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QTL ANALYSIS OF DEHYDRATION TOLERANCE AT SEEDLING STAGE IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Dehydration tolerant QTLs at the seedling stage in rice (*Oryza sativa* L.) was studied using green shoot length as a parameter, which was measured during the recovery period after dehydration stress. According to the results of pilot experiment carried out to evaluate the two parental rice cultivars for the level of dehydration tolerance, at 4-day and 5-day dehydration stress, *Japonica* parent Hyogokithanishiki (HGKN) showed more tolerance than that of Hokuriku-142 *Indica* parent (HOK). Quantitative trait loci (QTLs) associated with dehydration tolerance was identified using 163 recombinant inbred lines derived from these parents on a linkage map constructed with 95 simple sequence repeat (SSR) markers. Three dehydration tolerant minor QTLs were identified on chromosome 1, 2 and 3 at composite interval mapping by WinQTLCARTOGRAHER. According to the additive effect plots the QTLs on chromosome 1 and 2 have the allelic effect from the *Indica* parent Hokuriku-142 and QTL on chromosome 3 has allelic effect from Hyogokithanishiki *Japonica* parent. However, according to the 1000 time permutation test, these QTLs didn't exceed the threshold LOD value.

Key words: Dehydration tolerance, QTL, rice (*Oryza sativa* L.), SSR markers

INTRODUCTION

Drought stress is the most common adverse environmental condition that can seriously reduce crop productivity. Increasing crop resistance to drought stress would be the most economical approach to improve agricultural productivity and to reduce agricultural use of fresh water resources. Cultivated rice (*Oryza sativa* L.) demands tremendous amount of water during growth. Development of new rice cultivars with drought tolerance saves a great amount of water and also helps to increase yield stability. Drought resistance in rice depends on one or more factors of the following components: ability of roots to exploit deep soil water, the capacity for osmotic adjustment and the ability to control and reduce water loss (Ngueyen *et al*. 2004). Drought tolerant rice cultivars exhibit various adaptations such as early-maturity, flowering even under little or no rain, stop growing as soil moisture declines, leaf rolling and response of root distribution to soil drying (Azhiri-Sigari *et al.* 2000).

However, drought tolerance is unmanageable to molecular genetics studies mainly due to limited awareness of specific traits linked to drought tolerance. Furthermore, it is difficult to conduct drought stress treatments in a quantitative and reproducible way. These difficulties have significantly impeded research on plant drought tolerance. Limited awareness of plant phenotypes specifically conferred by drought stress has prevented researchers from using traditional genetics approaches to directly study drought stress tolerance (Tripathy *et al.* 2000). The biological basis for drought tolerance is still largely unknown and few drought tolerance determinants have been identified (Ludlow and Muchow, 1990; Bohnert *et al.* 1995; Araus *et al.* 2002; Bruce *et al.* 2002). Undisclosed drought tolerance mechanism has stagnated both traditional breeding efforts and use of modern genetics approaches in the improvement of drought tolerance of crop plants (Passioura 2010; Sinclair 2011).

Despite the lack of understanding of drought tolerance mechanisms, physiological and mo-

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lecular biological studies have documented several plant responses to drought stress (Bohnert *et al.* 1995; Blum, 1996; Ingram and Bartel, 1996; Bray, 1997; Schroeder *et al.* 2001; Luan, 2002; Sharp *et al.* 2004; Xiong *et al.* 2006). Molecular approaches to develop drought tolerant rice cultivar have been applied by QTL analysis. Integration of publicly available rice genetic map covered by microsatellite markers with trait data of diverse mapping populations has been successfully identified chromosomal regions associated with some secondary traits of drought response, such as osmotic adjustment (Lilley *et al.* 1996; Zhang *et al.* 1999, 2001; Robin *et al.* 2003), cell membrane stability (Tripathy *et al.* 2000), abscisic acid (ABA) content (Quarrie *et al.* 1994, 1997), stomatal regulation (Price *et al.* 1997), leaf water status, and root morphology (Champoux *et al.* 1995; Ray *et al.* 1996; Price and Tomos 1997; Yadav *et al.* 1997; Ali *et al.* 2000; Courtois *et al.* 2000; Zheng *et al.* 2000; Zhang *et al.* 2001; Kamoshita *et al.* 2002; Price *et al.* 2002), membrane stability and osmotic adjustment (Li *et al.* 2005), leaf size and ABA accumulation (Robbin *et al.* 2003).

Wang *et al.* (2004) showed the reduction in shoot height under dehydration stress in dehydration susceptible rice cultivar, CT9993 while dehydration tolerant IR2266 was not affected. Genomic regions of rice chromosomes with drought-resistant loci have been reported by Adam *et al.* (2002), Tripathy *et al.* (2000), Zeng *et al.* (2000).

In the present study we used F_6 generation of inbred line population for QTL analysis at the seedling stage. The *Indica* parental cultivar, Hokurikuriku-142 and *Japonica* parental cultivar, Hyogo-kithanishiki are genetically divergent types, which provides enough segregation of both molecular marker alleles and ergonomically important genes for QTL analysis. The drought tolerant data were collected according to Ranawake and Nakamura (2011). The objective of this study was to investigate the effectiveness of new breeding materials for rice dehydration tolerant through genetic recombination between *Indica* and *Japonica* rice that have no previous evidence on drought tolerant traits.

MATERIALS AND METHODS

F_6 generation of 163 recombinant inbred line (RIL) population derived from *Japonica* rice cultivar, Hyogo-Kithanishiki (HGKN), and an *Indica* rice cultivar, *Hokuriku-142* (HOK) were used for QTL analysis. Hokuriku was bred from a cross between a Korean cultivar, *`Milyang 21* and an *IRRI* line ` *IR-2061-214-31`* in the Hokuriku Agricultural Experimental station, Japan (Misawa *et al*. 2000).

Evaluation of inbred line population for dehydration tolerance: Two week old rice seedlings were undergone a 5 day drying period. Five-day after complete drying plants were watered and 10 days after recovery period plants were evaluated for drought tolerance (Ranawake and Nakamura 2011). Experiment was carried out according to the complete randomized block design and repeated three times. Average green shoot length was used as the parameter to evaluate the inbred line population on the 10th day of the recovery period followed by 5-day dehydration stress.

DNA extraction, marker genotyping and construction of linkage map: DNA extraction was done and primer pairs for rice microsatellites (simple sequence repeat: SSR) were surveyed for polymorphisms. The linkage map was constructed using MAPMAKER 3.0, for 100 SSR markers. Composite interval mapping was performed to identify significant QTLs with forward-backward regression. The experiment-wise LOD threshold level was determined by computing 1,000 permutations, as implemented by the WinQTLCARTOGRAHER (Wang *et al*. 2003 http://statgen.ncsu.edu/qtlcart/WinQTLCart). These permutations can account for non-normality in marker distribution and trait values. The level of significant LOD value for QTLs in this study was determined to be 3.01 at $p<0.05$.

RESULTS AND DISCUSSION

Evaluation of two parental rice cultivars for dehydration tolerance: Considering the fact that two parental cultivars showed the highest significant difference in the green shoot length at 5-day dehydration stress, this stress condition was decided to apply for the evaluation of RILs (Fig.1).

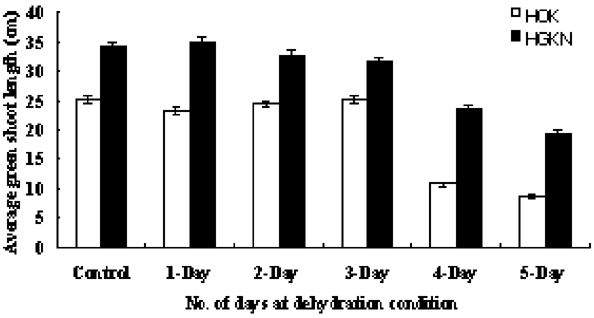


Fig. 1: Mean length of green shoot of seedlings survived after dehydration stress for 1-5 days in two parental cultivars. Data were taken at the 10th day of recovery period after the indicated period of dehydration stress. Twenty seedlings of each parent were subjected to the dehydration stress and the experiment was repeated three times. Bars indicate standard errors.

Evaluation of inbred line population for dehydration tolerance: The frequency distribution of average green shoot length in the RIL population on the 10th day of the recovery period after 5-day completely drying period showed a transgressive segregation in both directions (Fig. 2). This indicates that the alleles for drought tolerance in the population have donated from both drought tolerant parent and drought susceptible parent.

QTL analysis of dehydration tolerance in inbred line population: QTL analysis was performed using WinQTLCARTOGRAHER version 2.0 (Wang *et. al*, 2003). To understand the significance of these QTLs, composite interval mapping was performed. Minor dehydration tolerant QTLs with LOD score more than 2 were detected on chromosome 1, 2 and 3 at composite interval mapping of the present study (Fig.3, Fig. 4). A total of 24% of phenotypic variation has been explained by these 3 QTLs collectively (Table1). Drought tolerant QTLs on chromosome 1 and 2 have acquired drought tolerant alleles from drought susceptible parent Hokuriku while the reported strongest drought tolerant QTL on chromosome 3 possesses tolerant alleles from Hyogokithanishiki parent(Table1).

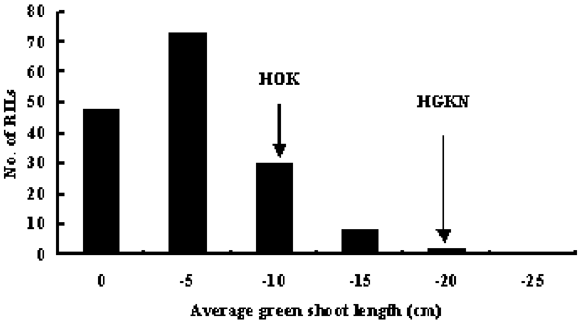


Fig. 2: Frequency distribution of the RILs after 5-day dehydration period. Data were taken on the 10th day of the recovery period after 5-day dehydration stress. Twenty seedlings of each parent were subjected to the dehydration stress and experiment was repeated three times

According to additive effect plots depicted in the composite interval map plot, the QTLs on chromosome 1 and 2 had the allelic effect from the *Indica* parent Hokuriku-142 and QTL on chromosome 3 had allelic effect from Hyogokithanishiki *Japonica* parent (Table 1).

There are many evidences on presence of drought tolerant candidate regions on chromosome 1, 2 and 3. RFLP linkage map for Co39 x Moroberekan recombinant inbred population which was used for QTL analysis for osmotic adjustment and lethal osmotic potential also indicated that chromosomes 1 and 3, possess the QTLs associated with root characters (Champoux *el al*., 1995). Wang, *et al.* (2005) has been selected rice chromosome 1 for identification of candidate genes for drought stress tolerance in rice. Price *et al.*, (2002) and Lafitte *et al.*, (2002) also give evidence on presence of drought tolerant QTLs on chromosome 1. In the region of RM472–RM104 on chromosome 1, drought tolerant QTLs for

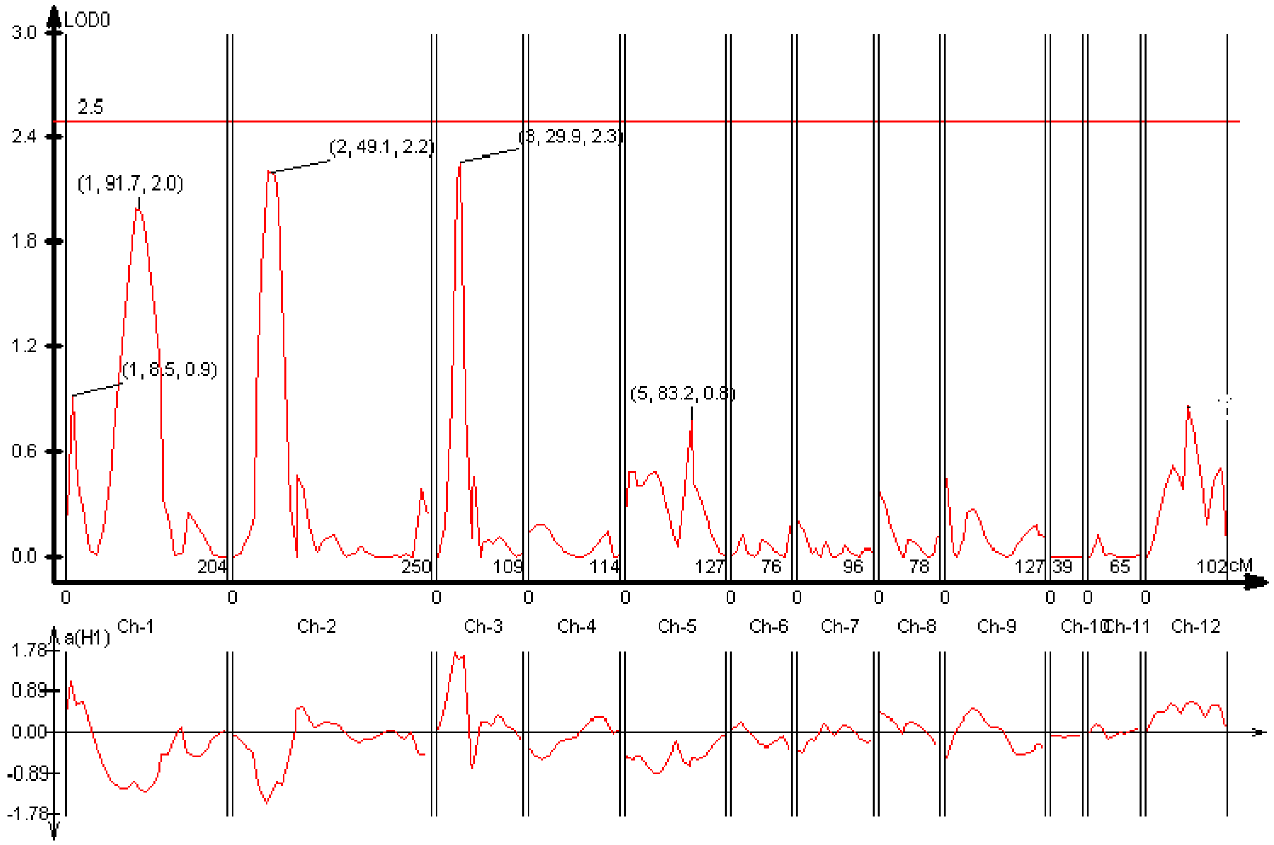
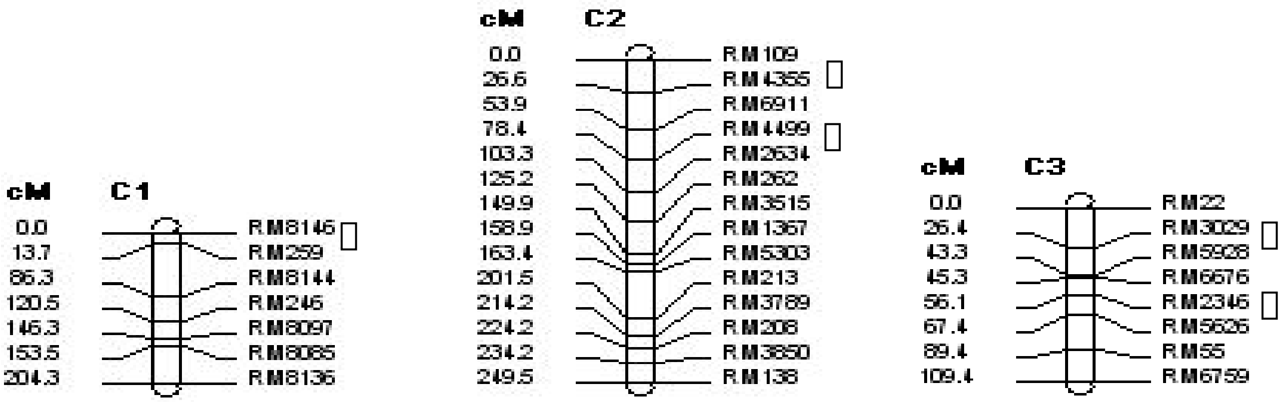


Fig. 3: QTLs controlling dehydration tolerance at the seedling stage identified by the composite interval mapping in the 5-day dehydration stress

root volume and number of days to leaf rolling were detected by Yue *et al.*, (2006). QTL were also identified for root thickness and root weight (Zheng *et al.* 2000), as well as for relative water content, leaf rolling, and leaf-drying score (Babu *et al.* 2003) under drought stress. Further, a total of three QTLs on chromosomes 2, 4, and 7 were detected for relative growth rate in rice (Kato *et al.* 2008).

Chromosome 2 was found to be associated with the grain yield or yield components un-



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der drought stress conditions by Yue *et al.* (2008). Yue *et al.* (2008) has also reported presence of drought resistant QTLs both on long arm and short arm of the chromosome 3 for flag leaf length, flag leaf width and plant height under drought stress. This chromosomal region on chromosome 3 coincides with the same chromosomal regions where we detected minor QTL on chromosome 3 (Table 1). These all findings align with the findings of our study at least proving that rice chromosomes 1, 2 and 3 are responsible for drought tolerance at the seedling stage.

Table 1: Dehydration tolerant QTLs found at composite interval mapping

Chromosome	Position (cM)	R^2	Additive effect	LOD score
1	91.7	0.0608	-1.1	2
2	49.1	0.1096	-1.437	2.2
3	29.9	0.0815	1.89	2.3

cM= Centimogon distance of chromosome

However considering background genetic effect, these QTLs do not exceed threshold LOD value computed by the 1000 time permutation test in WinQTLCARTOGRAHER version 2.5. According to results of QTL analysis there was a narrow variation in the detected QTLs for dehydration tolerance. This might be the reason that two parental rice cultivars had narrow but significant difference at the level of dehydration tolerance which was not wide enough to detect strong dehydration tolerant QTLs.

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