Korean J. Crop Sci. 51(6): 565~570(2006)

Mapping of Quantitative Trait Loci Associated with Viviparous Germination in Rice

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Abstract : The viviparous germination (VG) with lodging caused the yield reduction and quality deterioration in rice. We carried out the evaluation of VG tolerance (on the 40th day after heading) and mapping QTLs associated with VG tolerance using the recombinant inbred lines (M/G RILs) from a cross between Milyang 23 (japonica/indica) and Gihobyeo (japonica). The VG rates of Milyang 23 and Gihobyeo were 0.0 and 7.0%, respectively. The averaged VG rate of 162 M/G RILs was 7.7%, and their range was from 0.0 to 50.9%. Of the 162 RILs, 144 lines were tolerant less than 10%, and 18 lines were susceptible more than 10%. Using the M/G RIL Map, three QTLs associated with the viviparous trait were detected on chromosome 2 (qVG 2-1 and qVG 2-2) and 8 (qVG 8). qVG 2-1 was linked to RM 32D and RZ 166, and had LOD score of 2.97. qVG 2-2 was tightly linked to E13M59.119-P1 and E13M59.M003-P2, and showed higher LOD score of 3.41. qVG 8 was linked to RM33 and TCT116, and had LOD score of 2.67. The total phenotypic variance explained by the three QTLs was about 24.4% of the total variance in the population. The detection of new QTLs associated with VG tolerance will provide important informations for the seed dormancy, low temperature germination, or comparative genetics.

Keywords: quantitative trait loci, recombinant inbred line, rice, viviparous germination

Rice (*Oryza sativa L.*) is a stable food crop which is grown under rainy conditions in Asia and Africa. Recently, the heavy rains and typhoons are frequently occurred through the greenhouse effect called the earthworm in Asia. Sometimes, rice fields had been flooded and lodged by the heavy rains and typhoon which is often risen in Korea, China and Japan. Rice seeds at harvesting time leads to germination in the mother plant under rainy conditions during late summer and autumn. This phenomenon is known as viviparous germination (VG) or pre-harvest sprouting, and it can be happen at ripening stage. VG followed lodging is badly damaged to the rice yield and quality than other stresses during growth (Oh *et al.*, 1987). The viviparous seed could rapidly lose the seed viability after harvesting, because it leads to the reduction of grain weight and the promotion of fungi infection by accelerating the hydrolysis of endosperm starch in the viviparous grains (Castor & Frederiken, 1977).

VG tolerance is basically one of important traits in rice breeding as well as other cereals. The rainy weather condition at harvesting time is caused of VG and lodging in rice cultivars with low seed dormancy. It is widely known that VG tolerance is affected by the level of seed dormancy (Kim, 1995; Gubler *et al.*, 2005; Seshu & Sorrells, 1986). In rice, the seed dormancy is known the complex inheritance and environmental interaction, and it was reported to be govern by two genes (Seshu & Sorrells, 1986). Thus VG tolerance is the quantitative trait influenced by many environmental factors and controlled by several dominant genes (Bailey *et al.*, 1999; Oh *et al.*, 1987; Seshu & Sorrells, 1986).

In late 20th-century, molecular markers have also made it possible to identify individual genetic factors controlling quantitative traits such as seed dormancy and VG (Tanksley, 1993). Nowadays, the mapping of quantitative trait loci (QTLs) associated with the traits affecting the yield and quality is very important for the application of map-based cloning and marker-assisted selection in rice breeding programs. The mapping of QTLs associated with the VG tolerance has been reported by a few research groups compared to other agronomic traits in rice (Bailey *et al.*, 1999; Dong, *et al.*, 2003; Li *et al.*, 2004). In recent, the research associated with the VG tolerance has been actively performed in wheat (Gubler *et al.*, 2005; Knox et. al., 2005; Kulwal *et al.*, 2005; Lohwasser, *et al.*, 2005). However, the mapping of the QTLs associated with the seed dormancy which has the closest relationship with the VG tolerance has been reported in various materials (Cai & Morishima, 2000; Kato *et al.*, 2001; Lin *et al.*, 1998; Miura *et al.*, 2002; Wan *et al.*, 1997). Accordingly, It is necessary to detect the new major QTLs associated with the VG tolerance or seed dormancy in rice,

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and to compare the similarity of chromosome locations reported in the previous works.

The objectives of this work are to evaluate the VG tolerance, to detect the new QTLs associated with the VG tolerance, and to search new breeding materials for the VG tolerance using a population of recombinant inbred lines crossed between indica and japonica rice.

MATERIALS AND METHODS

Plant materials and cultivation

A population of 162 recombinant inbred lines (M/G RILs) was derived from a cross between Milyang 23 (indica/japonica) and Gihobyeo (japonica) via single seed descent through the F_{18} generation. The parents and their 162 M/G RILs were sown in a seeding tray on 25, April, and the 35 days-old seedlings were transplanted in spacing 30 × 15 cm with 30 hills per row in the paddy field of Wonkwang University (Iksan, Korea). The fertilizers of nitrogen, phosphorous and potassium were applied 110, 70 and 80 kg/ha, and nitrogen and potassium fertilizer were split-applied with urea as basal 50%, 25% at tillering stage and 25% at panicle initiation stage, respectively. The management of the experimental plot was based upon the standard cultivation method of Honam Agricultural Research Institute, NICS, RDA, in Korea.

Evaluation of VG tolerance

For evaluating the VG tolerance of two parents and 162 M/G RILs (F_{18}), the three panicles from healthy five plants per lines were randomly sampled on the 40th day after heading. The collected panicles were laid on the paper towel of two sheets saturated with the distilled water into the plastic tray (45 × 30 × 3 cm), sealed with rap after covering the saturated filter paper of two sheets, and incubated in the dark at 25°C for 6 days. The panicles were sprayed with the distilled water at intervals of 2 days. The treatments were placed in a randomized block design with five replications, and the germinated seeds were counted on the 7th day.

QTL identification

The M/G RIL Map was used for QTL analysis (Cho *et al.*, 1998). The linkage map consisting of 231 AFLPs), 212 RFLPs, 86 SSLPs, 5 isozyme loci, and 2 morphological mutant loci had an average interval size of 3.4 cM. Interval QTL mapping associated with VG 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. Genomic parameters containing explained variation and additive effect of each QTL explained by VG tolerance were also estimated. The designation of QTLs followed the QTL nomenclature by McCouch *et al.* (1997).

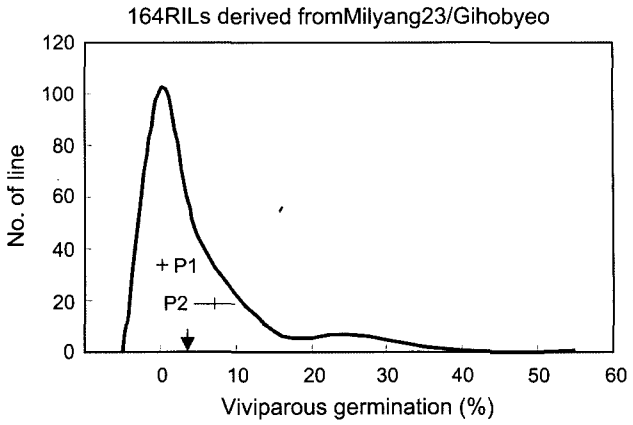

Fig. 1. Distribution of VG tolerance in the 162 recombinant inbred lines (M/G RILs, F_{18}) derived from the cross between Milyang 23 (japonica/indica) and Gihobyeo (japonica) on the 40th day after heading. The mean value of M/G RILs is indicated by arrow. P1, Milyang 23; P2, Gihobyeo.

RESULTS

The result of 162 M/G RILs to the VG tolerance on the 40th day after heading was shown in Fig. 1. The tolerance of VG tolerance between Milyang 23 (japonica/indica) and Gihobyeo (japonica) showed a significant difference, which those of Milyang 23 and Gihobyeo were 0% and 7.0%, respectively. The mean of VG rates in 162 M/G RILs showed the medium value between two parents (3.2%), and the range was from 0.0 to 50.9%. By Ju *et al.* (2000b) for the classification of VG tolerance, 102 lines were classified as the highly tolerant group (VG less than 1%), 42 lines fell into the moderately tolerant group (VG of 1-10%), 15 lines fell into the intermediate group (VG of 11-30%), and 3 lines fell into the susceptible group (VG of 30-60%). 18 of the 162 RILs, lines were susceptible than their male parent (japonica).

Mapping of QTLs affecting VG tolerance

The analysis of QTLs associated the VG tolerance of 162 M/G RILs is shown in Table 1 and Fig. 2. Using 'M/G RIL Map (standard map for QTL mapping with 571 markers)', three QTLs conferring the VG tolerance trait on the 40th day after heading were mapped to chromosome 2 and 8, respectively. There were two QTLs on chromosome 2. qVG

Table 2. QTLs for the VG tolerance of 162 M/G RILs on the 40th day after heading.

QTLs	Chr #	LOD score	Probability	Position of QTLs (cM)	Flanking markers	Variance explained (%)	Additive effect†
qVG 2-1	2	2.97	<0.001	96	RM32D~RZ166	8.00	-2.28
qVG 2-2	2	3.41	<0.001	154	E13M59.119.-P1~E13M59.M003-P2	9.13	-2.40
qVG 8	8	2.67	<0.001	88	RM33~TCT116	7.22	2.10
Total variation explained by the three QTLs						24.35	

†The positive additive effect indicates that the Milyang 23 allele increases tolerance, and the negative additive effect indicates that the Gihobyeo allele increases the tolerance.

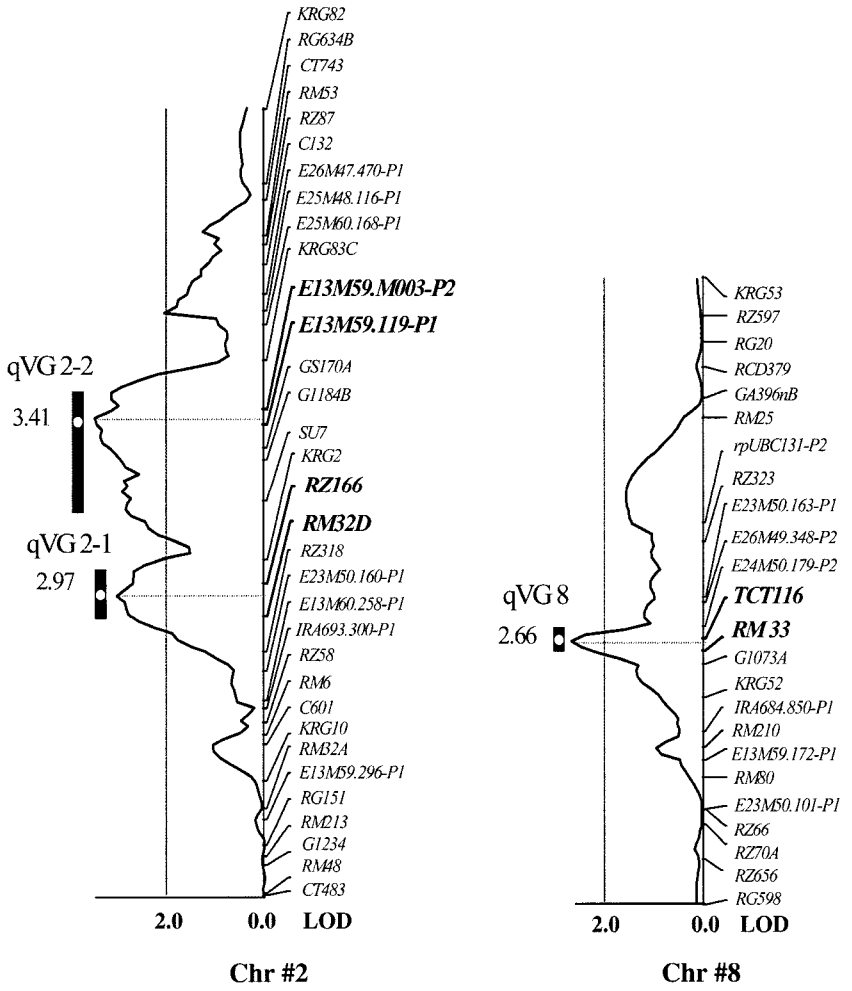

Fig. 2. QTL map for the VG tolerance of 162 M/G RILs on the 40th day after heading.

2-1 was linked to RM 32D and RZ 166, and had LOD score of 2.97. qVG 2-2 was tightly linked to E13M59.119-P1 and E13M59.M003-P2, and showed LOD score of 3.41. qVG 8 on chromosome 8 had LOD score of 2.67, and it was tightly linked to RM33 and TCT116. The phenotypic variation explained by the three QTLs was 24.4% of the total variance in the 162 RIL population. Three QTLs showed phenotypic variation of similar level. The phenotypic variation accounted by qVG 2-2 was 9.13% of the total variance, and qVG 2-1 and qVG 8 explained 8.0% and 7.22% of the total variance, respectively. In the parental additive effects of the three QTLs, qVG 2-1 and qVG 2-2 affected the Gihobyeo allele increased VG tolerance, and qVG 8, by the Milyang 23 allele increased VG tolerance.

DISCUSSION

VG tolerance is one of the important agronomic traits

which caused the yield reduction and quality deterioration in rice (Oh *et al.*, 1987). In rice, VG tolerance is evaluated at 40 to 50 days after heading in field or laboratory condition. The data showed the highly significance between field and laboratory condition (Ju *et al.*, 2000b). We conducted the evaluation of VG tolerance under the saturated water condition on the 40th day after heading based on the standard investigation system for crops of RDA (1993). Ju *et al.* (2000a) also reported that the evaluation of VG tolerance was the most efficient method which the panicle at 45 days after heading was investigated after incubating (day and night, 25/15°C) for 6 days under the saturated water condition.

VG tolerance shows a varietal difference, it is usually weak in japonica rice than in indica rice (Ju *et al.*, 2000b). The reason is that the level of seed dormancy is usually lower in japonica rice than in indica rice (Beachell, 1943; Kim, 1995; Suh & Kim, 1994). In this work, the female parent of M/G RILs, Milyang 23 showed no germination on the 40th day after heading, and it originated from the cross between indica and japonica variety. The male parent, Gihobyeo showed the moderate VG tolerance as the mean VG rate of 7%. The VG of 162 M/G RILs ranged 0 to 50.9%, and showed the medium value (the mean value of 3.2%) between two parents. 144 lines were the moderately tolerance showed the VG rate less than 10%. The result indicated that the VG tolerance of 162 M/G RILs was affected by both parents, Milyang 23 and Gihobyeo.

Above all, the breeding of high VG tolerance is important to incorporate a desired level of seed dormancy into japonica cultivars in Korea. It is, however, difficult to transfer the VG tolerance from indica to japonica rice in short duration. In a wide cross, the useful traits are mostly coinherited with non-desirable agronomic traits in their progenies, or target traits are often loss in the process of progeny selection through crossing between japonica and indica cultivar. So, we thought that the breeding strategy for the VG tolerance would like to practically utilize the Tongil type cultivar crossed between indica and japonica cultivar. In this work, M/G RILs (japonica/Tongil) with the high VG tolerance can be utilize as the mid-parent.

We thought that the quantitative traits such as VG tolerance, seed dormancy, and other stress tolerance is desirable to solve by the marker assistant selection based on QTL analysis along with approaches using quantitative genetics. In recent, mapping of QTLs associated with VG tolerance or seed dormancy was reported in rice by some researchers (Cai & Morishima, 2000; Dong, *et al.*, 2003; Guo *et al.*, 2004; Li *et al.*, 2004; Lin *et al.*, 1998; Miura *et al.*, 2002; Wan *et al.*, 1997; 2005; 2006). We identified three new QTLs associated with the VG tolerance in this work. These QTLs located on chromosome 2 and 8, respectively, and the phenotypic variation explained by the three QTLs was 24.4% of the total variance. The locations of these QTLs differed by researchers. Wan *et al.* (1997) detected four QTLs for seed dormancy (on the 35th day after heading) on chromosome 3, 6, 7 and 12, respectively. Lin *et al.* (1998) detected five QTLs for seed dormancy (on the 40th day after heading) on chromosome 3, 5, 7 (two QTLs) and 8, respectively. Dong *et al.* (2003) reported a total of six QTLs for VG tolerance on chromosome 1 (two QTLs), 4, 5, 7 and 8, respectively. These differences showed that VG tolerance was influenced by genetic background and environmental factors (Anderson *et al.*, 1993). VG tolerance is in close connection with not only seed dormancy but also low temperature germination (Kim, 1995; Gubler *et al.*, 2005; Seshu & Sorrells, 1986), because the temperature during the maturing time is low in Korea. Karrsen *et al.* (1983) and Frey *et al.* (2004) also reported that the seed dormancy in developing seeds is dependant on ABA which is synthesized in the embryo and not on maternal sources of ABA. Thus, the evaluation of VG tolerance can be conducted with a similar method in the evaluation of seed dormancy (Lin *et al.*, 1998; Wan *et al.*, 1997), and the location of QTLs for VG tolerance, seed dormancy and low temperature germinability can be similar according to the tested materials. Dong *et al.* (2003) reported that the QTLs for VG tolerance closely coincided with the QTLs seed dormancy (Lin *et al.*, 1998) and low temperature germinability (Miura *et al.*, 2002). Interestingly, one orthologous Vp1 gene transcribed from VIVIPAROUS-1 on maize chromosome 3 was also detected on rice chromosome 1 (Quarrie *et al.*, 1997; Bailey *et al.*, 1999).

However, it is generally difficult to compare the chromosomal locations and phenotypic variation of QTLs for a genetic trait because of different materials and molecular maps. In this work, the locations of QTLs associated with VG tolerance were different from the previously reported results (Dong *et al.*, 2003; Lin *et al.*, 1998; Wan *et al.*, 1997), and the phenotypic variation (24.4% of the total variance) explained by the three QTLs was also lower than the results reported by Lin *et al.* (1998), Dong *et al.* (2003) and Wan *et al.* (1997). We thought that the reason was due to the VG tolerance of high or moderate level in both parents. The VG tolerance of 162 M/G RILs was affected by both parents, and qVG 2-1 and qVG 2-2 showed the additive effect by Gihobyeo allele, and qVG 8, by Milyang 23 allele.

In rice, VG and seed dormancy are a mixture of process that influenced by multigenes and environmental factors. The molecular works based on QTL analysis for VG and seed dormancy have to actively perform in rice as well as wheat (Gubler *et al.*, 2005; Knox *et al.*, 2005; Kulwal *et al.*,

2005; Lohwasser, *et al.*, 2005). The mapping of common QTLs associated with VG tolerance, seed dormancy and low temperature germination will provide new strategies which the desired level of VG tolerance can be introduced into cereals according to the cultivation systems and environmental factors. It is not long before, QTL mapping will be the desirable tool for map-based cloning and marker-assisted selection in which the breeding of quantitative traits are influenced by environmental factors and polygenes. We think that our result will provided for the breeding strategy as the basic information of map-based cloning and marker-assisted selection, or as breeding resources for VG tolerance.

ACKNOWLEDGEMENTS

This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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