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Haplotype Diversity at *Sub1* Locus and Allelic Distribution Among Rice Varieties of Tide and Flood Prone Areas of South-East Asia

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Abstract: Single nucleotide polymorphisms and restriction digestion-based haplotype variations among 160 flood prone rice varieties were analyzed with enzymes *Alu* I and *Cac*8 I to generate polymorphisms at *Sub1A* and *Sub1C* loci (conferring submergence tolerance), respectively. Haplotype associated with phenotype was used to study the haplotype variations at *Sub1A* and *Sub1C* loci and to determine their functional influence on submergence tolerance and stem elongation. Three patterns at *Sub1A* locus, *Sub1A0* (null allele), *Sub1A1* (does not cut) and *Sub1A2* (one SNP), and four patterns at *Sub1C* locus, *Sub1C1*, *Sub1C2, Sub1C3* and *Sub1C4*, were generated. Both tolerant *Sub1A1* and intolerant *Sub1A2* had the same length, but the difference was presence of a restriction site in the *Sub1A2*, but absent at the *Sub1A1*. Further, two types of polymorphism were detected at the *Sub1C*, one included major length polymorphisms (165, 170 and 175 bp) and the other was a single restriction site at different position. Eight haplotypes (different combinations of the two loci), A1C1, A1C2, A1C4, A2C2, A2C4, A0C2, A0C3 and A0C4, were detected among 160 varieties. Haplotype A1C1 was comparatively more related to haplotypes A1C2 and A1C4, having the same *Sub1A* allele, and these haplotypes were found only in Bangladeshi, Sri Lankan and Indian varieties. Most tolerant varieties in A1C1 haplotype showed slow elongation, having tolerant specific *Sub1A1* and *Sub1C1* alleles. Further, the varieties Madabaru and Kottamali (A2C2) also showed moderate level of tolerance without *Sub1A1* allele. These varieties were different with FR13A and also suspected to carry different novel tolerant genes at other loci. These materials could be used for hybridization with *Sub1* varieties for pyramiding additional tolerant specific alleles into a single genotype for improving submergence tolerance in rice.

Key words: haplotype; *Sub1*; allele; single nucleotide polymorphism; submergence

Haplotype diversity analysis at a specific locus has the advantage to find new source of gene (McCartney et al, 2004). Haplotype associated with phenotype is also useful for grouping of genotypes based on presence and absence of a particular allele. However, information provided by single nucleotide polymorphisms (SNPs) is most useful, when gene-based haplotypes of a region are being examined (Rafalski, 2002). A linear arrangement of alleles (i.e., nucleotides) at different SNPs on a single chromosome, or part of a chromosome, is called

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a haplotype (Judson et al, 2002). The informative subset of SNPs is often referred to as haplotype tagging SNPs (htSNPs), or haplotype tags (Johnson et al, 2001; Chapman et al, 2003). Choice of a set of tagging SNPs is required to explain the highest amount of the total haplotype diversity (Daly et al, 2001; Judson et al, 2002; Hampe et al, 2003; Luca et al, 2003). Haplotype-based analysis provides higher power than single-locus test (Clark et al, 1998; Zhang et al, 2005; Liu et al, 2007). The haplotype analysis revealed that U.S. weedy rice retains large blocks of linkage disequilibrium for the multiple loci from the wild relatives and also incorporates haplotypes from cultivars (Mispan et al, 2013). Studies of comparative SNP and haplotype analysis for big window size haplotypes (3-SNP slide-window covering two 160 kb on average) reveal much higher genetic diversity than the 10 kb-window and gene-window haplotypes in maize (Lu et al, 2011).

The variety, FR13A, has been found to be relatively more tolerant to submergence, which is a farmer's variety from Orissa, India, and its local name is Dhullaputia (Mackill, 1986). Systematic screening of rice germplasm at International Rice Research Institute (IRRI) has confirmed that FR13A can survive for about 14 d under complete submergence. Under flash flooding, limited stem elongation growth was found to be associated with a cultivar's ability to survive. Rice, adapted to lowland condition, appears to be different to deep water. A negative correlation between survival rate and elongation growth was found, when tolerance is inherited from FR13A (Yamada, 1959; Jackson et al, 1987; Sardana, 1997; Sasaki et al, 2000a, b). Mechanisms associated with high submergence tolerance are reduced elongation and low synthesis or sensitivity to ethylene (Setter et al, 1997; Ella et al, 2003; Jackson and Ram, 2003). Reduced elongation under complete submergence is vital for survival, because elongating plants will collapse as soon as the water level recedes. A cluster of ethylene response factor (ERF) genes, i.e. *Sub1A*, *Sub1B* and *Sub1C*, have been cloned (Xu and Mackill, 1996; Xu et al, 2000, 2006). Furthermore, it was confirmed that *Sub1A* is the contributor for tolerance (Xu et al, 2006; Septiningsih et al, 2009), and the major determinant of submergence tolerance to mitigate the devastating wash out of rice plants due to flash floods (Xu et al, 2006; Septiningsih et al, 2009; Bailey-Serres et al, 2010; Singh et al, 2010). Two markers (close to *Sub1*) were converted to cleaved amplified polymorphism sequences (CAPS) markers, through digesting amplified products with respective restriction enzymes, and clear tolerant specific *Sub1A1* and *Sub1C1* alleles were found in tolerant accessions. Intolerance was found to be associated with poor submergence induced *Sub1A2* (or *Sub1C2*) or complete absence of *Sub1A* (only *Sub1C* allele).

Neeraja et al (2007) and Septiningsih et al (2009) described the marker-assisted backcrossing (MAB) procedures to transfer the *Sub1* allele into rice cultivars, which are widely grown in south and Southeast Asia. Using the MAB, *Sub1A* is introgressed to eight rice varieties (Collard et al, 2013). To enable more precise molecular breeding, a number of gene-based and tightly-linked markers in *Sub1* region have been developed (Neeraja et al, 2007; Septiningsih et al, 2009, 2013, 2015; Iftekharuddaula et al, 2011). A flood-resistant variety, Swarna Sub1, carries *Sub1* that enables it to survive for up to 14 d under complete submergence at the seedling stage. Maintaining genetic diversity is important, in terms of responding to evolutionary and environmental forces (Reed and Frankham, 2003). However, additional source of submergence tolerance is needed and crucial, because breeders are still striving to find additional sources of tolerance.

Xu et al (2006) analyzed haplotype diversity of rice and found identical submergence tolerant haplotype from submergence prone areas in Sri Lanka and eastern India. Bai et al (2003) and McCartney et al (2004) used previously identified markers that flank *Qfhs.ndsu-3BS* (a major QTL for *Fusarium* head blight resistance) to differentiate germplasms and identified wheat lines that putatively carry the QTL. Detecting novel QTLs, via haplotype-based comparisons, has greater advantage over expensive QTL mapping (McCartney et al, 2004). Further, mapping studies should be restricted to resistant lines that have a high probability of carrying novel resistance genes. A series of target genes identified in different parents are the best way to combine into a single genotype (gene pyramiding schemes) (Servin et al, 2004) and to utilize in gene expression analysis. Thus, haplotype analysis is useful to examine potential polymorphism of *Sub1*. This approach is also useful to find novel genes for submergence tolerance.

Genetic diversity analysis among flood prone rice varieties will be useful for identifying the varieties, having the maximum diversity with submergence tolerance. Genetic variation can also be assayed by haplotype analysis, based on detecting SNPs at the locus. A SNP is a single base change, which can occur within an allele/DNA sequence or within the whole