

QTL analysis of seed storability in rice

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Abstract

A double haploid population, which consists of 127 lines derived from anther culture of a typical indica and japonica hybrid 'ZYQ8'/'JX17', was used in this study. Seed storability was investigated by using the storage property measured by the difference of seed germination rates before and after treatment of the rice seeds under 40°C and 95% relative humidity for 10 days in a phytotron. Three QTLs related to rice seed storability were detected on chromosomes 9, 11 and 12, with the LOD scores 2.76, 4.83 and 2.54, respectively, together explaining 35.4% of the genetic variation. The 'JX17' allele at *qLS-9* and the 'ZYQ8' alleles at *qLS-11* and *qLS-12* could enhance the rice seed storability. The effects of the 'ZYQ8' alleles of *qLS-11* and *qLS-12* were also verified using chromosome segment substitution lines.

Key words: *Oryza sativa* — double haploid population — quantitative trait loci — seed storability

Storage properties of rice seeds would influence the frequency of germplasm regeneration, the amount of seed required for production, seedling vigour, and the risks of hybrid seed production and management (Chen 1994, Yamauchi and Winn 1996, Zhang et al. 1998), especially under wet weather conditions in South China. Although traditional measures involving, atmosphere, temperature and chemical products could improve rice storage properties, they have the shortcomings of high cost, chemical residues, and the difficulty of being used on small-scale farms (Jin 1996, Zeng and Qian 2001, Zeng et al. 2002).

Genetic research on seed storability in other crops has been reported (Piech and Supryn 1979, Singh & Ram 1986). In rice, reports also indicated that there were significant differences in storage properties among rice germplasm accessions from different geographical regions (Ellis and Hong 1994, Kameswara and Jackson 1996a,b, 1997, Redona and Macill 1996, Yamauchi and Winn 1996, Zeng and Qian 2001, Zeng et al. 2002). By studying 10 IRRI rice varieties, Juliano et al. (1990) reported that the phenol content in the hull was positively correlated with the storage properties. Using 16 Asian and one Africa cultivars, which represented three ecological types, indica, javanica and japonica. Kameswara and Jackson (1996a) indicated that the order of seed storability in different rice varieties was: indica > javanica > japonica. Using the backcross population of 'Nipponbare/Kasalath', Miura et al. (2002) identified three QTLs related to seed longevity, which were on chromosomes 2, 4 and 9, respectively. To evaluate seed storability, a relatively large number of seeds is needed,

but segregating populations such as an F₂ or a backcross have limited seed availability. Also, the triploid endosperm for the seeds and the diploid plant on which the seeds are set have different genotypes. Therefore, QTL-mapping of seed-related traits depends on the availability of permanent segregating populations such as recombinant inbreds (RIs) or doubled haploids (DHs). In each of these lines, triploid endosperm seeds and their corresponding diploid plants have a consistent genotype. Because each line is breeding true, multiple plants from each line can provide enough homozygous seeds for evaluation of seed storage properties. Most reports from China have been focused on hybrid seed viability and its evaluation, with only a few involved in rice seed viability and its genetic variation (Huang and Fu 1986, Yu et al. 1999, Zeng and Qian 2001, Zeng et al. 2002).

The availability of almost unlimited numbers of molecular markers and genetic maps of high resolution in rice provides powerful tools for quantitative trait locus mapping, near isogenic line development, and gene cloning (Yano 2001). In this study, an artificial ageing method was used to evaluate seed storability. QTLs for rice storage properties were located by using a DH population and their genetic effects were analysed with chromosome fragment substitution lines derived from DH lines.

Materials and Methods

DH population: A typical indica variety 'ZYQ8' and a typical japonica variety 'JX17' of rice, *Oryza sativa* L. were used as parents to make hybrids. The anthers from F₁ plants were collected and cultured on the inducing medium SK3 (Zhu et al. 1993). After natural doubling or treatment with colchicine, double haploid (DH) plants were obtained. Chromosome segment substitution lines (CSSL), CSSL45 and CSSL29, were also used in this study after three successive backcrosses of selected DH lines with 'JX17' and molecular assisted selection (Q. Qian and D. Zeng, unpublished data).

Generation of seeds for storability test: The two parents and their DH population were planted in Hainan in December, 2004. Six rows of each DH line were planted. The heading date was recorded, and seeds were harvested 40 days after flowering. The sample seeds were kept in an oven at 50°C for seven days, and then, at 40°C until the seed water content reached 11–13%.

Ageing treatment: Artificial ageing treatment was based on an improved method originally proposed by Zeng et al. (2002): The sample seeds were treated at 40°C and 95% relative humidity (RH) for

10 days by using a KCH-1000 (Japan) thermostatic moisture regulator. Fifty seeds from each DH line were treated, with three replications.

Seed germination test Seeds were germinated on paper toweling according to Zeng et al. (2002). The germination was conducted at 30°C, 100% RH and 8 h of light per day with a light intensity of 15 000 lx by using an FLI-301N incubator (Japan). The number of germinated seeds was counted on the 10th day. Seeds without ageing treatment, 50 from each DH line with three replications, were used as controls.

Statistical analysis The statistical software SAS was used for data analysis (SAS institute 1996). Storability is calculated as

$$\text{Storability} = G_t/G_u \times 100\%$$

where G_u is the germination rate for the untreated seeds and G_t is the germination rate for treated seeds. The higher the storability, then the better the storage properties.

QTL analysis With a previously constructed linkage map (Lu et al. 1996, Xu et al. 1998), interval QTL mapping was conducted by using the software of Mapmaker/QTL 1.1 for storability. The presence of a QTL was claimed when an LOD score was larger than 2.5. The genetic variance explained by each QTL and by all QTLs and QTL additive effect were calculated. QTLs were named by following McCouch et al. (1997).

Results

Seed storability in parents and DH population

Figure 1 shows the distribution of seed storability in the parents and the DH population. There were significant differences in storage properties between the two parental varieties, 'ZYQ8' and 'JX17', and the storability values for the parents were 81.5% and 24.3%, respectively. Thus the seed storage property of 'ZYQ8' was superior to that of 'JX17'. In the DH population, the storability ranged from 21.4 to 100, mainly around 65% or so, showing continuous variation. A certain number of DH lines segregated transgressively over their parents, indicating seed storability as a typical quantitative trait.

QTL for seed storability

A molecular linkage map, which consisted of 234 markers evenly distributed over all 12 rice chromosomes, has been constructed by using this population (Xu et al. 1998) and is suitable for QTL analysis. Interval QTL mapping for seed

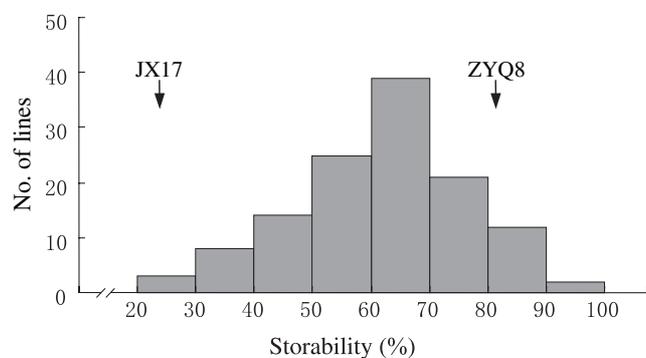


Fig. 1: Distribution of the storability in the DH population derived from an indica 'ZYQ8' and a japonica 'JX17'

storability identified three QTLs, $qLS-9$, $qLS-11$ and $qLS-12$, on chromosomes 9, 11 and 12, respectively (Fig. 2 and Table 1). The additive effect of $qLS-9$ was -9.21 and the QTL allele from 'JX17' could enhance the storability of rice seed, $qLS-11$ and $qLS-12$ had additive effects of 12.30 and 8.65, respectively, indicating that 'ZYQ8' alleles at these two loci could enhance the storability. In other words, 'ZYQ8' alleles resulted in increased germination. The variance explained by these QTLs ($qLS-9$, $qLS-11$ and $qLS-12$) were 12.2, 18.2 and 10.6, respectively. The total phenotypic variation explained by the three putative QTLs was 35.4% based on the multiple-QTL model in MAPMAKER/QTL.

Seed storability analysis with chromosome fragment substitution lines

Using the graphical genotype of the DH lines, the storage property of two chromosome fragment substitution lines, CSSL45 and CSSL29, were analysed (Fig. 3, Table 2). Each substitution line contained positive QTL alleles at only one of three QTLs for seed storability, i.e., CSSL45 had a positive allele ('ZYQ8' allele) at $qLS-11$, and CSSL29 had a positive allele ('ZYQ8' allele) at $qLS-12$. In CSSL45, the chromosome fragments around $qLS-9$ on chromosome 9 and around $qLS-12$ on chromosome 12 were both substituted by 'JX17'. Similarly in CSSL29, the positive allele ('ZYQ8' allele) for seed storability only existed at $qLS-12$. Although there were genetic differences between CSSL45 and CSSL29 on other chromosomes, no major QTL was detected for seed storability, so the influence of these chromosomes on seed storability could be ignored.

The water contents and the germination ratios of these CSSLs and the parents are shown in Table 2. The differences in water content among the lines were not significant. The seed storage property of these CSSLs and the parents could be estimated without taking the effect of water content into consideration. There were significant differences in seed storability among the two CSSL lines and parents. CSSL45, which contained positive alleles at $qLS-11$, had better seed storability at 41.3% than CSSL29 at 34.7%. Therefore, the results from the substitution lines confirmed the QTL locations determined by QTL mapping with the DH population.

Discussion

Rice storage properties have great impact on many aspects of rice production, such as the renewal of conserved germplasm seeds, the seed amount required in crop production, and seedling growth, especially the wet weather conditions in South China. In addition, direct-seeding requires high quality seeds. As reported by Yamauchi and Winn (1996), seeds with good storability grew better in the soil.

By comparing the germination rate of rice seeds treated with and without artificial ageing, three QTLs related to seed storability were identified among them, and $qLS-11$ on chromosome 11 can be considered as a major QTL related to seed storability. The 'ZYQ8' allele at this locus could enhance storage property by 12.3%, and this locus explained 18.2% of total phenotypic variation in DH. The 'ZYQ8' allele at $qLS-12$ could also enhance seed storability. While the 'JX17' allele at $qLS-9$ would increase seed storability, this was consistent with the reported differences in seed storability between indica and japonica (Kameswara and Jackson 1997).

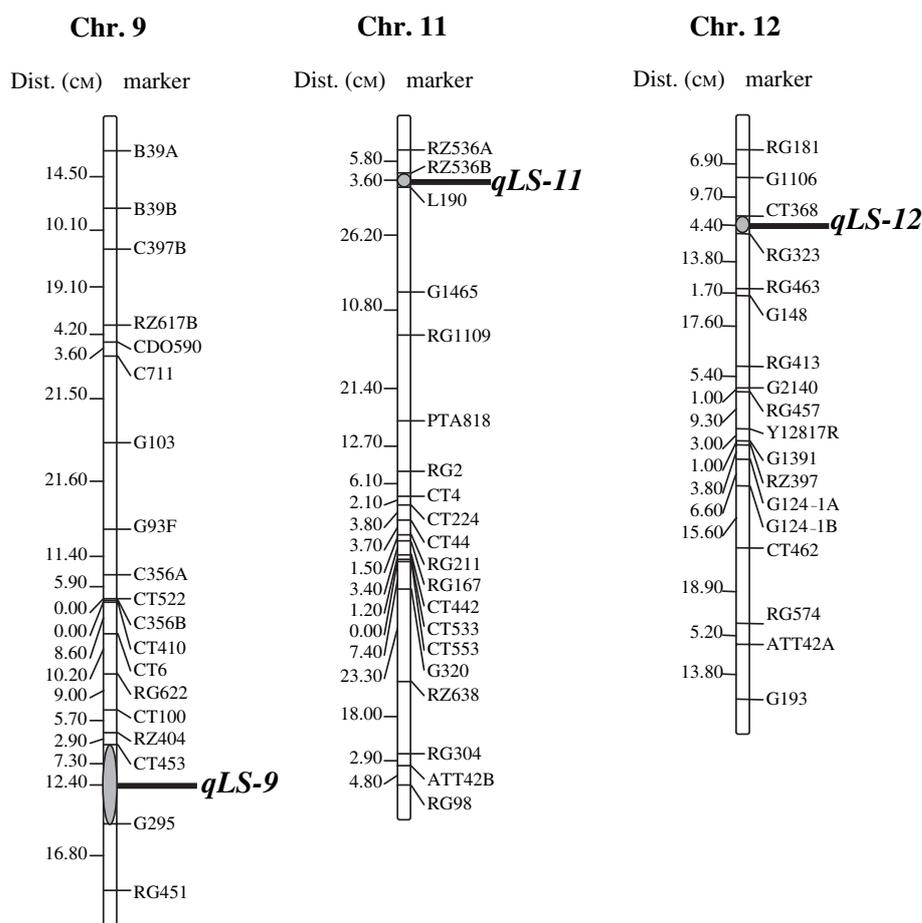


Fig. 2: QTLs for storage properties on the DH linkage map

Table 1: Parameters of QTL related to seed storability¹

| Locus | Chromosome | Marker interval | LOD score | Variation explained % | Additive effect |
|---------------|------------|-----------------|-----------|-----------------------|-----------------|
| <i>qLS-9</i> | 9 | GA65–G295 | 2.76 | 12.2 | -9.21 |
| <i>qLS-11</i> | 11 | RZ536B-L190 | 4.83 | 18.2 | 12.30 |
| <i>qLS-12</i> | 12 | CT368–RG323 | 2.54 | 10.6 | 8.65 |
| Total | | | | 35.4 | |

¹QTL nomenclature followed McCouch et al. (1997).

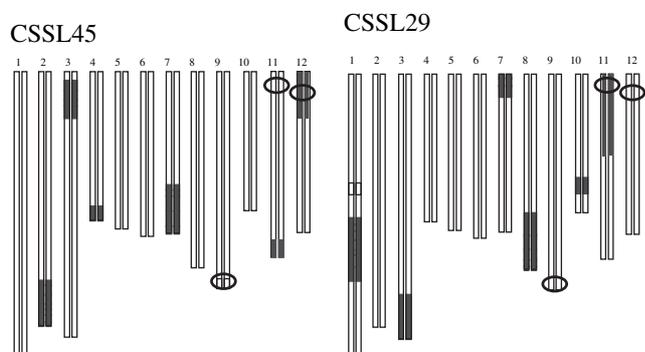


Fig. 3: Graphical genotypes of the two chromosome segment substitution lines developed from the 'ZYQ8'/'JX17' DH lines. 'JX17' was used as the recurrent parent. The white bar indicates 'JX17' segment, while the black bar indicates 'ZYQ8' segment

In this study, the seed storability in chromosome fragment substitution lines derived from the corresponding DH lines confirmed the effects of *qLS-11* and *qLS-12* identified using the DH population.

There are reports that seed dormancy and seed storability are controlled by different genetic mechanisms (Miura et al. 2002). This is consistent with the result reported by Guo et al. (2004), in which four QTLs identified for seed dormancy using the same DH population were located. To remove any interference from dormancy, seeds kept at 50°C for a week were used in this study.

Identification of seed storability is very complicated and also seed-dependent, and cannot be done with populations such as an F₂. In this study, true breeding lines developed by anther culture were used and their genetic consistency over generations or environments meets all the demands required for seed storability analysis. Using MAS, the 'ZYQ8' allele at *qLS-11*

| Lines | QTL | Water content (%) | G_u^1 | G_t | Storability (%) |
|--------|---------------|-------------------|------------|------------|-----------------|
| CSSL45 | <i>qLS-11</i> | 12.7 ± 0.3 | 91.5 ± 8.2 | 37.8 ± 2.6 | 41.3 |
| CSSL29 | <i>qLS-12</i> | 12.1 ± 0.2 | 93.7 ± 9.2 | 32.5 ± 3.1 | 34.7 |
| ZYQ8 | | 12.4 ± 0.5 | 89.4 ± 6.7 | 72.9 ± 7.0 | 81.5 |
| JX17 | | 11.5 ± 0.2 | 92.4 ± 5.9 | 22.5 ± 4.4 | 24.3 |

Table 2: Seed storability analysis of chromosome fragment substitution lines

¹ G_u is the germination rate for the untreated seeds, and G_t is the germination rate for artificially aged treatment seeds.

can be transferred to cultivated varieties, especially hybrid rice, to improve seed storability. In addition, there is diverse genetic diversity in seed storability in rice. The genetic basis of seed storability in different genetic materials or backgrounds could be different (Zeng et al. 2002). Genetic analysis of seed storability using diverse germplasm will help in the understanding of the genetic mechanisms involved in seed ageing, in developing methods for the genetic diagnosis of seed life, and in managing germplasm resources more effectively.

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