Homoeologous cloning of ω-secalin gene family in a wheat 1BL/1RS translocation

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ABSTRACT

Wheat 1BL/1RS translocations are widely planted in China as well as in most of the wheat producing area in the world for their good qualities of disease resistance and high yield. 1BL/1RS translocations are however poor in bread making, partially caused by a family of small monomeric proteins, ω-secalins, which are encoded by genes on 1RS. Based on published sequence of a rye ω-secalin gene we designed a pair of primers to cover the whole mature protein coding sequence. A major band could be amplified from 1BL/1RS translocations but not from euploid wheat. Using this primer set we conducted PCR amplification by using high fidelity Pfu polymerase on the genomic DNAs and cDNAs purified from a 1BL/1RS translocation Lankao 906. Sequencing analysis indicated that this gene family contains several members of 1150 bp, 1076 bp, 1075 bp, 1052 bp and 1004 bp genes, including two pseudogenes and three active genes. The gene transcripts were differentially expressed in developing seeds.

Keywords: wheat, 1BL/1RS translocation, ω-secalin, quality, homoeologous cloning.

INTRODUCTION

The short arm of rye 1R chromosome contains several disease resistance genes such as leaf rust, stem rust, stripe rust and powdery mildew. The 1BL/1RS translocation, involving the short arm of rye chromosome 1R and the long arm of wheat chromosome 1B was originally selected for its resistance to diseases [1, 2]. Since 1BL/1RS translocations were introduced into China during early 70s last century, they have been widely used in wheat breeding programs. Statistic data showed that among 179 wheat cultivars and recently bred wheat lines 38% are 1BL/1RS translocations. In some major wheat growing area 1BL/1RS translocations account for up to 59% of the total wheat cultivars [3]. Except of the advantage in disease resistance, the translocation is also useful for its positive effects on agronomic traits including yield performance, yield stability and wide adaptation [4-10]. Furthermore, 1BL/1RS translocations carrying new disease resistance characteristics have been developed via wide crosses between wheat and rye or triticale. The 1BL/1RS translocation became one of the most frequently used alien introgressions in wheat breeding programs throughout the world [11]. However, serious defects in bread processing such as poor mixing tolerance, superficial dough stickness and low bread volume have been brought about by the translocation [12-16]. Recently a correlation between the translocation and the poor qualities in noodle processing has also been reported [17].

Locus *Sec*-1 in 1RS chromosome arm encodes two kinds of seed storage proteins ω-secalins and 40K γ-secalins [18, 19]. ω-secalins and 40K γ-secalins are separated groups of genes although they are tightly linked to each other [20]. It is believed that the poor quality of 1BL/1RS translocations in bread processing is partially caused by the expression of ω-secalins, which are a family of small monomeric proteins related to wheat ω-gliadin [14]. Up to date only three ω-secalin genes, two from a rye cv and one from a wheat/rye translocation have been reported [21, 22]. Lankao 906 is a high yield wheat 1BL/1RS translocation with high resistance to rust and powdery mildew. Its bread making quality is however poor. The objective of the present study is to use homoeologous cloning method to clone the ω-secalin gene family from Lankao 906 and to analyze the

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gene family from molecular level. This will provide useful information for the future production of quality wheat 1BL/1RS translocations.

MATERIALS AND METHODS

Plant materials

Three wheat 1BL/1RS translocations Lankao 906, Bobwhite, R59 and six non 1BL/1RS translocations Chinese Spring, Zhongyou 9507, Gaocheng 8901, Yangmai 158, Yangmai 10 and Wenmai 6 were used.

Extraction of DNA and RNA

CTAB method [23] was used to isolate total DNA from young leaves. Total RNA was extracted from developing seeds about 15 d post anthesis with TRIzol method (Tianweishidai Company, China).

Synthesis of cDNA

cDNA was synthesized from above total RNA by using the Super Script^{TM} II first strand synthesis system (Invitrogen).

PCR

PCR primers was designed using DNAStar software based on the published sequence from a ω-secalin gene present on the short arm of 1RS [22]. The primers covered the whole mature protein coding sequence and most of the signal peptide coding region. The primer sequences were as follows: ω-sec-P1 (sense) 5'-accttcctcatctttgtcct-3', ω-sec-P2 (antisense) 5'-ccgatgcctataccactact-3'. PCR reaction was carried out by using Pfu PCR Kit KP-101 (Tianweishidai company) and PTC-100^{TM} Programmable Thermal Controller (MJ Research) with the following program: 3 min at 94ºC (initial denaturation), and 30 cycles of 45 sec at 94ºC, 45 sec at 65ºC, and 1.5 min at 72ºC. Additional 7 min extension was followed after the 30 cycles. The 50 µl reaction volume contained 20 mM Tris-HCl (pH8.8), 10 mM KCl, 2 mM $MgSO_4$, 0.2 mM of each dNTP, 0.4 µM of each sense and antisense primers, 3.75 U Pfu DNA polymerase and about 100 ng of template DNA or 1 µl cDNA from the cDNA synthesis system. Partial PCR products were subjected to electrophoresis in 1×TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) on 1% agarose gel, and visualized by ethidium bromide staining.

Molecular cloning and DNA sequencing of PCR products

PCR products were purified with PCR product purification kit DP-204 (Tianweishidai Company). The purified PCR products were then cloned into pGEM-T Easy vector with blunt end DNA fragments cloning kit VT405 (Tianweishidai Company). Transformation was performed with TOP10 competent *E. coli* cells by heat shock method. Positive clones were selected by Colony PCR and their plasmids were extracted with plasmid extraction kit DP-103 (Tianweishidai Company). Sequencing of PCR products was carried out from both ends of the inserted fragments with T7 and SP6 primers respectively. Nucleotide sequences of the cloned fragments were finally determined with a 3730 DNA Sequencer (ABI) and the sequence data were analyzed with a computer program DNAStar.

Southern and Northern blotting

For southern blot analysis 15 µg genomic DNA was digested with *BamH* I and *EcoR* I respectively. For northern blot analysis 10 µg total RNA was used. DNA was transferred to nylon membrane from agarose gel as described by Read and Mann [24]. The transfer of RNA from agarose gel to the membrane was performed with formaldehyde method according to Sambrook and Russell [25]. One of ω-secalin sequences 1076 bp-1 was used to prepare probes with oligo labeling method as described by Feinberg and Vogelstein [26].

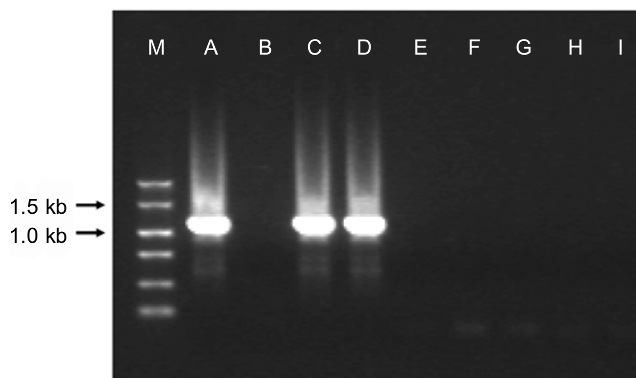

Fig. 1 PCR amplification of 1BL/1RS and non 1BL/1RS wheat cultivars with primers ω-sec-P1 and ω-sec-P2. M: DNA marker; A: Lankao 906 (1BL/1RS); B: Chinese spring; C: Bobwhite (1BL/1RS); D: R59 (1BL/1RS); E: Zhongyou 9507; F: Gaocheng 8901; G: Yangmai 158; H: Yangmai 10; I: Wenmai 6.

RESULTS

Primer specificity

Based on the sequence of published ω-secalin gene [22], a 1076 bp fragment should be amplified with primers ω-sec-P1 and ω-sec-P2 as described in Materials and Methods. As expected a major band above 1 kb was amplified from genomic DNAs of all of the three 1BL/1RS translocations and no amplification occurred in all of the six non 1BL/1RS wheat cultivars (Fig. 1), indicating that the primers could be used to amplify the ω-secalin genes specifically.

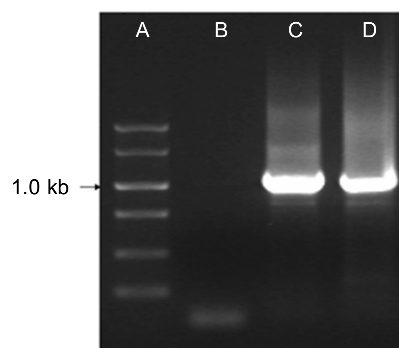

Fig. 2 PCR amplification from genomic DNA and cDNA of Lankao 906 with primers ù-sec-P1 and ù-sec-P2. A: DNA marker; B: negative control without template DNA; C: genomic DNA; D: cDNA.

Tab. 1 Outline of amplified fragments from genomic DNA and cDNA of Lankao 906

Item	Genome DNA # (% of total)	cDNA # (% of total)
Total sequences	42	69
Target sequence	1076bp	1076bp
The longest	1150bp	1076bp
The shortest	311bp	158bp
Longer than 1000bp	27 (64.3%)	45 (65.2%)
Shorter than 1000bp	15 (35.7%)	24 (34.8%)
1150bp	1	0
1076bp	13 (30.9%)	21 (30.4%)
1075bp	5 (11.9%)	0
1052bp	2 (4.8%)	5 (7.1%)
1051bp	0	1
1004bp	6 (14.3%)	18 (26.1%)
1000-800bp	3	1
800-600bp	4	8
600-400bp	3	4
400-200bp	5	10
200-100bp	0	1

Sequences of ω-secalin genes from Lankao 906

The genomic DNA and the cDNA from Lankao 906 were used as templates to conduct PCR amplification by using high fidelity Pfu polymerase. The PCR products were checked by electrophoresis (Fig. 2), purified and cloned into pGEM-T Easy plasmid. Forty two genomic and sixty nine cDNA clones were selected for sequencing. Among these clones except of those 1076bp fragments that have the same size of the target sequence, there were many clones containing shorter insertions and only one clone with longer insertion. The shortest fragment amplified from cDNA was only 158 bp, while the longest fragment was 1150 bp amplified from genomic DNA. Except of those 1076 bp fragments, the clones containing fragment of 1004 bp represent the main population (Tab. 1).

Sequence comparisons between different length genomic clones

In comparison with the 1076bp target sequence, all of the shorter clones contain a deletion in different regions.

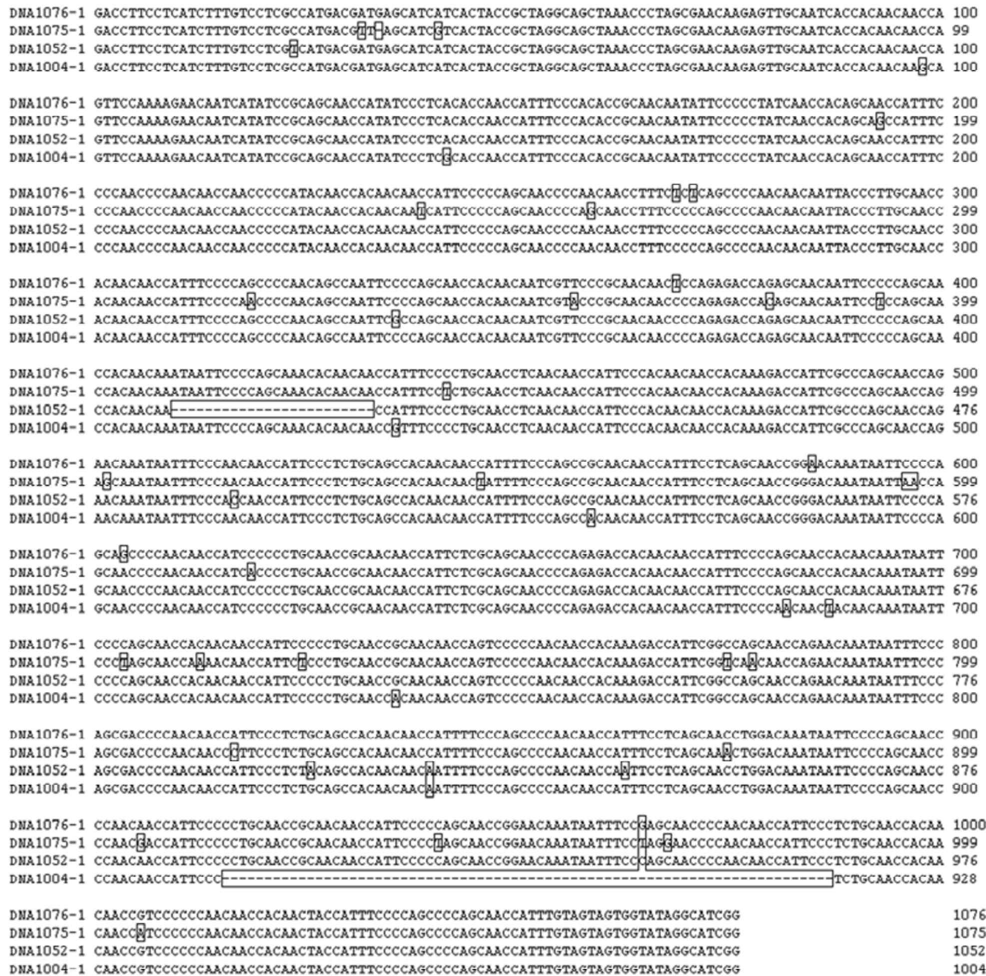

Fig. 3 Comparison of representative genomic sequences.

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DNA1076-1 GACCTTCCTCATCTTTGTCCTCGCCATGACGATGAGCATCATCACTACCGCTAGGCAGCTAAACCCTAGCGAACAAGAGTTGCAATCACCACAACAACCA 100
DNA1150   GACCTTCCTCATCTTTGTCCTCGCCATGACGATGAGCATCGTCACTACCGCTAGGCAGCTAAACCCTAGCGAACAAGAGTAGCAATCACCACAACAACCA 100

DNA1076-1 GTTCCAAAAGAACAATCATATCCGCAGCAACCATATCCCTCACACCAACCATTTCCCACACCGCAACAATATTCCCCCTATCAACCACAGCAACCATTTC 200
DNA1150   GTTCCAAAAGAACAATCATATCCGCAGCAACCATATCCCTCACGCCAACCATTTCCCACACCGCAACAATATTCCCCCTATCAACCACAGCAACCATTTC 200

DNA1076-1 CCCAACCCCAACAACCAACCCCCATACAACCACAACAACCATTCCCCCAGCAACCCCAACAACCTTTCTCTCAGCCCCAACAACAATTACCCTTGCAACC 300
DNA1150   CCCAACCCCAACAACCAACCCCCATACAACCACAACAACCATTCCCCCAGCAACCCCAACAACCTTTCCCCCAGCCCCAACAACAATTACCCTTGCAACC 300

DNA1076-1 ACAACAACCATTTCCCCAGCCCCAACAGCCAATTCCCCAGCAACCACAACAATCGTTCCCGCAACAACTCCAGAGACCAGAGCAACAATTCCCCCAGCAA 400
DNA1150   ACAACAACCATTTCCCCAGCCCCAACAGCCAATTCCCCAGCAACCACAACAATCGTTCCCTCAACAACCCCAGAGACCACAGCAACAATTCCCCCAGCAA 400

DNA1076-1 CCACAACAAATAATTCCCCAGCAAACACAACAACCATTTCCCCTGCAACCTCAACAACCATTCCCACAACAACCACAAAGACCATTCGCCCAGCAACCAG 500
DNA1150   CCACAACAAATAATTCCCCAGCAAACACAACAACCATTTCCCCTGCAACCTCAACAACCATTGCCACAACAACCACAAAGACCATTCGCCCAGCAACCAG 500

DNA1076-1 AACAAATAATTTCCCAACAACCATTCCCTCTGCAGCCACAACAACCATTTTCCCAGCCGCAACAACCATTTCCTCAGCAACCGGAACAAATAATTCCCCA 600
DNA1150   AACAAATAATTTCCCAACAACCATTCCCTCTGCAGAAACAACAACCACTTTCCCAGCCGCAACAACCATTTCCTCAGCAACCGGGACAAATAATTCCCAA 600

DNA1076-1 GCAGCCCCAACAACCATCCCCCCTGCAACCGCAACAACCATTCTCGCAGCAACCCCAGAGACCACAACAACCATTTCCCCAGCAACCACAACAAATAATT 700
DNA1150   GCAACCCCAACAACCATCCCCCCTGCAACCGCAACAACCATTCTTGCAGCAACCCCAGAGACCACAACAACCATTTCCAAAGCAACCACAACAAATAATT 700

DNA1076-1 CCCCAGCAACCACAACAACCATTCCCCCTGCAACCGCAACAACCAGTCCCCCAACAACCACAAAGACCATTCGGCCAGCAACCAGAACAAATAATTTCCC 800
DNA1150   CCC-AGCAACCACAACAACCATTCCCCGTGCAACCGCAACAACCAGTCCCCCAACAACCACAAAGACCATTCGGCCAGCAACCAGAACAAATAATTTTCC 799

DNA1076-1 AGCGACCCCAACAACCATTCCCTCTGCAGCCACAACAACCATTTTCCCAGCCCCAACAACCATTTCCTCAGCAACCTGGACAAATAATTCCCCAGCAACC 900
DNA1150   AGCGACCCCAACAACCATTCCCTCTGCAGCCACAACAACCATTTTCCCAGCCCCAACAACCATTTCCTCAGCAACCTGGACAAATAATTCCACAGCAACC 899

DNA1076-1 CCAACAACCATTCCCCCTGCAACCGCAACAACCATTCCCCCAGCAACCGGAACAAATAATTTCCGAGCAACCCCAACAACCATTCCCTCTGCAACCACAA 1000
DNA1150   CCAACAACCATTCCCCAGCAACCGGAACAAATAATTTCCCAGCAACCCCAACAACCATTCCCTCTGCAACCACAACAACCGTCCCCCCAACAACCACAA 999

DNA1076-1 CAACCGTCCCCCCAAC---------------------------------------------AACCACAACTACCATTTCC------------CCAGCCCC 1043
DNA1150   CTACCATTTCCCCAGCCCCAACAAGCATTCCCCCAAAAAGCAGAACAAATAATTCCCCAGTGACCCCAACAACCATTCCCTCTGCAGCCACACCAACCTC 1099

DNA1076-1 AGCAACC------------------ATTTGTAGTAGTGGTATAGGCATCGG                                                  1076
DNA1150   AGCAACCTTATCCACAGCAGCAACCATTTGTAGTAGTGGTATAGGCATCGG                                                  1150
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Fig. 4 Comparison of sequences between the 1076 bp and the 1150 bp genomic clones.

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DNA1076-1 TFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYSPYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFSQPQQQLPLQP 100
DNA1075-1 TFLIFVLAMTLASSLPLGS.TLANKSCNHHNNQFQKNNHIRSNHIPHTNHFPHRNNIHPINHSSHFPNPNNQHPYNHNNHSPSNPSNLSPSPNNNYPCNH 100
DNA1052-1 TFLIFVLVMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYSPYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFPQPQQQLPLQP 100
DNA1004-1 TFLIFVLAMTMSIITTARQLNPSEQELQSPQQAVPKEQSYPQQPYPSHQPFPTPQQYSPYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFPQPQQQLPLQP 100

DNA1076-1 QQPFPQPQQPIPQQPQQSFPQQLQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQPQQPFSQPQQPFPQQPRQIIPQ 200
DNA1075-1 NNHFPNPNSQHPSNHNNRTRNNPRDHSNNSSSNHNK.FPSKHNNHFLCNLNNHSHNNHKDHSPSNQSK.FPNNHSLCSHNNYFPSRNNHFLSNRDK.LTS 200
DNA1052-1 QQPFPQPQQPIRQQPQQSFPQQPQRPEQQFPQQPQ--------QPFPLQPQQPFPQQPQRPFAQQPEQIISHQPFPLQPQQPFSQPQQPFPQQPGQIIPQ 192
DNA1004-1 QQPFPQPQQPIPQQPQQSFPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQPQQPFSQPQQPFPQQPGQIIPQ 200

DNA1076-1 QPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQQPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQPFSQPQQPFPQQPGQIIPQQP 300
DNA1075-1 NPNNHHPCNRNNHSRSNPRDHNNHFPSNHNK.FPSNQNNHSPCNRNNQSPNNHKDHSVNNQNK.FPSDPNNPSLCSHNNHFPSPNNHFLSKLDK.FPSNP 300
DNA1052-1 QPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQQPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQQFSQPQQPIPQQPGQIIPQQP 292
DNA1004-1 QPQQPSPLQPQQPFSQQPQRPQQPFPQQLQQIIPQQPQQPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQQFSQPQQPFPQQPGQIIPQQP 300

DNA1076-1 QQPFPLQPQQPFPQQPEQIISRQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV.AS                                           358
DNA1075-1 NDHSPCNRNNHSPSNRNK.FPRNPNNHSLCNHNNHPPNNHNYHFPSPSNHL..WYRHR                                           358
DNA1052-1 QQPFPLQPQQPFPQQPEQIISQQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV.AS                                           350
DNA1004-1 QQPFPLQPQQ----------------------PSPQQPQLPFPQPQQPFVVVV.AS                                             334
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Fig. 5 Protein sequence comparisons among some representative genomic clones.

Fig. 3 shows comparison of some representative sequences. All of the 1075bp clones lacked one base at the position 34. All of the 1052 bp clones were deletions of a 24 bp fragment from 410 to 423 base pairs. Most of the 1004 bp clones lacked a 72 bp fragment from nucleotides 916 to 987, except that one clone contains deletion of nucleotides 906-977. All the clones less than 1000 bp contain deletions at different positions. The 1150bp clone contains one base pair deletion at nucleotide 704 and three insertions of 45, 12 and 18 base pairs at positions of 1016, 1035 and 1050 respectively (Fig. 4).

There is no switch of reading frame for clones 1052 and 1004 bp in comparison with the 1076 bp target sequence. However the deletion of the one base pair in both 1075 bp and 1150 bp clones caused premature protein termination (Fig. 4 and 5).

The genomic clones containing similar lengths are nearly identical in sequences. For example, eleven sequences from thirteen 1076 bp clones showed 97 to 99.9% sequence identity in nucleotides. The minor difference may be contributed by errors occurred during PCR amplification.

Comparison of cDNA sequences to the genomic sequences

Four groups of cDNA clones have been obtained, ranging from 1076 bp, 1052 bp, 1051 bp to 1004 bp. As

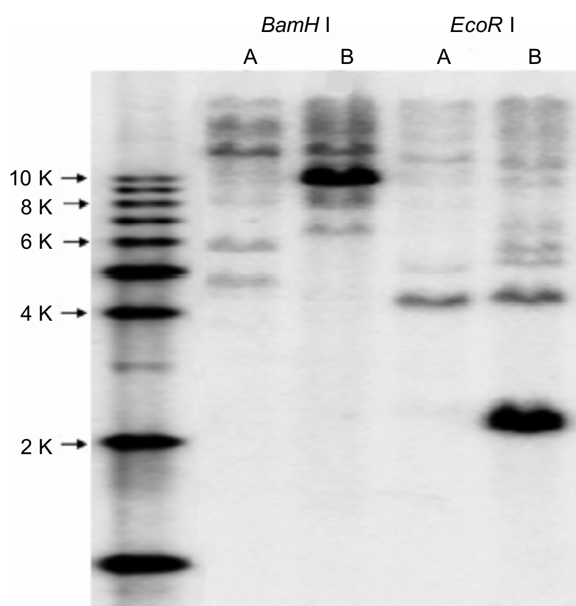

Fig. 6 Southern blot analysis on genes related to ω-secalins. A Chinese Spring—non 1BL/1RS; B Lankao 906—1BL/1RS.

expected, the target 1076 bp cDNA clone is identical to its corresponding genomic clone. Furthermore, all of the 1052 bp and 1004 bp cDNA clones have identical respective genomic clones, suggesting that these clones are representing the real genes. Compared to the 1052 bp clone, the 1051 bp sequence contained one more deletion at position 395. Since only one 1051 bp cDNA clone but no corresponding genomic clone has been found, it is likely that the 1051 bp cDNA clone is a PCR product occurred during amplification rather than a real gene transcript.

The 15 genomic clones less than 1000 bp do not have any corresponding cDNA clones, suggesting that these short sequences may be produced during PCR amplification. Indeed, when the 1076 bp target gene was used as a template for PCR reaction, about 6% of the PCR products are incomplete sequences. Similarly, cDNA clones shorter than 1 kb do not show any corresponding genomic clones. Thus the clones shorter than 1 kb are not the real gene products of ω-secalin family.

Southern and Northern blotting analyses

To further characterize ω-secalin gene family in 1BL/1RS translocation lines, Southern blot was performed on Lankao 906. As shown in Fig. 6, in 1BL/1RS translocation line Lankao 906 three to four major bands ranging from 2.2 kb to 10 kb could be detected from *BamH* I and *EcoR* I digestions respectively, indicating that there are at least three ω-secalin related genes in 1RS. The 10 kb and the 2.2 kb fragment from respective *BamH* I and *EcoR* I digestions show higher intensity than other bands (Fig. 6). Non 1BL/1RS translocation line Chinese Spring was used as a control (Fig. 6A).

Total RNAs from seeds of 1BL/1RS translocation line Lankao 906 (Fig. 7, line B) and non 1BL/1RS translocation line Chinese Spring (Fig. 7, line A) were analyzed on Northern blot, probed with the 1076 bp ω-secalin gene sequence. At least two populations of transcripts can be found in Lankao 906, while the transcript with higher molecular weight exhibits higher expression level (Fig. 7, line B). In Chinese Spring, a non 1BL/1RS translocation line, two bands above the molecular weight of transcripts detected in Lankao 906 can also be found, which may be contributed by wheat ω-gliadins that have higher molecular weight than rye ω-secalins (Fig. 7). Compared to Chinese Spring, Lankao 906 gave stronger bands hybridized to ω-secalin sequence as shown by Southern blot (Fig. 6) but weaker gene expression as shown by Northern blot (Fig. 7). Whether ω-secalin gene is preferentially expressed during different developmental stages needs to be further analyzed.

DISCUSSION

In this report, we use homoeologous cloning method to clone ω-secalin gene family from a 1BL/1RS wheat translocation line. At present, three 1076 bp ω-secalin genes have been reported [21, 22]. Except of the 1076 bp sequences, we have found additional clones containing 1150 bp, 1075 bp, 1052 bp and 1004 bp sequences. Among these clones, the ones of 1076-, 1052- and 1004-bp se-

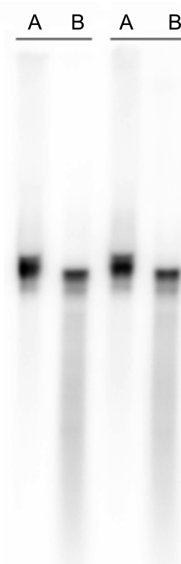

Fig. 7 Northern blot analysis of total RNA at seed development stage. A Chinese Spring—non 1BL/1RS; B Lankao 906—1BL/1RS.

quences may represent the real genes of ω-secalin family since there are corresponding genomic and cDNA clones. The 1075 bp and the 1150 bp genomic clones may be pseudogenes since no corresponding cDNA clones can be found. Identical 1150 bp sequence has been also obtained by Clarke BC (personal communication), it is thus believed to be a real ω-secalin gene. In comparison with the 1076 bp ω-secalin sequence, the ORF in the 1150 bp clone has been changed, due to one base pair deletion that leads to a premature termination.

On the other hand, a single 1051 bp cDNA sequence obtained has no corresponding genomic clone, suggesting that it may be produced during PCR amplification instead of a real gene.

To support the homoeologous cloning results, our Southern blot analysis demonstrates that there are probably at least three ω-secalin genes located on 1RS. At least two ω-secalin genes are actively transcribed in the developing seeds of Lankao 906. The transcript with higher molecular weight expressed more.

The published three 1076 bp ω-secalin gene sequences are not 100% matched to our 1076 bp sequences, which may be due to the difference in materials used by different groups. Two sequences published by Hull [22] were from rye cv Gazelle and another sequence published by Clarke [21] was from a wheat translocation line containing a small interstitial segment from rye cv Imperial. The wheat 1BL/1RS translocation line we used is a progeny of a wide cross between a wheat cultivar with a hexaploid triticale cv Mzalenod Beer.

The 1004 bp and 1052 bp ω-secalin gene sequences have not been reported. It is unlikely that these sequences are derived from PCR amplification based on our observations that these sequences are discovered from both the genomic and cDNA clones with high frequencies up to 33% of the total clone sequenced. Besides, we could never detect these sequences when the 1076 bp clone was used as template. It is very much likely that the 1004 bp and the 1052 bp sequences are the real genes of ω-secalin family. Furthermore, proteins translated from both the 1004 bp and the 1052 bp genes share about 97% sequence identity to that from the 1076 bp gene, suggesting that they belong to the ω-secalin gene family and may play similar function.

In summary, by homoeologous cloning we have found five populations of ω-secalin genes, representing three actively transcribed genes, the 1076 bp, 1052 bp, 1004 bp sequences and two pseudogenes, the 1150 bp and the 1075 bp sequences. By Southern and Northern analyses, different populations of ω-secalin gene family have been found. The ω-secalin genes are differentially expressed in developing seeds. The biological function of the differential expression of ω-secalin genes needs to be further analyzed. An open question is whether suppression of ω-secalin gene expression would improve the wheat quality of 1BL/1RS translocations in bread making.

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