Identifying and mapping cDNA fragments related to rice photoperiod sensitive genic male sterility

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Abstract The differentially expressed cDNA fragments have been obtained by differential screening with cDNA-RAPD technique in photoperiod sensitive genic male sterile (PGMS) rice. Some of them have been reassessed with Northern blot hybridization, from which a PGMS-related positive fragment, *RPG43*, has been identified. Further analysis on *RPG43* with Southern blot and RAPD indicates that the fragment is a single-copy sequence and its mRNA has been processed after transcription. Sequence analysis reveals that *RPG43* is 744 bp in length and contains a 60 bp region (from 126th to 185th bp) showing 72% homology to a human DNA sequence, pac pDJ-356d6, on chromosome 11. So it is a new sequence found in plant and its GenBank access number is AF126027. In addition, *RPG43* has been mapped to a position 3.8 cM away from RFLP marker R1553 on chromosome 5 of rice.

Keywords: photoperiod sensitive genic male sterility Rice, cDNA-RAPD, sequence analysis, chromosome location.

Photoperiod sensitive genic male sterile (PGMS) rice is male sterile when grown under long-day conditions, whereas its pollens are fertile under short-day conditions at the photoperiod sensitive stage of fertility transformation. Therefore, comparing differentially expressed mRNAs is helpful to analyzing differential expression of sterile genes and reaching the goal of isolating the genes in the end. There are mainly 5 ways to identify differentially expressed mRNAs, which are mRNA subtractive hybridization[1] developed from genomic subtraction[2], DD-PCR[3], cDNA RDA[4] developed from genomic RDA[5], cDNA-RAPD[6] and SSH[7]. All the methods mentioned above have their advantages and disadvantages. In our previous reports, cDNA-RAPD was first employed to analyze differentially expressed mRNAs from fertile and sterile young panicles during the sensitive stage of fertility transformation under conditions of short day and long day, and some specific cDNA fragments were detected [8]. In this study, those specific fragments were reassessed on molecular level, and one of them was identified to be relative to PGMS and was mapped to chromosome 5.

1 Materials and methods

PGMS rice Hs-1 used in this study was developed by Fujian Agricultural University and was certified by the National Science and Technology Committee of China in 1995. Hs-1 is a good material for conducting a fertility transformation due to its obvious sensitivity to photoperiod. Furthermore, false positive may be avoided for molecular research because Hs-1, developed by rice anther culture, has homozygous genotype.

Differentially expressed cDNA fragments were detected by cDNA-RAPD technique described in ref. [8]. These fragments were recovered with Gel Extraction Kit (Qiagen) and cloned with pGEM-T

Easy Vector Systems (Promega).

After the specific cDNA fragments were labeled using the Prime-Gene System (Promega), Northern blot and Southern blot were conducted according to the method of Sambrook et al.[9].

RAPD reaction. 10-mer RAPD primers were obtained from University of British Columbia, Canada. PCR reaction mixture consisted of 100 mmol/L Tris-HCl (pH 8.3), 2.0 mmol/L $MgCl_2$, 0.1 mmol/L dNTPs, 0.12 μmol/L 10-mer random primer, 40 ng genomic DNA and 1 U Taq DNA polymerase (Promega), and the final volume was adjusted to 25 μL with sterilized water. After being predenatured for 5 min at 94℃, PCR was carried out for 40 cycles: at 94℃ for 45 s, at 37℃ for 1 min and at 72℃ for 2 min, followed by an elongation period for 10 min at 72℃. Amplification was completed in the PC-100TM programmable thermal controller, MJ Research, Inc., USA.

Electrophoresis and rapid silver staining. RAPD products were separated by electrophoresis in 5% polyacrylamide gels. Subsequently, gels were stained with rapid silver method and then recorded. The rapid silver method according to Sanguinetti et al.[10] was optimized and simplified. The procedure consisted of an initial pre-stain fixation for 3 min in 10% ethanol, 0.5% acetic acid, staining for 6 min in fixing solution plus 0.2% $AgNO_3$, washing of gels in water for 3 min and a development for 5—10 min in 3% NaOH and 0.5% formaldehyde.

Differentially expressed cDNA fragments were sequenced by CyberSyn Company. In order to make the sequence accurate, the fragment was sequenced from two ends with Sp6 and T7 primers respectively.

The molecular marker linkage map for chromosome location was constructed by Tang et al.[11], and the map was based on a recombinant inbred population (RI, F_8, 131 lines) established from a cross between two *indica* rice varieties H359 and Acc8558. The linkage map is 1435.8 cM in whole length, consisting of 147 RFLP and 78 AFLP markers. Mapping data were analyzed with Mapmarker (Ver. 3.0)[12] with the linkage criteria of minimum LOD score 3.0 and maximum recombination fraction 0.4. The order "Try" was used to determine the chromosome location of cDNA fragments and the function kosambi was employed to measure the distance between markers.

2 Results

(i) Northern blot analysis. 6 differentially expressed cDNA clones were randomly selected and used as probes for Northern blot analysis. Fertile and sterile total RNAs were transferred to nitrocellulose and hybridized to those probes. Northern blot hybridization indicated that only one clone presented positive result; that is, the clone detected specific band was only in sterile total RNA, and no signal was found in fertile total RNA (fig. 1). The clone was an amplified product appeared only in sterile cDNA pool in the cDND-RAPD reactions in which fertile cDNA and sterile cDNA pools were used as templates and primer 43 as 10-mer arbitrary primer. The clone was about 750 bp in length and named *RPG43* (fig. 2).

(ii) Cloning of *RPG43*. *RPG43* was cloned with pGEG-T easy vector systems kit (Promega), and its white clones were about 60%. From the restriction map of the recombinant clone, we concluded that *RPG43* had been cloned into the vector.

(iii) Southern blot analysis. Total DNAs of Hs-1, H359 and Acc8558 were digested by *Eco*R I or *Eco*R V, and then transferred to nitrocellulose and then hybridized to the ^{32}P-labeled *RPG43* probe. The

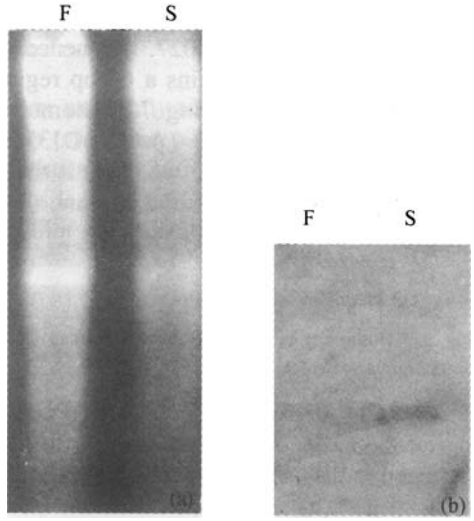

Fig. 1. Northern blot hybridization of *RPG43*. (a) Electrophoresis patterns of fertile (F) and sterile (S) young panicles total RNA; (b) *RPG43* was labeled to hybridize to F and S total RNA.

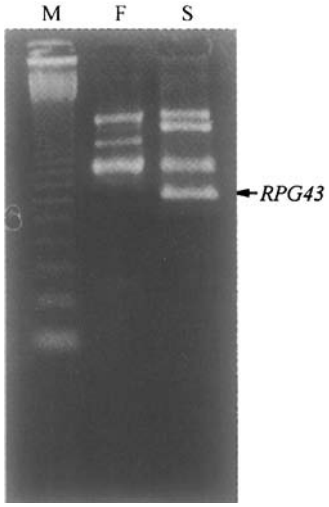

Fig. 2. cDNA-RAPD fragment patterns of arbitrary primer 43. F, Fertile young panicles; S, sterile young panicles; M, 123 bp molecular weight marker.

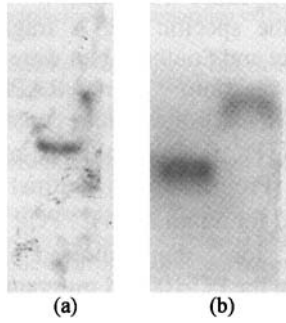

Fig. 3. Southern blot hybridization of *RPG43*. (a) Hs-1 genomic DNA was digested by *Eco*R I and transferred to nitrocellulose; (b) H359 and Acc8558 genomic DNA were digested by *Eco*R V and transferred to nitrocellulose.

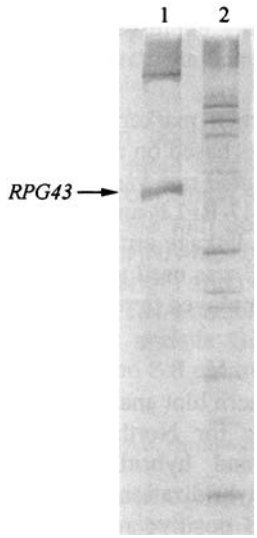

Fig. 4. RAPD fragment patterns of arbitrary primer 43. 1, Recombinant plasmid for inserting *RPG43* was digested by *Eco*R I ; 2, RAPD fragment patterns using Hs-1 genomic DNA as template.

result showed that *RPG43* might be a single-copy sequence (fig. 3). Among amplified products in the RAPD reaction using Hs-1 genomic DNA as template and primer 43 as 10-mer arbitrary primer, the product with the same molecular weight as *RPG43* was not found (fig. 4). Analysis on *RPG43* by Southern blot and RAPD indicated that the mRNA of *RPG43* was processed after transcription.

(iv) Sequence analysis of *RPG43*. *RPG43* has been sequenced. The sequence was compared to Genbank database by the internet, and its Genbank access number is AF126027. Sequence analysis revealed that *RPG43* contains a 60 bp region (from 126th to 185th bp), showing 72% homology to a human DNA sequence, pac pDJ356d6, on chromosome 11 (fig. 5). So we suggest that *RPG43* should be a new sequence found in plant.

RPG43 185CACAGCGGTGAAAATGCAGATCAGTGTGGCAATACTGTGTCCGGTTCAAAAAATGTGATT 126

Sbjct 29103CACAGAATTGATACTGCAGATCAGTGGGGAATTACAGTCTTTCAATTAAACATACTGATT29162

Fig. 5. Homology between *RPG43* and human DNA sequence pac pDJ356d6 on chromosome 11. The shadows indicate homologous sequence.

(v) Chromosome mapping of *RPG43*. RI population was constructed with two parents H359 and Acc8558. Genomic DNA of the two parents were digested, transferred to nitrocellulose and then hybridized to the ^{32}P-labeled *RPG43* probe. Result indicated that *RPG43* was polymorphic between two parents (fig. 3(b)). Then *RPG43* was labeled for RFLP analysis with all lines of RI segregating population (partial result is shown in fig. 6). The RFLP data were analyzed by Mapmaker (Ver. 3.0), and *RPG43* was mapped to a position 3.8 cM away from RFLP marker *R1553* on chromosome 5 of rice (fig. 7).

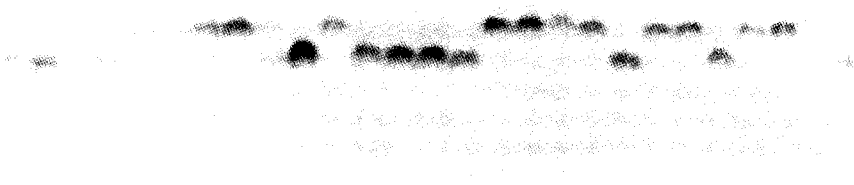

Fig. 6. Autoradiography of the DNA hybridization between the probe *RPG43* and partial lines of RI population developed by two parents H359 and Acc8558. Genomic DNAs were digested by *Eco*R V and transferred to nitrocellulose.

3 Discussion

RPG43 is a differentially expressed cDNA fragment detected only in the sterile young panicle at the photoperiod sensitive stage of fertility transformation of PGMS rice Hs-1, and its mRNA is processed after transcription. For that there is no difference between the two panicle representative populations analyzed except the regions of sterile genes, we conclude that *RPG43* is a cDNA clone related to PGMS. Screening cDNA libraries using *RPG43* as probe is under way.

RPG43 was mapped on chromosome 5 using molecular marker linkage map constructed with RI population. The result is identical to that of the previous studies using marker gene lines as marker materials reported by Zhang et al.[13], Qian et al.[14] and Lin et al.[15]. It is the first report that both the molecular marker and morphological markers linked to PGMS were mapped on the same chromosome. However, owing to the bad integration of classical genetic maps with molecular genetic maps[16], we cannot determine whether the locus of PGMS mapped using morphological markers is tightly linked to *RPG43*.

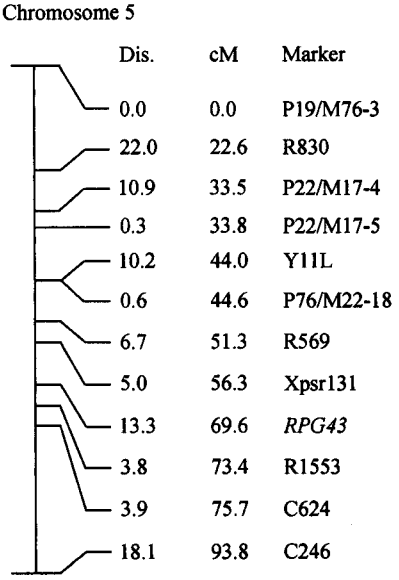

Fig. 7. Molecular marker linkage map of rice chromosome 5 showing the location of *RPG43* and its linked markers.

In this report, 6 differentially expressed cDNA clones were randomly selected and then labeled for Northern blot hybridization. The result indicated that only in one clone positive signal was detected. The ratio of false positive reached 83%. We suggest that both instability of RAPD due to short arbitrary primer and different efficiency of reverse transcription may lead to false positive. Hence, specific cDNA fragments should be further testified by Northern blot hybridization in mRNA differential display.

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