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Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.)

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Abstract Construction of introgression lines using cultivated rice as recipient and wild rice is a novel approach to explore primitive and broad genetic resources in rice breeding. We recently generated a set of 159 introgression lines via a backcrossing program using an elite Indica cultivar rice Guichao 2 (*O. sativa* L. ssp. *indica*) as recipient and a common wild rice Dongxiang accession (*O. rufipogon* Griff.) as donor. In this study, we have evaluated the previously constructed 159 introgression lines for drought-tolerance. A total of 12 quantitative trait loci (QTLs) related to drought tolerance were mapped. Furthermore, a drought tolerant introgression line, IL23, was identified and characterized. Genotype analysis of IL23 demonstrated that IL23 contained two QTLs associated with drought tolerance, *qSDT2-1* and *qSDT12-2*, which were located on chromosome 2 and 12 within the two introgressed segments derived from the common wild rice, respectively. Physiological characterization, including measurement of water loss, osmotic potential, electrolytical leakage, MDA content, soluble sugars content and the leaf temperature, revealed that IL23 showed the characteristics associated with drought tolerance. Identification and characterization of IL23 would provide a useful basis for isolation of novel genes associated with drought tolerance and for molecular breeding of drought tolerant rice. Furthermore, the results in this study indicated that construction of introgression lines from common wild rice should be an appropriate approach to obtain favorable genetic materials.

Keywords Common wild rice (*O. rufipogon* Griff.) · QTL · Introgression lines · Polyethylene glycol (PEG) · Drought tolerance

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Introduction

Plant growth is greatly affected by abiotic stresses such as drought, excessive salinity, and low temperature. In a typical year, abiotic stresses can decrease crop yields by about 15% in Asia, more than twice the damage caused by biotic stresses (Dey and Upadhaya 1996). Drought stress was found to be one of the major causes that limit the yield instability (Ozturk et al. 2002), and great efforts have been made to breed drought tolerant crop variety.

As the most important world food crop, the cultivated rice (*Oryza sativa* L.) demands tremendous water during growth, which makes its production encounter a lot of challenges. Breeding of new rice cultivar with drought tolerance not only saves a great amount of water but also helps to increase and stabilize the yield.

It is therefore scientifically significant to study the genetic basis of drought tolerance and also economically important to explore and utilize new genetic resources of drought tolerance in rice breeding. In view of complex drought tolerant mechanism in rice, attentions were mainly focused on localizing genes that contribute to drought response in a quantitative way through marker-assisted selection. Identification of genomic regions with drought-resistant loci, which are useful for marker-assisted breeding of drought tolerance, has been well documented (Crasta et al. 1999; Frova et al. 1999). The QTL mapping associated with drought tolerance in rice was mainly focused on root traits such as root thickness, ratio of root and shoot, root dry weight (Champoux et al. 1995). In addition, some QTLs associated with aerial traits were also mapped, including osmotic adjustment, dehydration tolerance, stoma tolerance, leaf rolling and leaf drying (Lilley et al. 1996; Price and Tomos 1997; Price et al. 2002).

Common wild rice (*O. rufipogon* Griff.), as the progenitor of cultivated rice (*O. sativa* L.), constitutes the primary gene pool for rice genetic improvement (Second 1982; Oka 1988; Wang et al. 1992). During the course of domestication from wild rice to cultivated rice, profound changes of agronomic traits and genetic diversity occurred, and the number of alleles of cultivated rice was only 60% that of wild rice, suggesting that many alleles of wild rice were lost during the course of domestication, which led to lower genetic diversity of the cultivated rice (Sun et al. 2001). Because of the narrow genetic base of cultivated rice, it is necessary to explore primitive and broad genetic resources. The wild rice may offer abundant genetic resources in drought tolerance research as it has more novel alleles than cultivated rice.

Although wild rice is speculated to have more favorable alleles that might be lost or weakened in cultivated rice, few QTLs associated with drought tolerance in wild rice were reported. In order to identify novel alleles from wild rice, we recently generated a BC_4F_4 introgression population of 159 lines via a backcrossing program using the cultivated rice as recipient and the common wild rice as donor (Tian et al. 2005). In this study, the previously constructed 159 introgression lines were evaluated for drought-tolerance, and 12 QTLs related to drought tolerance were mapped. Furthermore, a drought tolerant introgression line, IL23, was identified and characterized. The IL23 line was found to contain two positive QTLs from wild rice and to steadily exhibit strong drought-tolerance. These results would be helpful for cloning and utilizing the drought-responsive genes derived from wild rice.

Results

Evaluation of phenotypes and QTL mapping for drought-tolerance

Construction of introgression lines using cultivated rice as recipient and wild rice is a novel approach to explore primitive and broad genetic resources in rice breeding. The introgression lines (ILs) were theoretically identical to the entire genome of the cultivated rice except for few introgressed segments of the wild rice, and all the phenotypic variation in the ILs should be associated with the introgressed segments (Fridman et al. 2004). Based on this strategy, we constructed a set of 159 introgression lines (the BC_4F_4 population), using the common wild rice Dongxiang accession (*O. rufipogon* Griff.) as donor and the cultivated rice Guichao2 (GC2) as recipient in a backcrossing program (Tian et al. 2005).

The cultivated rice GC2 plants (recipient parent) at the two and half-leaf-stage showed discrepant performance in responsive to different concentration of PEG treatment (Fig. 1). After treated with 35% PEG solution for 1 day, all the leaves of GC2 seedlings became rolled and the first leaves at the bottom were yellow and wilt. The first leaves of some plants became yellow and most of second leaves became rolled in 30% PEG, and the tips of partial second leaves got rolled in 25%

Fig. 1 Phenotype of GC2 seedlings in different concentration of PEG (from 0 to 35% PEG) treatment for 1 day

PEG solutions, but very little changes occurred in 20% PEG solution. Along with the increase of treatment time, seedling growth was severely affected when treated with 30 or 35% PEG solutions. After treated for 8 days, the seedlings treated with 30 or 35% PEG solutions were injured seriously or could not survive.

Thirty percent PEG solution was further used to create drought condition in evaluation for drought tolerance of the previously established introgression population of 159 ILs (Tian et al. 2005). Our results showed that most of introgression lines with two and half leaves exhibited drought sensitivity similar to GC2: their seedlings leaves were rolled and the first leaves at the bottom became yellow and wilt after treated for 1 day; they were completely killed or injured seriously 8 days later. However, a few lines showed steadily strong drought-tolerance compared to GC2, indicating drought-tolerant QTLs in these introgression lines. Some drought-tolerant ILs were only wilt on the leaf apex after treated with 30% PEG for 1 day, whereas the whole second leaf of GC2 got curled completely; some ILs only showed wilted on the second leaf and were able to hold the growth trend after treated with 30% PEG for 6 days, while the growth of GC2 was obviously inhibited and all the leaves got yellow which indicated the GC2 seedlings were damaged seriously. The results from IL23, one of the drought-tolerant introgression lines, were representatively shown in Fig. 2.

A total of 12 QTLs associated with drought tolerance were located on chromosomes 2, 4, 5, 6, 8, 9 and 12, respectively, after analysis of this introgression population of 159 ILs for genetic linkage of the PEG stress tolerance-related QTLs with the 126 SSR polymorphic markers previously identified in our lab (Tian et al. 2005; and data not shown). All of the QTLs were detected at least twice among three replicates and the phenotypic variances explained by these QTLs ranged from 3 to 14% (Table 1, Fig. 3). Of the 12 QTLs detected, 8 QTLs could be detected at the first day, suggesting that these loci might be the earlier expressing genes when the seedlings underwent drought stress. Seven QTLs could be detected for 8 days, indicating these regions expressed stably in drought stress condition. These four QTLs, *qSDT2-1* near SSR marker RM279 on chromosome 2, *qSDT6-1* near RM253 on chromosome 6, *qSDT6-2* near RM217 on chromosome 6, and *qSDT12-2* near RM17 on chromosome 12 had strong positive additive effect, further indicating the alleles from Dongxiang wild rice could increase drought tolerance. It is worthy to point out that *qSDT12-2* might be an important locus associated with drought tolerance as it was detected for three times, and that the drought-tolerant line IL23 carried the two QTLs, *qSDT2-1* and *qSDT12-2*. The IL23 line was therefore chosen for further characterization.

Fig. 2 Different phenotypes of IL23 and GC2 after 30% PEG treatment for 1 day (**A**) and 6 days (**B**)

Growth characteristics of IL23

In normal condition without PEG treatment, the growth of young IL23 seedlings was somewhat quicker than GC2 (Fig. 4A), IL23 exhibited a higher plant height than GC2 (108.6 cm for IL23 plants after the heading date and 97.8 cm for GC2 plants at the same stage). Furthermore, IL23 showed more grain yield per plant than GC2 (24.7 g for IL23 and 22.0 g for GC2) due to more filled grains and a higher seedset ratio.

When treated with PEG at various concentrations, the IL23 seedling all exhibited drought tolerance compared to GC2. The results showed that the IL23 seedlings had a quicker growth rate than GC2 during PEG treatment for 6 days, as indicated by shoot fresh weight of young seedlings (Fig. 4B, C) and by root fresh weight (Fig. 5B, C).

When the seedlings, which have been treated with various concentration of PEG for 6 days, were trans-

Table 1 QTLs associated with drought tolerance detected in seedling stage

Locus	Chromosome	SSR marker	*P*-value[a]	PV[b] (%)	Add[c]
qSDT2-1	2	RM279	0.00311	7	1.2
qSDT4-1	4	RM335	0.01592	9	–0.70
qSDT5-1	5	RM249	0.03053	5	–0.64
qSDT5-2	5	RM13	0.03015	5	–0.91
qSDT6-1	6	RM253	0.04797	5	1.25
qSDT6-2	6	RM217	0.04563	4	1.05
qSDT8-1	8	RM342A	0.01159	9	–1.15
qSDT9-1	9	RM342B	0.03215	5	–1.33
qSDT9-2	9	OSR28	0.04441	5	–0.73
qSDT9-3	9	OSR29	0.02622	9	–0.74
qSDT12-1	12	RM277	0.03126	7	–1.33
qSDT12-2	12	RM17	0.00182	14	1.38

[a]*P*-value, the probability that the marker genotype had no effect on the trait, *P*-value is the minimum of three *P*-values detected in three phenotypic evaluations

[b]PV, phenotypic variance explained by the QTLs

[c]Add, additive effect of allele from *O. rufipogon*. All of the QTLs were detected at least twice among three replicates

ferred to the PEG-free solution, the IL23 seedlings got recovered more quickly from PEG stress than GC2. After recovered in the PEG-free solution for 2 days, the growth rate of the IL23 seedlings is obviously quicker than GC2 as indicated by shoot fresh weight (Fig. 6A) and by root fresh weight (Fig. 6B) indicating that seedlings of IL23 may be injured less seriously. Furthermore, IL23 showed less curled degree of leaf than GC2 (Fig. 7), which, from another point of view, testified that the seedling of IL23 has better drought tolerance than GC2.

In order to investigate the effect of PEG stress on seed germination, we adopted different concentration of PEG treatment during seed soaking of IL23 and GC2. In the absence of PEG, germination rates of IL23 and GC2 seeds were typically close to 100%. In 20% PEG, IL23 and GC2 showed significant difference ($P < 0.01$) in germination rates (96.67% for IL23 and 83.33% for GC2). The similar results were obtained when treated with 25 and 30% PEG treatment. For example, when treated with 30% PEG treatment 36.67% of IL23 seeds were able to germinate while GC2 seeds had only 13.33% germination rate (Fig. 8). These results suggested that IL23 had stronger drought tolerance than GC2 at the stage of seed germination.

Physiological characteristics of IL23

Many physiological characteristics, including water loss rate, osmotic potential, electrolytical leakage,

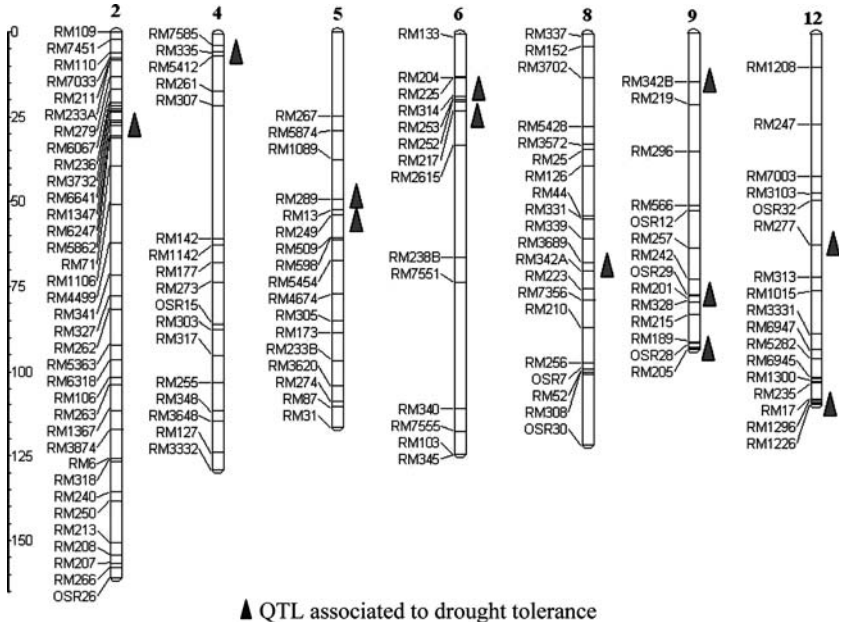

Fig. 3 The molecular genetic linkage map of IL23. Chromosome numbers are indicated above each chromosome. The positions of QTLs are indicated by triangle bars beside chromosomes

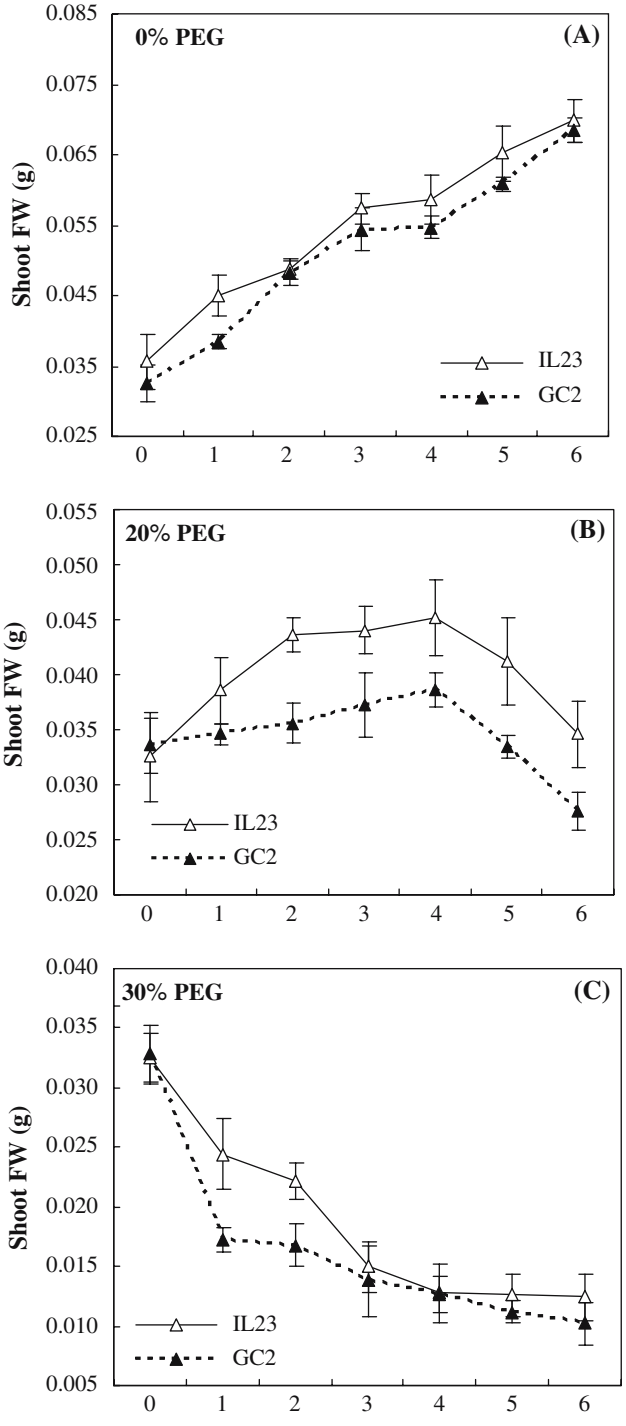

Fig. 4 Effect of different concentration of PEG on shoot fresh weight (FW) of IL23 and GC2. Plants were grown in greenhouse condition. Seedlings of IL23 and GC2 at the two and half-leaf-stage were treated in different concentrations of PEG form the 1st day to the 6th day and then rewatered for the following 2 days

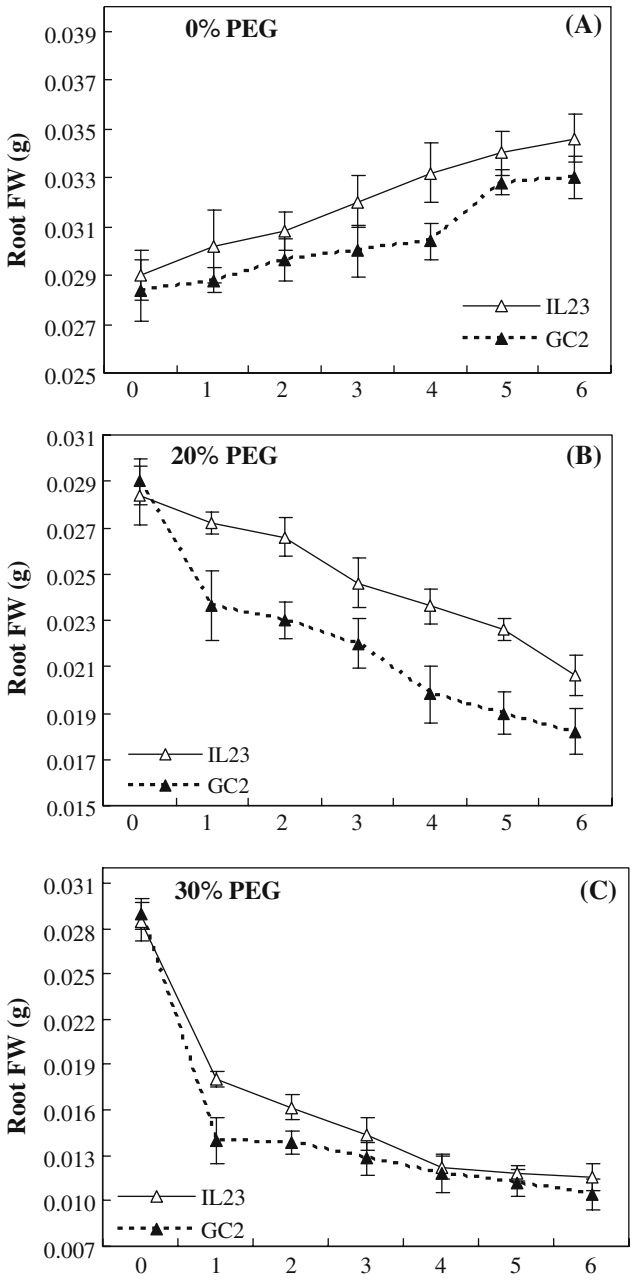

Fig. 5 Effect of different concentration of PEG on root fresh weight (FW) of IL23 and GC2. Seedlings of IL23 and GC2 at the two and half-leaf-stage were treated in different concentrations of PEG form the 1st day to the 6th day and then rewatered for the following 2 days. Roots were harvested and weighed at different treatment time

malondialdehyde content and soluble sugar content, are indicative of drought tolerance (Chen et al. 2005; Inan et al. 2004). We found that the water loss of IL23 was always significantly lower than that of GC2 in responsive to the 30% PEG treatment for 1, 2, 4 and 6 h ($P < 0.05$) (Fig. 9A). We also noted that IL23 and GC2 showed significant difference ($P < 0.01$) in the leaf osmotic potential (–0.4 MPa in IL23 and –0.34 MPa in GC2) (Fig. 9B) when treated with 30% PEG for 24 h. Furthermore, the IL23

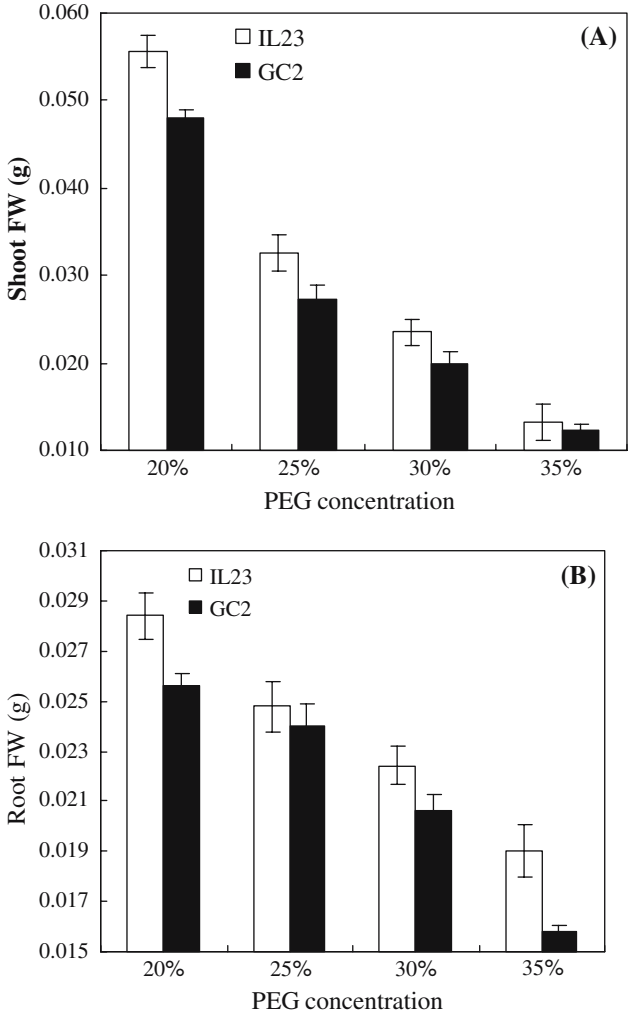

Fig. 6 Shoot fresh weight (**A**) and root fresh weight (**B**) of seedlings under treatment for 6 days with PEG at concentrations of 20, 25, 30 and 35%, respectively, followed recover treatment for 2 days in PEG free solution

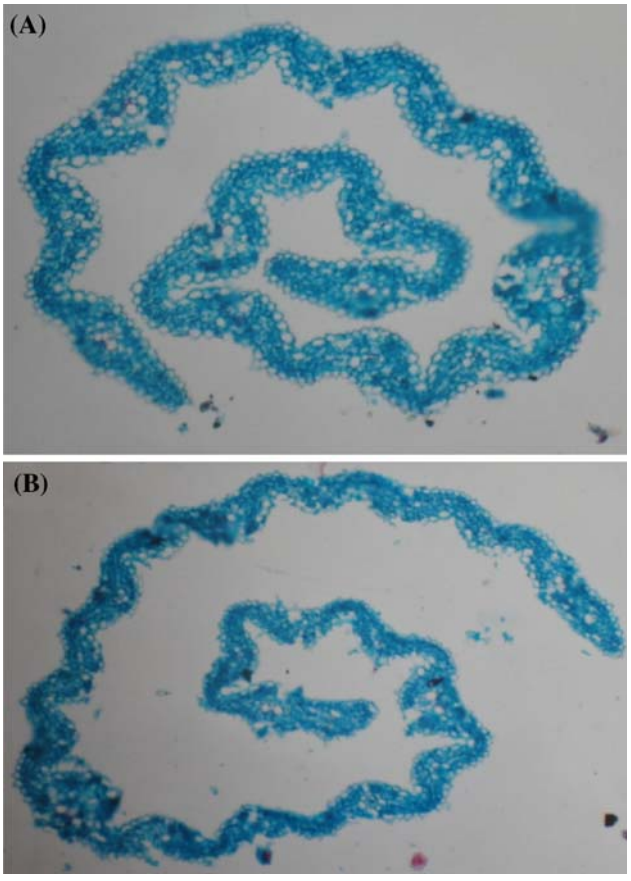

Fig. 7 Light microscopy of transverse sections of curled leaves (200×) from GC2 (**A**) and IL23 (**B**) seedlings at the two and half-leaf-stage after 30% PEG treated for 1 day

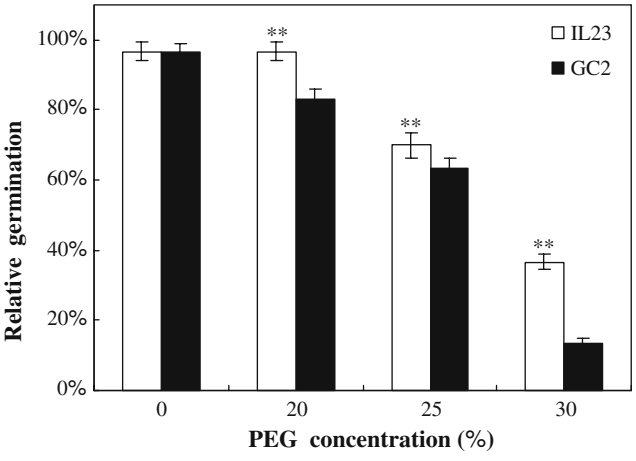

Fig. 8 Effect of different concentration of PEG on the germination rates of IL23 and GC2 after 2-day imbibitions

seedlings were found to distinctly exhibit less electrolytical leakage and less malondialdehyde (MDA) content under PEG stress, compared with GC2 (Fig. 9C and D). We also determined the content of soluble sugars, another class of compatible osmolytes, and demonstrated that the content of soluble sugars was higher in IL23 than in GC2 (Fig. 9E). In addition, we investigated the seedlings thermal images of IL23 and GC2 after 30% PEG stressed for 3 days. As shown in Fig. 10, the leaf temperature of IL23 was approximately 1°C higher than that of GC2, indicating that IL23 was faster to close its stomata than GC2 in responsive to PEG stress.

Taken together, all the results implied that IL23 was less injured than GC2 during PEG stress, further indicating the drought tolerance of the IL23 line.

Genotype analysis of IL23 and transcript analysis of introgression regions

In order to examine whether IL23 were near identical to the entire genome of GC2 except for the *qSDT2-1*

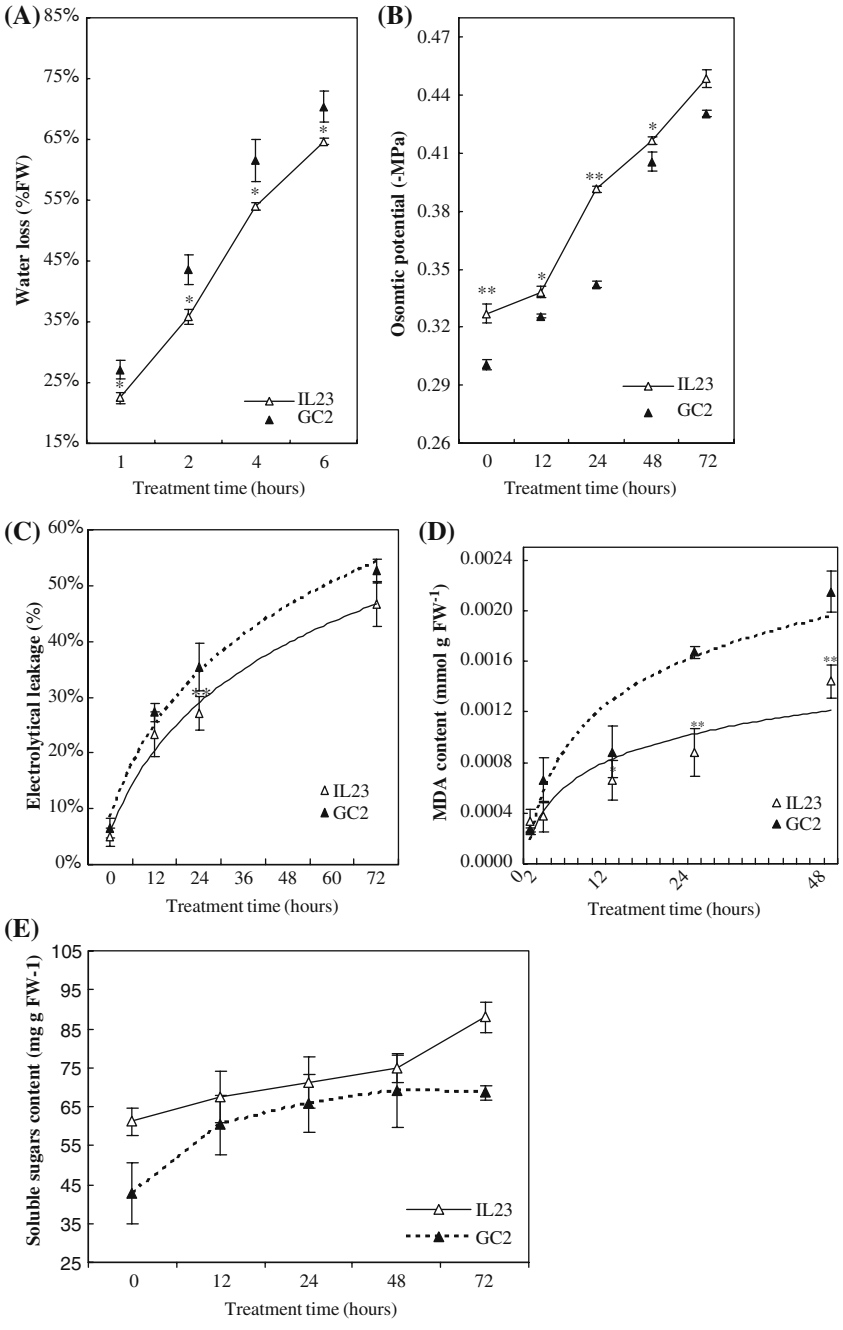

Fig. 9 Leaf water loss of explants (**A**), osmotic potential of the same leaves (**B**), electrolytical leakage (**C**), MDA content (**D**) and soluble sugar content (**E**) taken from IL23 and GC2 at the two and half-leaf-stage after 30% PEG treatment. Vertical bars show the standard error of the mean. Asterisks indicate significant differences at 5% level. Some error bars are not visible because of small standard deviations

or *qSDT12-2* containing segments introduced from the Dongxiang common wild rice, we further genotyped IL23 using our previously identified 254 SSR markers, which covered 1457.5 cM of 12 rice chromosomes and showed polymorphism between GC2 and the Dongxiang common wild rice (Tian et al. 2005). Among the 254 SSR polymorphic markers, 238 showed GC2 genotype patterns, only 16 exhibited patterns of the Dongxiang common wild rice, including 12 SSR markers (RM6225, RM7033, RM7562, RM211, RM6616, RM279, RM3188, RM6067, RM1285, RM236, RM3732, RM6641), which located together within the genetic distance of 15.9 cM on chromosome 2, and 4 SSR markers (RM235, RM270, RM4552 and RM17) within 6.7 cM on chromosome 12. These results demonstrated that IL23 is near identical to the entire genome of GC2 except for these two introgressed segments of Dongxiang common wild rice (data not shown). The drought-tolerant QTLs, *qSDT2-1* and *qSDT12-2*, were found to locate within these two introgressed segments as *qSDT2-1* was mapped near SSR marker RM279 on chromosome 2 and the QTL *qSDT12-2* near RM17 on chromosome 12. Taken together, these data suggested that the IL23 was a near

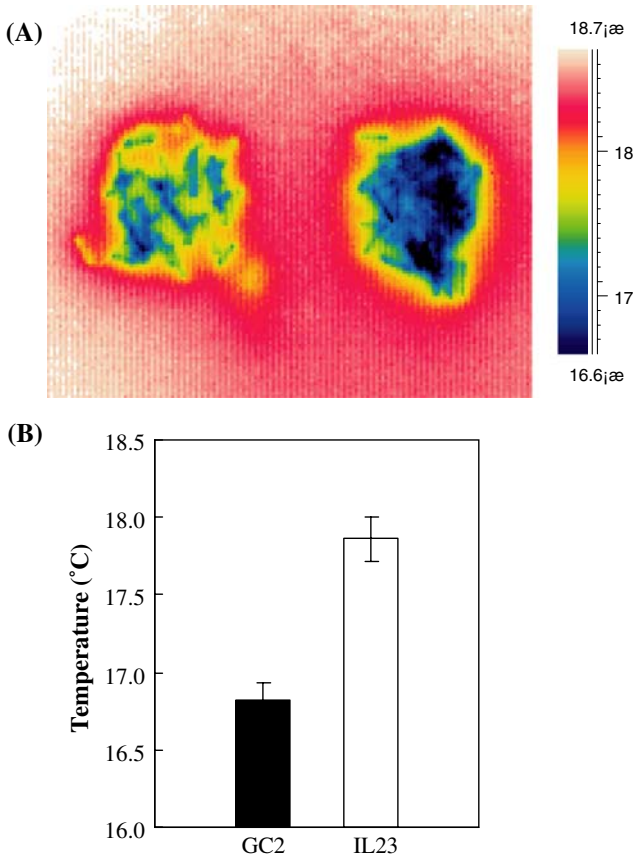

Fig. 10 Infrared thermography in IL23 and GC2. (**A**) Color infrared image of drought-stressed IL23 explants (left) and GC2 explants (right). Two and half-leaf-stage seedlings were subjected to 30% PEG stress for 3 days. (**B**) Leaf temperatures of drought-stressed explants. The leaf temperatures were calculated by quantification of the image shown in (**A**). Values presented are mean ± SD of measurements on 5000 pixels

isogenic line of drought resistance, and that the two QTLs, *qSDT2-1* and *qSDT12-2*, contributed to strong drought tolerance in IL23.

Database analysis with the newly released TIGR rice gene model version 3 (http://www.tigr.org/tigr-scripts/e2k1/irgsp_chromo_list.spl?db=osa1) has identified 600 gene models for the *qSDT2-1*-associated introgressed segment on chromosome 2, and 157 gene models for the *qSDT12-2*-associated introgressed segment on chromosome 12.

Among these 757 genes, three genes were previously reported for response to drought and other stresses, which encode thiosulfate transferase (LOC_Os02g07040), potassium transporter (LOC_Os12g42300), and thaumatin-like protein precursor (LOC_Os12g43380) (Kreps et al. 2002; Walia et al. 2005; Zhu 2002). We further examined the expression levels of these three genes in GC2 and IL23 by Real-time quantitative-PCR. The results in Fig. 11 clearly showed that all the three transcripts highly expressed in IL23. For example, the expression level of thiosulfate transferase, LOC_Os02g07040 located in the region of *qSDT2-1*, in IL23 was dramatically induced and accumulated at the level 20 times higher than that in GC2 treated with PEG for 2 h (Fig. 11A). Similar expression data was obtained for potassium transporter, LOC_Os12g42300 located in the region of *qSDT12-2* (Fig. 11B). And, the thaumatin-like protein precursor gene, LOC_Os12g43380 located in the region of *qSDT12-2*, also had higher expression level in IL23 than in GC2 (Fig. 11C). It remains to be elucidated whether significant accumulation of these three genes contributed to the strong drought tolerance of IL23.

Discussion

Construction of introgression lines derived from wild rice

Owing to the domestication of rice over 10,000 years severely limiting the gene pool, which can be utilized to improve rice, more and more attentions were given to the wild species of *Oryza*. Wing et al. (2005) also emphasized that the wild rice contained a wealth of genetic diversity that must be uncovered. Then how to utilize the novel wild rice resource is becoming a challenge. Introgression line is a powerful tool for identification of favorable genes from wild species. Eshed and Zamir (1994) constructed a population consisting of 50 introgression lines originating from a cross between the wild species *Lycopersicon pennellii* and the cultivated tomato. Each of the lines contained single wild tomato chromosomal segment in the background of cultivated tomato. Using this set of introgression lines as resource materials, they have successfully identified and cloned several genes underling quantitative traits (Frary et al. 2000; Fridman et al. 2004).

In our laboratory, Tian et al. (2005) constructed a set of 159 introgression lines derived from the cross between an Indica cultivar Guichao 2 and an accession of common wild rice collected from Dongxiang county, Jiangxi province, China. Dongxiang common wild rice, as the donor in the introgression lines, originated from the northernmost habitat of common wild rice (28°14′N) and tolerant to various abiotic stresses. Using the introgression lines, several QTLs with yield-related traits were mapped (Tian et al. 2005). In this study, we mapped several QTLs related to drought tolerance, which were consistent with previous studies. For example, the QTL, *qSDT12-2*, near the SSR

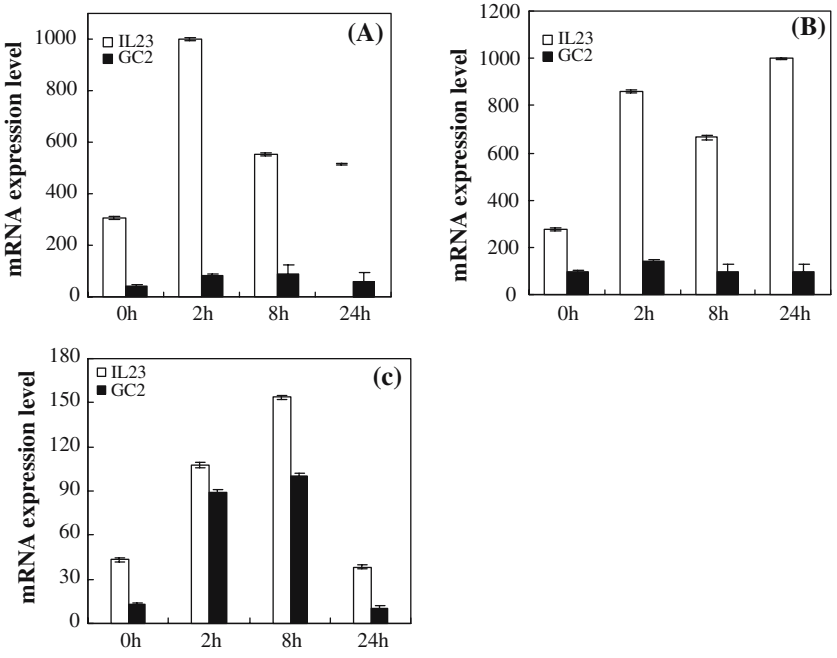

Fig. 11 Q-PCR result of three genes, LOC_Os02g07040 (**A**), LOC_Os12g42300 (**B**), LOC_Os12g43380 (**C**) in IL23 and GC2 during 30% PEG treatment

marker RM17 on chromosome 12 we mapped, was in the similar location to that of the leaf-rolling-score-related QTL (RM235-MRG5454) mapped by Yue et al. (2005). The QTL, *qSDT5-1*, we mapped near RM249 on chromosome 5, was alike in the same position to that of the QTL (RM509-RM430) related to the leaf-rolling-trait reported also by Yue et al. (2005). However, another important QTL near RM279 on the short arm of chromosome 2, *qSDT2-1*, showing strong positive additive effect, was a new QTL for no QTL was mapped in this region before.

We also identified a drought tolerant introgression line, IL23, by the phenotypic identification and analysis of physiological characteristics of drought tolerance using the same introgression lines. The line IL23 not only showed less curled degree of leaf than GC2 upon PEG treatment, but also showed better growth state under direct drought stress than GC2 at the seedling stage (data not shown). The IL23, carrying two positive QTLs derived from wild rice, is quite useful to identify drought related genes in further research. All the results demonstrated that introgression line is a powerful tool for identification of new genes.

PEG treatment can be an appropriate substitute for drought stress

For crop plants, it is troublesome to treat drought stress because of the difficulty of maintaining water supply, humidity and steady temperature. Recently, Lanceras et al. (2004) applied a line source sprinkler system to assess different level of water supply in drought stress research, but this system is costly. So we want to develop a new assay to appraise drought tolerance of rice seedlings. It has been disputed for a long time whether PEG treatment can be a substitute for drought stress, and some breeders deemed PEG treatment only as an osmotic stress, not drought stress. But as mentioned by Zheng et al. (2004), PEG-inducible water stress had a lot of transcripts overlapped with drought stress, and most transcripts had similar expression patterns during the two stresses. From our evaluation results, it appears that PEG stress simulating drought stress in greenhouse is an effective, convenient and economical assay to identify elite drought tolerant lines and it is easy to maintain humidity and temperature at vegetative growth phase in green house.

Physiological mechanisms for drought tolerance in the elite drought tolerant line

It is accepted that tolerant plants maintain the water content of tissues, or survive a reduction in tissue water content, or recover more completely after rewatering (Cabuslay et al. 1999). In this study, we examined the drought tolerant introgression line IL23 through multi-physiological assays. The result showed that IL23 had a superior ability to conserve water content than GC2, although PEG stress produced a significant decline in shoot and root fresh weight in both lines. And IL23 recovered more quickly and completely than GC2 after rewatering. This is maybe a result of different ability to close stomata between IL23 and GC2 in response to

PEG stress. For IL23, it had a higher leaf temperature than GC2, suggesting that IL23 probably closed more stomata than GC2 (as shown in the infrared thermography analysis), so IL23 could have less water loss.

Besides less water loss, plants could also survive the effects of drought physiologically through decreased loss of cell turgor (Lu and Neumann 1999). Then, maintenance of turgor by osmotic adjustment is an important physiological adaptation for minimizing the detrimental effects of water deficit (Morgan 1977). In our study, the line IL23 had lower osmotic potential after PEG stress treatment. At the same time, IL23 had more soluble sugars content than GC2 after 30% PEG stress. This could explain the lower osmotic potential in IL23 for soluble sugars can act as a compatible osmolyte to lower the seedlings osmotic potential. It suggested that IL23 increased the capacity for osmotic adjustment, which allowed the plant to increase the retention of water and maintain turgor under water deprivation condition.

Other than the above effects drought stress brought, water stress also generates secondary stresses, so-called oxidative stress, which can easily damage macromolecules on plasma membranes and increase electrolytical leakage. Commonly, tolerant plants have less injury on the membranes during stress treatment, and so have less electrolytical leakage. In addition, MDA content is another index to evaluate the injured degree of plants after stress treatment, which is a major cytotoxic product of lipid peroxidation and has been used extensively as an indicator of free radical production (Kunert and Ederer 1985). In this study, IL23 had lower electrolytical leakage level and MDA content than those in GC2. It implied that IL23 had less oxidation and injury on the plasma membranes after drought stress. Probably IL23 had less injury since it could close its stomata and lower its osmotic potential timely.

In summary, we speculate that IL23 probably has two main mechanisms to survive drought-stress caused by PEG. The first is to close more stomata during PEG stress to reduce water loss, and the second is to accumulate a class of compatible osmolyte, soluble sugars, to lower its osmotic potential and maintain high water retention. These two mechanisms may coordinately contribute the drought tolerance in the IL23 line.

Transcript analysis of introgression regions

To further discover the essence of drought tolerance of IL23, we gave a preliminary investigation of the expressional profile of the introgression QTL located. Previously, it is reported that plants would accumulate proteins to confer tolerance to drought stress (Smirnoff 1998), and thaumatin-like protein (TLP) family is among these. TLP is a pathogenesis-related (PR)-protein, which are also defined as proteins that are induced in response to stress and other abiotic stimuli (Zhu 2002). Jung et al. (2005) also mentioned that the production of TLP transcripts in the carrot seedlings increased dramatically with dehydration treatment but was not affected by abscisic acid, salicylic acid, or jasmonic acid. In this introgression line IL23, it was found that LOC_Os12g43380, thaumatin-like protein precursor, in *qSDT12-2* mapping region, was induced by PEG treatment. The transcript level increased dramatically within 8 h after treatment initiation, and dropped after 24 h treatment. Another gene, the potassium transporter of LOC_Os12g42300, in *qSDT12-2* region, also responded to PEG treatment. The two genes were already well documented by Kreps et al. (2002). They conducted a GeneChip microarray to perform a global expression profiling of Arabidopsis to osmotic, salt and cold stress, and found that the two genes were induced after 200 mM mannitol which brought osmotic stress, just like PEG stress.

Considering the complexity of drought stress response, it is important to identify a tolerant line to drought stress for understanding the genetic basis of the traits related to drought tolerance and expression profiling for drought related genes. We developed a novel strategy to construct introgression line with wild rice segment and evaluate the drought tolerance at the stage of seedling by PEG treatment in greenhouse. It is obvious that the strategy is effective and feasible. Identification and characterization of IL23 capable of drought tolerance would provide a useful basis for isolation of novel genes associated with drought tolerance derived from wild rice and for molecular breeding of drought tolerant rice.

Materials and methods

Plant materials

The Dongxiang common wild rice introgression lines in the genetic background of Indica cultivar, Guichao 2 (GC2), constructed in a previous study (Tian et al. 2005) were used to QTL analysis for drought tolerance and screened for drought tolerant lines.

PEG stress treatment and evaluation

Rice seeds were surface-sterilized in 5% sodium hypochloride for 20 min and washed in distilled water,

then germinated on Whatman paper saturated with water for 2 days at 37°C. The seedlings were transferred to hydroponic growth conditions in greenhouse (28°C day/25°C night temperatures, 12 h/12 h light/dark cycles, and 83% relative humidity). After about 2 weeks, ten regular seedlings with two and half leaves were chosen and placed into a glass tube (5 cm long in diameter, 12 cm in height).

To characterize the drought tolerance score of the introgression lines, the seedlings of GC2 were treated with 0, 20, 25, 30 and 35% polyethylene glycol (PEG, molecular weight 6000) solution as a control. Lines with same leaf rolling degree as Guichao 2 in 0% PEG solution were marked as ''Score 9'', and lines with leaf rolling degree more serious than Guichao 2 in 35% PEG solution were marked as ''Score 0''. Similarly, lines with same leaf rolling degree as Guichao 2 in 20, 25, 30 and 35% PEG solution were regarded as Score 7, 5, 3 and 1, respectively. Observation and evaluation were performed for 8 days after treatment. All the evaluations were repeated three times.

Plant growth and seed germination

The fresh shoots and roots were harvested after different concentrations of PEG treatment, washed with distilled water. Plant growth was determined by fresh weight of shoots and roots.

For seed germination assays, at least 50 seeds from IL23 and GC2 were sterilized and treated in different concentrations of PEG after 2-day imbibition. The germinated seeds (emergence of radicals) were counted after 1 day.

Measurement of physiological characteristics of introgression lines

To investigate the effect of PEG treatment on the degree of drought stress, the water loss of leaves and osmotic potential under 30% PEG treatment were measured according to Chen et al. (2005). The electrolytical leakage of seedlings was measured according to Zhang et al. (2004). MDA contents were measured using a thiobarbituric acid reaction (Heath and Packer 1968). Soluble sugars content was measured according to Chen et al. (2005). Each experiment was replicated three times. All effects discussed in this study were significant at $P \leq 0.05$.

Thermal imaging

Thermal images were obtained using a Thermacam PM250 infrared camera (Inframetrics, FLIR Systems) equipped with a 16° lens and a 256 × 256 pixels PtSi focal plane array detector responsive to the short wave infrared (3.5–5.0 μm) and a sensitivity of 0.1°C.

DNA extraction and SSR analysis

Fresh leaves were collected and ground in liquid nitrogen. DNA was extracted from the ground-tissues by the CTAB (Cetyltrimethyl ammonium bromide) method (Rogers et al. 1988). SSR primers were synthesized according to the sequences published by Temnykh et al. (2000). Amplification, 8% polyacrylamide denaturing gels and the sliver-staining protocol were described by Panaud et al. (1996).

Data analysis

QTL analysis for drought tolerance was conducted using the genotypic data from BC_4F_4 population (Tian et al. 2005) and phenotypic data obtained in this study with Map Manager QTXb17 (Manly et al. 2001) by single-marker regression method. QTLs were declared significant if the regression gave a significance of 5%. For the drought tolerant IL23 screened from the introgression lines, a total of 254 SSR markers of polymorphism were obtained and employed to further reveal its genotype.

Reverse transcription and real-time quantitative PCR

About 10 μl samples containing total RNA (2 μg), random hexamers (Invitrogen) were heated at 70°C for 2 min to denature the RNA, and then chilled on ice for 2 min. The reaction buffer and M-MLV (Invitrogen) were added to a total volume of 20 μl containing: 500 μM dNTPs, 50 mM Tris–HCl, 75 mM KCl, 3 mM $MgCl_2$, 5 mM dithiothreitol, 200 U of M-MLV, and 0.1 μg/μl random hexamers. The samples were then heated to 42°C for 1.5 h.

Real-time PCR amplifications were carried out with four genes selected from the QTLs regions. The 18s gene (forward primer, 5′-GCTTTGGTGACTCTAGATAAC-3′; reverse primer, 5′-GTCGGGAGTGGGTAATTTGC-3′) was used as a control in the PCR. All real-time PCR assays contained 10 mM Tris (pH 8.3), 50 mM KCl, 0.2 units of Taq DNA Polymerase (Sigma), 3 mM $MgCl_2$, 2.5 mM dNTPs, 1:100,000× SYBR Green I (Molecular Probes), and 4 nM of each primer in 10 μl. The real-time PCR primers were designed using Primers3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

Real-time PCR was performed in MJ sequence detection system starting with 3 min of pre-incubation at 95°C followed by 40 cycles. The threshold cycle (Ct) was determined by use of the maximum-second-derivative function of the software. Formation of expected PCR product was confirmed by agarose gel electrophoresis (2%) and melting curve analysis.

The Q-PCR primers for three genes were as followings:

The Q-PCR primers for LOC_Os02g07040: 5′-CAGAATGGGAAGCTCTACCG-3′; 5′-AATCCATCATTTCGGAGCTG-3′; the Q-PCR primers for LOC_Os12g42300: 5′-CCGAAGACAACACCAAGACA-3′; 5′-TGATGCATTATGGGCAGAAG-3′; the Q-PCR primers for LOC_Os12g43380: 5′-ACAACGT CGCCATGAGCTTC-3′; 5′-GATGATGCATTATG GGCAGAAG-3′.

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