

Mapping QTLs associated to germination stability following dry-heat treatment in rice seed

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Abstract Using 164 recombinant inbred lines (RILs) derived from a cross between Milyang 23 (indica/japonica) and Gihoby eo (japonica) in rice, dry-heat tolerance was evaluated for the seeds of parents and RILs, whose dormancy was naturally broken in six months after harvesting. Mapping QTLs associated to dry-heat tolerance was carried out through interval mapping using Qgene 3.0. Seed germination after dry-heat treatments (90 °C for 24 h) showed a significant difference between the two parents, when evaluated for percentage germination and mean germination time. Milyang 23 was highly tolerant to the dry-heat treatment, while Gihoby eo was sensitive. Three QTLs (qDHT 1, qDHT 5, and qDHT 7) conferring the dry-heat tolerance were mapped to chromosomes 1, 5 and 7, respectively. qDHT 1 on chromosome 1 was tightly linked at 4 cM from ME1-1. The phenotypic variation explained by the three QTLs was 27.18% of the total variance in the 164 RIL populations, and the parental additive effects of three QTLs affected the Milyang 23 allele increased dry-heat tolerance. The detection of new QTLs associated with dry-heat tolerance will provide important information for disease and insect control, using dry-heat treatment in organic or low input sustainable agriculture.

Keywords Dry-heat tolerance · Quantitative trait loci · Recombinant inbred line · Rice · Seed germinability

Introduction

In cereal breeding, such as rice, barley and wheat, large-scale dry-heat treatment is a convenient and effective method for controlling the external and internal seed-borne pathogens. Dry-heat treatment also breaks the seed dormancy of freshly harvested cereal seeds in breeding program.

Rice (*Oryza sativa* L.) varieties are classified as japonica, tropical japonica, or indica, depending on the varietal differences in morphological and physiological characteristics (Morishima and Oka 1981). Among agronomic characteristics, seed dormancy, longevity, and dry-heat tolerance also showed varietal difference, with most japonica rice varieties having a lower seed dormancy, longevity, and dry-heat tolerance than indica rice varieties (Seshu and Sorells 1986; Lee et al. 2002; Guo et al. 2004). Recently, significant correlation was seen between seed dormancy and dry-heat tolerance of rice seed (Lee et al. 2006a). Indica varieties were highly tolerant of dry-heat treatment, whereas japonica varieties were sensitive (Lee et al. 2002). Dry-heat treatment (65 °C, 7 days) resulted in considerably lower fungal incidence, and did not have any adverse effect on seed germination and seedling vigor before or after storage (Dadlani and Seshu 1990). This treatment was better than HNO₃ or hulled treatments in breaking rice seed dormancy (Zhang 1990). In dry-heat treatment, seed viability and seedling vigor are largely reduced with high temperature, but seed germination and seedling emergence are promoted as breaking seed dormancy under the optimum range of dry-heat temperature.

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The tolerance of rice seeds to high dry-heat temperature showed a varietal difference, along with positive correlation to seed dormancy and seed longevity (Lee et al. 2002, 2006a). However, the molecular identity associated with dry-heat tolerance has not yet been reported, and dry-heat tolerance has a complex mechanism which is affected by physical and chemical factors concerned with the hull and pericarp (Dadlani and Seshu 1990; Detry 1993). Recently, molecular markers have made it possible to identify individual genetic factors controlling complex traits (Tanksley 1993).

Accordingly, there is a necessity to detect the major quantitative trait loci (QTLs) controlling dry-heat tolerance of rice seeds. The objectives of this work were to investigate the relationship between seed dormancy and dry-heat tolerance in rice seed, and to detect the QTLs tagged to germination stability following dry-heat treatment. A recombinant inbred population derived from a cross between Milyang 23 (indica/japonica) and Gihoby eo (japonica) was used as a mapping population.

Materials and methods

Materials and cultivation

A population of 164 recombinant inbred lines (M/G RILs) was derived from a cross between Milyang 23 (indica/japonica) and Gihoby eo (japonica) via single seed descent through the F_{18} generation (Cho et al. 1998). The parents and the M/G RILs were planted and seeded on 25th April, and 35-day-old seedlings were transplanted with spacing of 30×15 cm, at the Wonkwang University paddy fields (Iksan, Korea). The fertilizers containing nitrogen, phosphorous and potassium were applied at 110, 70 and 80 kg/ha, respectively. The seeds were harvested from five individuals for each RIL on October 10, and stored at room temperature for 6 months. The seeds of parents and M/G RILs, whose dormancy was broken naturally, were used to evaluate dry-heat tolerance.

Evaluation of dry-heat tolerance

The selected seeds were taken in paper bags (5×8 cm) with five replications of 100 seeds, and pretreated to remove seed moisture for 48 h at 40 °C. The moisture content of seeds was about $13 \pm 1\%$ before pretreatment, with a mean of $8.0 \pm 0.2\%$ after pretreatment. The seeds of two parents, Milyang 23 and Gihoby eo, were exposed to different dry-heat temperatures (70, 75, 80, 85, 90, 95, and 100 °C) for 24 h. The seeds of M/G RILs were treated at 90 °C for 24 h, after which were stored at room temperature for 5 days. To evaluate dry-heat tolerance, the seed

germinability was measured at 25 °C in the dark. The seeds were placed on two sheets of filter paper (Whatman Inc., Madestone, England) in 9-cm Petri-dishes. The filter paper was moistened with 10 mL of distilled water. After sowing, the percentage germination was recorded every day for 20 days. Mean germination time (MGT) and T50 (the time at which 50% of the seeds germinated) were calculated as described by Scott et al. (1984) and Coolbear et al. (1984). Percentage germination was used to determine the degree of dry-heat tolerance.

All treatments were tested in a completely randomized design, and data analyses were carried out using the SAS software (V 9.1, SAS Institute Inc.). Averages were compared by analysis of variance (ANOVA) and the latest significant difference (LSD) at 1 or 5%.

QTL identification

An M/G RIL Map which had been integrated with a total of 1300 RFLP, microsatellite (SSLP), AFLP, isozyme, morphological and QTL markers, was used for QTL analysis (Cho et al. 1998). Interval QTL mapping related to dry-heat tolerance was conducted using the software Qgene 3.0 (Nelson 1997). A logarithm of odds (LOD) score of 2.0 was used as the threshold for the identification of putative QTLs in a given genomic region (Kim et al. 2004; Kwon et al. 2001). The total phenotypic variation and the additive effect of each QTL explained by dry-heat tolerance were also calculated; however, the dominant effects could not be evaluated. The QTL nomenclature followed was that of McCouch et al. (1997).

Results

Effect of dry-heat treatment on seed germination

The effect of dry-heat treatment on seed germination depends on the temperature and exposure time. In the two parents, dry-heat tolerance showed a significant difference with respect to temperature, under the dry-heat treatment for 24 h (Table 1). In Milyang 23 (indica/japonica, male parent), the germinability followed by dry-heat treatments of 70–90 °C for 24 h did not significantly differ compared to the control, and it showed a germination rate of over 90% under dry-heat treatments of 95 °C. MGT and T_{50} of Milyang 23 followed by dry-heat treatments of 70–80 °C for 24 h did not significantly differ compared to the control, but were significantly different in dry-heat treatments over 85 °C. The germination capacity of japonica rice was severely reduced by higher dry-heat temperatures compared to the control. The germination rate of Gihoby eo (japonica, female parent) showed a significant difference

Table 1 Effect of dry-heat treatments on the seed germination of Milyang 23 (*indica/japonica*) and Gihoby eo (*japonica*) in 6 months after harvesting

Dry heat (DH, °C) ^a	Percent germination		MGT (days)		T50	
	Milyang 23	Gihoby eo	Milyang 23	Gihoby eo	Milyang 23	Gihoby eo
Control	100.0a	99.4a	3.0e	3.1d	2.5e	2.6c
70	100.0a	96.8a	3.0e	3.7cd	2.5e	3.1c
75	100.0a	94.8ab	3.0e	4.0c	2.5e	3.4bc
80	100.0a	89.8b	3.1de	4.2c	2.5e	3.5bc
85	98.6a	81.6c	3.4d	4.9b	2.9d	4.2b
90	96.2a	37.8d	3.9c	9.2a	3.4c	8.1a
95	90.8b	0.4e	4.9b	–	4.3b	–
100	13.0c	0.0e	10.1a	–	9.5a	–
Variety	$P < 0.001$		$P < 0.001$		NS	
DH	$P < 0.001$		$P < 0.001$		$P < 0.001$	
Variety × DH	$P < 0.001$		$P < 0.001$		$P < 0.001$	

MGT mean germination time, T50 the time at which 50% of the seeds germinated

^a Seeds were treated for 24 h in a dry oven; water content of seeds was $10.3 \pm 0.2\%$ after predrying for 48 h at 40 °C. Mean separation within columns by the latest significant difference at 1% level

with increase in temperature under dry-heat treatments over 80 °C. MGT and T₅₀ of Gihoby eo, followed by dry-heat treatments over 85 °C, showed significant difference. Both parents showed varietal differences in percent germination and MGT after dry-heat treatments. Also, the seed germination followed by dry-heat treatments between two parents showed a significant difference under dry-heat treatment of 90 °C for 24 h. Milyang 23 was highly tolerant to the dry-heat treatment, while Gihoby eo was sensitive.

Based on these results, the seeds of 164 M/G RILs were treated at 90 °C for 24 h. The result of 162 M/G RILs to the dry-heat tolerance in 6 months after harvesting is

shown in Fig. 1. Percent germination of 164 M/G RILs ranged very widely from 0 to 100%, and the mean value showed the median (72.4%) between two parents. The distribution of seed germinabilities of 164 M/G RILs followed by dry-heat treatments showed a marked leaning toward Milyang 23 (*indica*, female parent). The number of RILs which had higher percent germination than the mean value of 164 M/G RILs was 104, and the number of RILs which had higher percent germination than the value of Milyang 23 with high dry-heat tolerance among them was 44. We conclude from these results that the dry-heat tolerance of Milyang 23, *indica* female parent was extremely dominant.

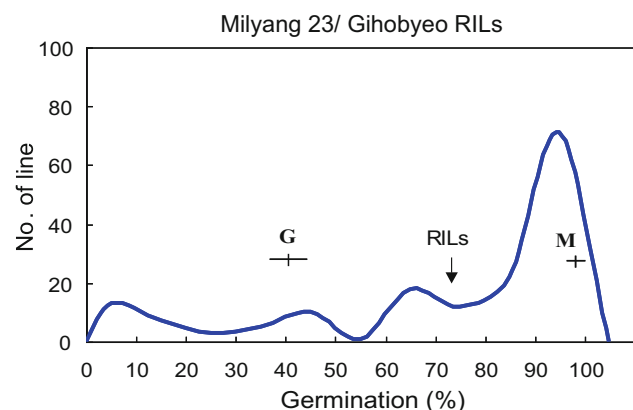


Fig. 1 Distribution of dry-heat tolerance on the seed germinability in 162 recombinant inbred lines (F₁₈) derived from the cross between Milyang 23 (M, *japonica/indica*) and Gihoby eo (G, *japonica*) in 6 months after harvesting. The mean value of RILs is indicated by arrow

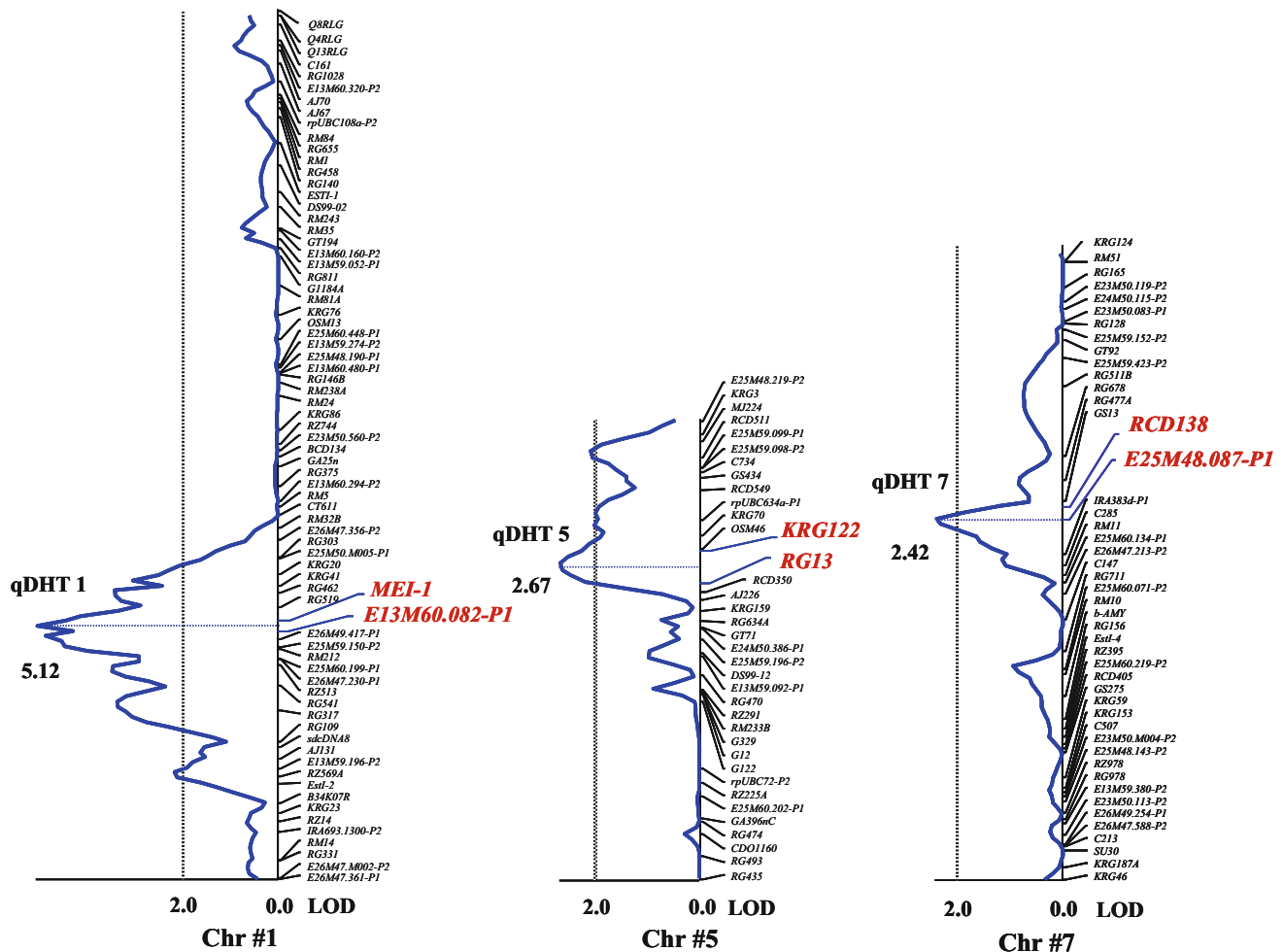
Mapping of QTLs related to dry-heat tolerance

The seed germinability of 164 M/G RILs followed by dry-heat treatments (90 °C, 24 h) was used as the basis for interval QTL mapping related to dry-heat tolerance on M/G RIL Map (Cho et al. 1998). The QTLs associated with dry-heat tolerance are shown in Table 2 and Fig. 2. Three QTLs conferring the dry-heat tolerance trait were mapped to chromosome 1, 5 and 7, respectively. qDHT 1 on chromosome 1 was tightly linked to E13M60.082-P1 and ME1-1, and had LOD score of 5.12; it is located at 4 cM from ME1-1 marker on chromosome 1. qDHT 5 on chromosome 5 was also tightly linked to RG13 and KRG122, and showed LOD score of 2.67. qDHT 7 on chromosome 7 had LOD score of 2.42, and it was linked to E25M48.087-P1 and RCD138. The phenotypic variation explained by the three QTLs was 27.18% of the total variance in the 164

Table 2 QTLs for the dry-heat tolerance of seeds in 162 recombinant inbred lines (F_{18}) derived from the cross between Milyang 23 (japonica/indica) and Gihobyeeo (japonica) in 6 months after harvesting

QTLs	Chr#	LOD score	Probability	Position of QTLs (cM)	Flanking markers	Confidence interval (cM)	Variance explained (%)	Additive effect ^a
qDHT 1	1	5.12	<0.001	100	E13M60.082-P1–ME1-1	92–104	13.39	12.06
qDHT 5	5	2.67	<0.001	100	RG13–KRG122	94–120	7.22	9.43
qDHT 7	7	2.42	<0.001	122	E25M48.087-P1–RCD138	114–124	6.57	8.19
Total variation explained by the three QTLs							27.18	

^a The positive additive effect indicates that the Milyang 23 allele increases tolerance

**Fig. 2** QTL position for dry-heat tolerance of seeds on the germination of 162 recombinant inbred lines (F_{18}) derived from the cross between Milyang 23 (japonica/indica) and Gihobyeeo (japonica) in 6 months after harvesting

RIL population. The phenotypic variation accounted by qDHT 1 showed the highest phenotypic variation (13.39%), and qDHT 5 and qDHT 7 explained 7.22 and 6.57% of the total variance, respectively. In the parent strains, the additive effects of the three QTLs (qDHT 1, qDHT 5, and qDHT 7) increased the dry-heat tolerance of the Milyang 23 allele. This result was in accordance with the data shown in Table 1 and Fig. 1.

Discussion

Dry-heat treatment for cereal seeds is used to control the seed-borne pathogens and to break the seed dormancy. It is a convenient and effective method for large-scale treatment in various crops (Nakagawa and Yamaguchi 1989; Zhang 1990; Seshu and Dadlani 1991; Detry 1993). Dadlani and Seshu (1990) reported that dry-heat treatment at 65 °C for

7 days had no adverse effects on germination and vigor, before and after storage in rice seeds. In other crops, lower temperatures (65–76 °C) had no adverse effects on seed viability (Zeigler et al. 1987; Nakagawa and Yamaguchi 1989; Fourest et al. 1990). However, higher temperatures severely reduced the germination capacity of rice seeds. We thought that dry-heat tolerance is a concern with the moisture content and hull structure of seeds. Especially, the lethal temperature is largely affected by the moisture content of seeds when seeds are exposed to higher temperature. It may be related to certain physical and chemical factors associated with the covering structure such as hull and pericarp under the same moisture content. Lee et al. (2002) reported that there was no difference in the percentage germination of hulled rice seeds between tolerant and sensitive varieties, when treated by dry-heat at 75 °C. The lethal temperature of germinability followed by dry-heat treatments differed between indica and japonica varieties having the same condition of moisture content. In this study, the lethal temperature of Gihobyeeo, japonica, was at 95 °C for 24 h, while that of Milyang 23, indica, was over 100 °C for 24 h, at 7.8% moisture content. The data is similar to the result reported in rice by Lee et al. (2002) and Hagiwara et al. (1990). Sacheti and Sacheti (1996) also concluded that dry-heat treatments at more than 95 °C for a week, was lethal for some leguminous species. Thus, a safe level of dry-heat temperature is an essential component for the control of seed-borne pathogens and the break of seed dormancy.

Conversely, dry-heat tolerance showed a sharp contrast at 90 °C for 24 h between the two parents, Milyang 23 and Gihobyeeo. When the seeds of M/G RILs were treated at 90 °C for 24 h, the dry-heat tolerance of Milyang 23, indica/japonica female parent was extremely dominant compared to that of Gihobyeeo, japonica female parent. The traits for physiological stresses are quantitative traits controlled by polygene. In late twentieth century, molecular markers have also made it possible to identify individual genetic factors controlling quantitative traits (Tanksley 1993); also, the mapping of QTLs associated with the traits affecting the environmental stresses is very important for the application of map-based cloning and marker-assisted selection in breeding programs. In this work, the germinability of M/G RILs followed by dry-heat treatment was used for interval QTL mapping related to dry-heat tolerance on M/G RIL Map (Cho et al. 1998). We identified three new QTLs controlling the dry-heat tolerance of 162 M/G RILs: qDHT 1, 5 and qDHT 7, located on chromosome 1, 5 and 7, respectively, and the phenotypic variation explained by the three QTLs was 27.18% of the total variance. The qDHT 1 had LOD scores of 5.12, and explained 13.39% of the total variance. qDHT 1 was also tightly linked to ME1-1 (4 cM) on chromosome 1. All of

three QTLs showed the parental additive effect which affected Milyang 23 alleles to increase the dry-heat tolerance. The ME1-1 marker that was closely linked to qDHT 1 for the dry-heat tolerance will be useful for introducing a high degree of dry-heat tolerance by marker-assisted selection.

In a previous report (Lee et al. 2006a, b), the dry-heat tolerance of rice seeds found a significant correlation to the seed dormancy or seed longevity, and we reported that three QTLs associated with viviparous germination (the seed dormancy at 40 days after harvesting) were detected on chromosome 2 and 8 (two QTLs on 2 chromosome) using the same materials. These result showed that the QTL locations between dry-heat tolerance and seed dormancy differ from each other. However, the relationship between dry-heat tolerance and seed dormancy were not consistent in these results. The relationship between seed dormancy and seed longevity is not indefinite, in spite of some studies being conducted to solve this problem (Roberts 1963; Siddique 1988; Juliano et al. 1990). We thought that a key component of dry-heat tolerance in rice seeds is hardly understood, but seed dormancy is one of the important factors when the seed was exposed under stress environments. In this work, the dormancy of tested seeds was completely broken under natural conditions in 6 months after harvesting, but there was a difference in the dry-heat tolerance of M/G RILs or their parents. The result indicated that dry-heat tolerance is a complex character influenced by many genetic and environmental factors. Accordingly, we thought this might be related to certain physical and chemical factors associated with the covering structures, such as hull and pericarp, as mentioned above. In future, the detection of new QTLs associated with dry-heat tolerance will provide important information for the relationship between dry-heat tolerance and seed dormancy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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