angela-2, suggesting that the new band of 99L2 might be derived from transposon activation.

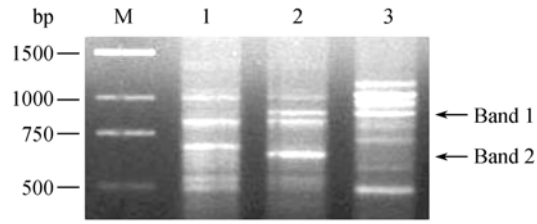

Fig. 5. Results of RAPD amplification using marker OPO-10. M, Marker; 1, Shinchunaga; 2, 99L2; 3, Qinling rye (Band 1 appeared in both 99L2 and Qinling rye; Band 2 appeared in 99L2 with smaller molecular size than in Shinchunaga).

2.5 SSR comparison between 99L2 and its donor parents

58 SSR markers located on 21 chromosomes of wheat were used in this study[11]. Among them, 4 markers (number 1—4) amplified no products both in 99L2 and its donor parents and the remainder 54 markers produced amplification in 99L2 and Shinchunaga. Among the 54 markers, 23 amplified no products in Qinling rye. This suggested that many SSR markers (27/58 = 47%) had no transferability between wheat and rye, which could not be used in rye.

Among the 54 markers with amplification products, 31 markers (number 5—35, 31/54 = 57%) respectively amplified same bands between 99L2 and Shinchunaga.

The remainder 23 markers (number 36—58, 43%) respectively amplified differences between 99L2 and Shinchunaga. This suggested that DNA sequences in 99L2 revealed by the corresponding 23 SSR markers located on wheat 17 chromosome arms were changed in comparison with that of Shinchunaga. These changes included: (i) the SSR bands in Shinchunaga disappeared in 99L2; (ii) additional bands appeared in 99L2; (iii) molecular sizes of bands were different between 99L2 and Shinchunaga (Fig. 6).

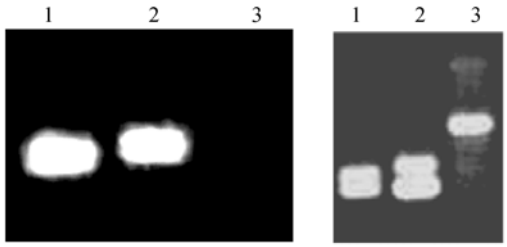

Fig. 6. SSR amplification by markers *Xgwm*337 (left) and *Xgwm*234 (right). 1, Shinchunaga; 2, 99L2; 3, Qinling rye.

Marker X*gwm*6-4BL produced one band with same size between 99L2 and Qinling rye, which did not exist in wheat parent Shinchunaga (Fig. 7). However, the sequencing results showed that the DNA sequence of the band in 99L2 (197 bp, GenBank Accession No. AY952872) was different from that of Qinling rye (197 bp, GenBank Accession No. AY954981), indicating that the band in 99L2 was not derived from Qinling rye. This band in 99L2 was probably caused by the increment of 12 additional GA repeats of SSR sequences of wheat parent Shinchunaga since the sequences of the corresponding band (173 bp, GenBank Accession No. AY952871) in Shinchunaga were different from that of 99L2 only in a reduction of 12 GA repeat units.

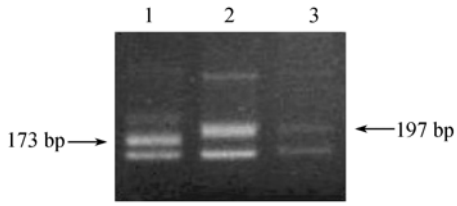

Fig. 7. SSR amplification by marker *Xgwm*6. 1, Shinchunaga; 2, 99L2; 3, Qinling rye.

3 Discussion

3.1 Rye DNA segments were introduced into 99L2

Compared with its donor parents, 99L2 showed some agronomic characteristics similar to rye parent, such as late maturity, higher plant height, tough glumes, longer ears and good resistance to stripe rust[6]. Lukaszewski et al.[14] showed that tough glumes were controlled by Gt gene located on 2RS. Higher plant height was also related to 2R[15]. From these morphological data, it was concluded that genetic materials on 2RS of QInling rye were transferred into 99L2. In this study, SDS-PAGE analysis indicated that 75K *γ-secalins* encoded by *Sec-2* locus on 2RS was also transferred into 99L2. *Sec-2* locus was close to the terminal C-bands of 2RS while Gt gene was located in the sub-terminal region. There was a genetic distance of about 10 cM between *Sec-2* and Gt[13,14]. However, no rye telomeric C-band was detected in 99L2, thus indicating the missing of rye telomeric C-band on 2RS. It was surprising that no rye chromosome segments were detected by GISH. Similarly, Lukaszewski et al.[14] unexpectedly found in the study of 2R that rye genetic materials could not be detected by GISH even though their presence was obvious based on the C-banding patterns and mapping with DNA markers. It seemed that the phenomenon could not be due to too small alien DNA segment introgression. Specific behaviors of chromosome 2R have been observed. For example, (i) 2RS arm was seldom paired with wheat chromosomes even when Ph1 gene was in deficiency[16]; (ii) loss of telomeric heterochromatin from 2RS in wheat background was easy to happen[17]; (iii) chromosome 2R was easiest subjected to rearrangements[18]; (iv) chromosome 2R had the most frequent to misdivide[8]. However, it is unclear whether or not the particular behaviors of chromosome 2R are related to the default of detecting 2RS fragments in wheat background by GISH.

Compared with its rye parent, 99L2 had the high-molecular-weight secalin encoded by *Sec-3* locus located on 1RL[13], which indicated that the corresponding genetic materials from 1RL of rye were introduced into 99L2. In addition, a kind of Qinling

rye-specific subtelomeric repeat sequence was also incorporated into 99L2. But in this study we still cannot tell that the repeat sequence was derived from which rye chromosome(s).

3.2 Mechanism for rye DNA introgression into 99L2 and early generation stability

DNA fragments at least in two different rye chromosomes were simultaneously transferred into wheat Shinchunaga and produced the stable wheat line 99L2 in early self-pollinated generation. This is difficult to be explained by the traditional mechanism of genetic recombination, by which alien genetic materials were transferred into wheat through translocations derived from homoeologous chromosome pairing in meiosis and exchange between wheat and alien species. One reason is that our results did not find the loss of wheat chromosome arms (or big segments) in 99L2 according to standard Giemsa C-bands[7] and microsatellite map of wheat[11]. According to the traditional mechanism of genetic recombination, if wheat-rye pericentric (or big-segment) translocation happened, there would be a consequence of loss of corresponding wheat chromosomal arms (or big segments). Another reason is that frequency of homoeologous chromosome pairing between Shinchunaga and Qinling rye was very low and no ring bivalents existed (data not provided), so it was almost impossible to obtain small segment translocations involved in more than two different chromosomes from early self-pollinated generation. Previous studies have found that alien genetic materials could also be introduced and stable lines could be obtained in wider crosses between maize[5,19], rice[20] and their alien species though there were no chromosome pairs in meiosis. For example, from the progenies of wide cross between maize and *Tripsacum*, new maze lines with chromosome number of $2n = 20$ same as normal cultivars were obtained, which showed some characteristics from *Tripsacum*[19]. In addition, alien DNA segments can be introduced into crops by using other special methods, such as (i) chromosome elimination system in wide crosses[4,5,21−23]; (ii) asymmetric somatic hybridization[24−26]; (iii) chromosome structural changes by gametocidal gene[27]; (iv) anther culture[28,29]; (v) chromosome instability by monosomic addition[15,28,29]; (vi) pollen tube channel technique, etc. The above methods for alien DNA introgression cannot be reasonably explained with the traditional mechanism of genetic recombination too. Zhou et al.[20] provided a new hypothesis under the chromosome level. The hypothesis suggested that partial DNA segment hybridization between crop and its distant species was a mechanism of introgression alien DNA into crops. Besides wide crosses, stable F_2 plants in genetic could be obtained from the cross of different rice cultivars[2,3]. These stable plants carried the DNA both maternal and paternal parents, with a bias of DNA bands to female or male. Wang et al.[3] have suggested the mechanism of somatic chromosome paring and recombination followed by loss of heterozygosity and non-identical daughter cells.

Compared with others, wheat-rye wide cross was special. For example, Qi et al.[30] found that the hybrid F_1 between Chinese wheat landrace Huixianhong and rye cultivar Jingzhou showed very high spontaneous seed sets, which produced 13 F_2 plants with chromosome number ranging from 42 to 51, respectively. Luo et al.[31] found that the hybrid F_1 plants between Chinese wheat landrace Xiaoyuermai and rye cultivar Winter also showed high spontaneous seed sets. Four F_2 plants with chromosome number ranging from 40 to 44, respectively were obtained, which was probably related to the occurrences of cytomixis and cell fusion. The above studies suggested that it was possible to obtain stable wheat lines with 42 chromosomes from the self-pollinated progenies of wheat-rye wide crosses. In fact, the wheat-rye 1BL/1RS translocation line Riebesel 47—51, which has been widely used in world wheat breeding with a tremendous contribution, was obtained from the self-pollinated progenies of crosses between wheat “Lembkes Obotriten” and rye cultivar Petkus Roggen[32].

Though the mysterious mechanisms for the origin of 99L2 were still unclear, the producing process of 99L2 probably included several steps. First of all is sexual wide hybridization between wheat parent Shinchunaga and rye parent Qinling and obtained haploid hybrid F_1 with genome ABDR ($2n$=28). Then,

small fragments of Qinling rye chromosomes were transferred into wheat Shinchunaga and other chromatins of Qinling rye were eliminated, thus resulting in the formation of haploid genome ABD (2*n*=21) with introgressed rye chromatin. Finally, through chromosome doubling 99L2 with genome AABBDD (2*n*=42) incorporated with small rye DNA fragments was obtained. Anyway, 99L2 was obtained in early hybrid generations; a mysterious mechanism for chromosome doubling should exist for the origin of 99L2. The chromosome doubling results in completely stable genetic constitution, which is very desirable for genetic research, especially at the molecular level to reveal minor modifications.

3.3 Changes of wheat DNA sequences in 99L2 with incorporated rye DNA

Polyploidy has been found to be common in plants. Bread or common wheat (*Triticum aestivum* L., 2*n* = 42) is a good example of allopolyploid made up of three diploid genomes A, B and D, formed ca. 8500 years ago by crossing between *T. turgidum* (maternal) and *Ae. tauschii* (paternal) and then chromosome doubling. It was found by mimicking the origination of common wheat that changes of some kinds of DNA sequences occurred at the early stages of artificial allohexaploid during which D genome of *Ae. tauschii* were introduced into tetraploid wheat (genome AABB)[33,34]. The changes might be favorable to rapid evolution of newly formed species. Rapid changes of DNA sequences from diverged genomes will lead to further rapid differentiation among the divergent genomes in the early generation of polyploidization, which is favorable to chromosome diploidization. That is, meiotic pairing of homoeologous chromosomes is suppressed, thus leading to a pairing pattern similar to that of diploids with exclusive bivalent pairing of homologous chromosomes. This is contributed to strict chromosome pairing and segregation and further ensuring normal chromosome behavior. At the same time, rapid changes have occurred at the gene expression level[33−36], which is probably favorable to genetic diploidization of gene expression and makes the genetic behavior more harmonious. These kind changes were also a widespread phenomenon in other allopolyploids.

By the study on a stability wheat line derived of early self-pollinating generation from wide cross between common wheat and rye, we found that DNA sequences of wheat itself were changed during introgression of partial rye DNA segments rather than all the rye R genome into wheat genomes. Comparing 99L2 with its wheat parent Shinchunaga at the biochemical and molecular level, changes were shown as follows: (i) changes were detected by 43% SSR markers at different locations of chromosome arms, which may be at any location such as terminal, middle, near-centromere according to the microsatellite map of wheat[11]. The changes included band disappearance of Shinchunaga (SSR band number reduced in 99L2) and additional band appearance in 99L2 (SSR band number increased). This phenomenon was also observed in artificial allopolyploid triticale, derived from cross between wheat and rye[37], and artificial allohexaploid wheat between tetraploid wheat and *Ae. tauschii*[38]. This kind of change was probably related with the changes of SSR primer sequences. Another change of SSR was in molecular size. This kind of changes resulted from the repeat unit variance in SSR repeat regions, which was probably related to the DNA replication slippage (data not provided); (ii) changes were revealed by RAPD markers, which was probably related with transposon activity; (iii) changes in high-molecular-weight subunits and gliadins may be related to corresponding DNA sequences of protein expression, which was probably triggered by wide cross. Studies have shown that intergenic regions of glutenin subunits were mainly made up of transposons[39−41], which would lead to the occurrence of DNA sequence changes. High recombination frequencies have occurred in the chromosomal region of gliadin loci[42]. Changes of DNA sequences in wheat itself might also be related to genetic harmony and genetic stability of wheat after incorporation of alien DNA segments.

Acknowledgements We thank Dr. Shin Taketa of Okayama University for providing the wheat Shinchunaga seeds and Prof. Shu Huanlin of Sichuan Agricultural University for technical assistance in C-banding and GISH hybridization. This work was supported by the Program for New Century Excellent Talents in University (Grant No. NCET-04-

0908), Changjiang Scholars and Innovative Research Team in University (Grant Nos. PCSIRT & IRT0453), National Natural Science Foundation of China (Grant Nos. 30270804 & 30070462), Education Committee, and Science and Technology Committee of Sichuan province.

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