

Molecular Breeding For Submergence Tolerance In Rice (*Oryza sativa* L)

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Abstract

Flooding is a serious problem for rice production in South and Southeast Asia affecting over 22 M ha. Improved rice varieties are not adapted to submergence and therefore farmers are left with no option but to cultivate the stress tolerant low-yielding landraces or grow modern rice varieties and risk substantial crop loss. Conventional breeding efforts for developing submergence tolerant rice varieties with high yields have some limitations. Submergence tolerance at the vegetative stage was identified to be controlled by a single major quantitative locus (QTL) on chromosome 9 along many minor QTLs. This major QTL SUB1 identified in FR13A, a landrace from Odisha, India provides tolerance to complete submergence for up to two weeks. The SUB1A transcription factor was identified as the major determinant of submergence tolerance and was present only in indica varieties. In genotypes with submergence tolerance, ethylene activates SUB1A-1 allele limiting ethylene response and promoting repressors of gibberellins, thereby reducing elongation and carbohydrate consumption under submerged conditions. Using a marker assisted backcrossing (MABC) approach, the SUB1 locus was introgressed into several popular varieties of South and South East Asia. Under control and field conditions, no differences were observed between SUB1 introgression lines and their recurrent parents in grain yield or quality aspects. Under submergence, considerable enhanced grain yield was realized in SUB1 introgression lines with a yield advantage of up to 3.8 t/ha over parental lines. Under flooding at vegetative and reproductive stages, two situations are generally observed: semideep stagnant water (30-50 cm of water level) and deep water rices (> 1 m). For deep water, the adaptation is the rapid elongation of internodes in rice maintaining the leaves and panicle afloat above the water. Two genes viz, SNORKEL1 and SNORKEL2 encoding ethylene responsive factors on chromosome 12 were identified for deep water rice. Experiments are being conducted to identify donors and their response to stagnant flooding. The tolerance to flooding during seed germination or anaerobic germination (AG) trait is also critical for proper seedling establishment in direct-seeded rice. Out of several QTLs for AG tolerance, two major QTLs were detected on chromosomes 7 and 9 explaining ~30% each of the phenotypic variations. These QTLs are being introgressed into SUB 1 introgressed as well as other varieties. Attempts are being made in many national and international institutes to pyramid the identified QTLs for submergence tolerance across different stages of rice plant together with other QTLs for salinity and drought tolerance based on needs of the ecology and variety and to evaluate these breeding lines.

Keywords: Rice, flooding, submergence tolerance, QTLs

Introduction

Flash flooding and the resulting submergence of plants in different parts of the world adversely affect at least 16% of the rice fields (Khush 1984), especially in the flood-prone rainfed lowlands in South and Southeast Asia. Over 22 million hectares of rice Asia are considered submergence-ranging from flash flood to deepwater situations (Mackill et al 2012). The submergence situations are extremely unpredictable and can be of short duration (7 to 14 days) to several months leading to partial or complete failure of the crop. With

the change in climate, heavy rains during the critical crop season have increased making the rice crop more vulnerable to submergence (Takeda and Matsuoka 2008). Amongst various cereal crops, rice is the only crop that can withstand water logging. However, under submergence stress, based on the duration and height of the water stagnation the productivity reduces to 50% to complete loss of the rice crop.

Submergence stress to rice crop can be at different growth stages viz,

- flash flooding at vegetative stage
- stagnant water due to flooding at vegetative and reproductive stage and
- submergence during germination

Sometimes there are combinations of the three stresses in addition to other abiotic stresses. Since the damage due to submergence is considerable and has direct impact in the resource poor farmers of fragile ecosystems, submergence stress has always been a research priority in rice improvement programs (Mackill et al 2008).

Flash flooding at vegetative stage

Short term flooding (up to 2 weeks) or flash flooding is the most common submergence stress, where the crop (< 2 months old) is exposed to submergence resulting in rice plants decay and death. The extent of yield loss varies based on the duration of the submergence, water depth, age of the plant and water temperature. While the submergence affects almost all the rice varieties, a few genotypes were observed to be less affected. These varieties revive after the flood water recedes, and continue to grow normally. Attempts have been made through conventional breeding approaches to combine this submergence tolerance with high yielding varieties during the 1990's (Mackill 1986; Mohanty and Chaudhary 1986; Singh and Dwivedi 1996, Mackill et al 1993). Thus bred varieties were not very popular due in the absence of the desired level of submergence tolerance and high yield.

Genetic analysis of progeny from crosses between tolerant and intolerant parents (Suprihanto and Coffman 1981) indicated that submergence was controlled by one or a few genes with major effects and minor modifiers (Mohanty and Khush 1985). A single dominant locus for submergence tolerance was identified by QTL mapping and designated as *SUB1* locus on short arm of chromosome 9 in a derivative of FR13A, a submergence tolerant landrace (Xu and Mackill 1996). The *SUB1* locus accounted for 69% of the variation in tolerance to submergence. This finding was the results obtained in different mapping populations with FR13A parentage (Mishra et al 1996; Kamolsukyounyoung et al 2001; Nandi et al 1997; Siangliw et al 2003; Sripongpangkul et al 2000; Toojinda et al 2003). In addition, minor QTLs affecting submergence tolerance were mapped on chromosomes 1, 2, 5, 6, 7, 10 and 11 (Nandi et al 1997; Toojinda et al 2003). The important plant traits associated with tolerance to flash flooding at vegetative stage are high non-structural carbohydrate content before submergence, slower underwater shoot extension and optimum anerobic respiration, and limited leaf chlorosis (Ella et al 2003; Jackson and Ram 2003; Ram et al 2002; Setter et al 1997). Rice plants with submergence tolerance exhibit only limited elongation

during submergence and thus conserve energy ensuring early recovery for the production of sufficient biomass after de-submergence (Das et al 2005; Jackson and Ram 2003; Sarkar et al 2006).

Stagnant water from flooding at vegetative and reproductive stage

Stagnant water from flooding describes flooding which occurs at depths of up to 30 to 50 cm in the field, and may occur for a few weeks to several months. Stagnant water as a result of flooding often occurs in Eastern India, especially in Odisha, West Bengal and Assam. The plants under this situation are partially submerged most of the time, thus grain production is greatly reduced due to poor tiller initiation and greater susceptibility to lodging. It is important to note that *SUB1* does not confer any tolerance to flooding induced stagnant water (Singh et al 2011). To date, there is a complete lack of information on the genetic control and physiological basis of tolerance to stagnant water from flooding. In All India Coordinated Rice Improvement (AICRIP) program, several rice varieties with moderate yields have been identified for semi-deepwater situation after screening the genotypes with water level at depth of 75 cm during crop growth period (Rani et al 2008). Generally, these varieties are pureline selections from tall landraces or derived from local landraces adapted to semi-deepwater situation.

In some regions, the flood waters remain in the rice field for more than 2 weeks to several months with depth of several meters. 'Deep water rices' or 'floating rices' are usually cultivated here which show rapid internode elongation (up to 7 m) along with rising flood water with the uppermost leaves and panicles above the surface of the water (Catling 1992). Using the landraces of this ecology, several varieties with moderate yields were developed for the deep water situations in India (Rani et al 2008). Based on the water depth at vegetative and reproductive stage of the crop, flooding is categorized into deepwater (1 m and above) and flood water remaining stagnant as semideep water (20-50 cm).

Submergence during germination

In direct seeded rice areas, flash floods severely hamper germination and damage rice seedlings forcing farmers either to re-sow or re-plant. Poor germination is also observed in irrigated ecologies when the leveling of land is poor and in flood-prone rainfed ecosystems when rainfall occurs within a few days following seeding (Ismail et al 2009; Angaji et al 2010). Poor germination by excessive water can occur in any type of land including upland; therefore the trait of anaerobic germination in rice varieties was also considered important. However, in the absence of accurate screening techniques and clear understanding of

genetics of associated traits, the efforts to improve germination under submergence were impeded (Mackill and Xu 1996).

Review of recent advances in research

Flash flooding at vegetative stage

The major locus for submergence *SUB1* was found to have a cluster of three putative ethylene response factor (ERF) genes viz, *SUB1A*, *SUB1B* and *SUB1C*. Further, *SUB1A* was identified as the major determinant of submergence tolerance (Xu et al 2006). *SUB1A* gene is present only in a few *indica* accessions and has two alleles (*SUB1A-1* and *SUB1A-2*) distinguished by two single nucleotide polymorphisms (Fukao and Bailey-Serres 2008a; Fukao and Bailey-Serres 2008b; Fukao et al 2009). An allelic survey of the *SUB1* ERF genes in a range of tolerant and intolerant germplasm revealed tolerant-specific alleles for *SUB1A* (*SUB1A-1*) and *SUB1C* (*SUB1C-1*). The tolerant and intolerant alleles (*SUB1A-2*; *SUB1C-2* to 8) show differences in several putative phosphorylation sites and are differentially expressed during submergence. Whereas *SUB1A-1* is highly expressed in tolerant lines and expressed at a very low level in intolerant accession, *SUB1C* shows the opposite expression pattern (Fukao et al 2009; Xu et al 2006; Singh et al 2010). The phylogeny of the *SUB1* genes of the domesticated varieties and wild accessions suggests that *SUB1A* arose from duplication of *SUB1B* (Fukao et al 2009). The *SUB1A* gene has been detected in some wild *Oryza rufipogon* accessions (Li et al 2011).

Under submergence, ethylene rapidly accumulates in tissues of submerged rice plants leading to increase in shoot elongation, adventitious root formation and carbohydrate metabolism (Fukao et al 2006). The accumulated ethylene affects the contents of gibberellic acid (GA) and abscisic acid (ABA) accordingly with the reaction of the genotype. In deepwater rices, ethylene promotes internode elongation during submergence, thus allowing the leaves above the water level for photosynthesis. By contrast, in lowland rice, ethylene production is restricted by the action of the *SUB1A1* allele. The submergence inducible *SUB1A1* is an ERF which increases the production of GA repressors, thus conserving the carbohydrates for the rejuvenation of the plant after the recession of the floods (Fukao and Bailey-Serres 2008a; Fukao and Bailey-Serres 2008b; Fukao et al 2009).

Marker assisted backcrossing (MABC) of *SUB1*

Since *SUB1* is a major locus explaining ~69% of the phenotypic variation, several attempts were made to transfer this locus even prior to identifying the *SUB1A* gene (Siangliw et al 2003; Toojinda et al 2005). At Kasetsart University, Thailand, *SUB1* locus was

introgressed into Thai jasmine rice KDML105 retaining its photoperiod sensitivity, aroma and amylose content (Siangliw et al 2003). At International Rice Research Institute (IRRI), Philippines, using MABC approach, six mega-varieties of Asia were introgressed with *SUB1* locus. The recipient parents were Swarna, Samba Mahsuri, IR64, TDK1, CR1009 and BR11 and the donor parent was IR49830-7 or IR40931-33 derived from FR13A. Foreground, recombinant and background selections were made using candidate gene based markers and rice microsatellite markers at each backcross. In addition, two more varieties, PSB Rc18 from Philippines and Ciherang from Indonesia were also introgressed with *SUB1*. The size of the introgressed fragment from the donor ranged from 1.2 to 6.3 Mb (Neeraja et al 2007; Septiningsih et al 2009; Iftekharuddaula et al 2011).

In several field trials across South and Southeast Asia, there was no difference in most of the parameters between the original varieties and varieties with *SUB1* (Manzanilla et al 2011; Sarkar et al 2009). On submergence, *SUB1* introgressed lines showed a yield advantage of 1 to 3.5 t/ha based on the duration and conditions of flood situation. There was a general delay in flowering in all the genotypes after submergence, which was expected as the surviving plants take additional time to recover and resume normal vegetative growth (Singh et al 2009). Farmers' field studies also showed that *SUB1* is effective even during reproductive stage. Several varieties containing the *SUB1* locus were developed and some of them were officially released in various countries, including India, the Philippines, Bangladesh, Nepal, Indonesia, Myanmar, and Cambodia.

Deepwater rices

QTLs for internode elongation and number of elongated internodes – two traits useful in deepwater rice have been identified (Sripongpangkul et al 2000; Nemoto et al 2004; Hattori et al 2007; Kawano et al 2008). Through positional cloning, two genes *SNORKEL1* and *SNORKEL2* on chromosome 12 were identified responsible for internode elongation in deepwater rice. Under deepwater situations, these two genes encoding ERFs trigger internode elongation through gibberellins (Hattori et al 2009). Three QTLs associated with internode elongation in deep water rice were introgressed into non-deepwater rice through markers and demonstrated to grow well under deepwater situation (Hattori et al 2008).

Anaerobic germination

Anaerobic germination (AG) indicates tolerance of flooding at seed germination and is a key trait for proper seedling establishment in direct-seeded rice in both

rained flood-prone and irrigated ecosystems. Several QTLs for AG tolerance were reported on chromosomes 1, 2, 5, 7 and 9 (Jiang et al 2004, 2006; Angaji et al 2010). Out of these, two QTLs with major effects explaining ~30% of the phenotypic variation on chromosomes 7 and 9 are being targeted for fine mapping and introgression into popular varieties (Septiningsih pers comm).

Stagnant water due to flooding

Realizing the impact of stagnant flood water stress in farmers' fields, now experiments are being conducted at Central Rice Research Institute (CRRI) and IRRI to evaluate the response of the varieties and landraces. The screening techniques are still being standardized. Studies at CRRI, Cuttack on stagnant flood water indicated the better performance of Varshadhan, IR 85085-SUB 17-3-3-2, and IR 49830-7-1-2-3 (Reddy pers comm). Preliminary research at IRRI indicated that three varieties viz, IRRI 119 (PSBRc68), Inpara-3 and IRRI 154 (NSICRc222) to possess high levels of tolerance to stagnant flooding (Collard pers comm), in addition to many breeding lines (Mackill et al 2010). All varieties were bred using conventional methods. Both IRRI 119 and Inpara-3 contain *SUB1* and are therefore suitable for a range of flood-prone areas. At present, new breeding lines are produced at IRRI using early generation screening for submergence tolerance in the field followed by screening for stagnant-flooding tolerance in the field situation. Lodging resistance, plant height and intermediate elongation appear to be some of the key traits in stagnant water due to flooding conditions. For the development of new varieties with stagnant flood water tolerance in the future, identification of new QTLs, characterization of physiological basis for tolerance, and the identification of new donor parents for this trait are being attempted.

Innovative studies underway

- In addition to the *SUB1* locus, another novel major QTL locus *qSUB1.1* on chromosome 1 for submergence tolerance with 52% phenotypic variance has been identified from new sources (Septiningsih et al 2012). Several genetic studies have already identified minor QTLs that can affect the level of submergence tolerance independent of *SUB1* (Nandi et al 1997; Toojinda et al 2003; Singh et al 2011; Septiningsih et al 2012). At IRRI, pyramiding of these major and minor QTLs is in progress.
- At IRRI, a major QTL *AG1* is being introgressed into IR64, IR64-Sub1, Ciherang-Sub1, PSB Rc18-Sub1, and PSB Rc82 and introgression for another major QTL from chromosome 7 is also attempted (Septiningsih pers comm).

- The development of *Sub1-Plus* varieties with tolerance of flooding at vegetative stage and submergence during germination is in progress. Prototype breeding lines with *SUB1* and AG tolerance have demonstrated that the two traits can be combined in the same line (Vergara et al 2010) as the expression of *SUB1* does not apparently begin until four days after germination.
- Pyramiding of genes for biotic stress tolerance viz, bacterial blight and blast is also attempted in several national and international programs.
- The *SUB1* gene has been introduced into salt-tolerant varieties, and tolerance to the two stresses is expressed well in these plants (Mercado et al 2010).
- Attempts are also made to introgress *SUB1* gene into drought tolerant varieties (Verulkar et al 2010).
- Under India – IRRI collaborative project, several popular Indian varieties are being introgressed with *SUB1*, *Saltol* and QTLs for drought viz, *DTY1.1*, *DTY 2.2*, *DTY3.1*, and *DTY 9.1*, and their combinations based on the ecology (Thomson et al 2010; Vikram et al 2011).
- Currently at IRRI and CRRI in collaboration (STRASA project), a top priority is given to develop new varieties with *SUB1* and stagnant flood water tolerance.
- Under the hypothesis that *SNORKEL* genes or QTLs identified from deep-water rices can be deployed for enhancing stagnant flood water tolerance, Nagoya University, Japan is collaborating with IRRI to test the effect of *SNORKEL* genes for stagnant flood water tolerance.
- Attempts are also underway to introgress *SUB1* into taller varieties withstanding stagnant flood water targeting the areas where there is simultaneous or sequential occurrence of submergence and stagnant water from flooding.
- Studies are in progress for the identification of new donor parents and QTLs for stagnant flood water tolerance and characterization of physiological basis for tolerance at IRRI.

Prospects of achieving the breeding goal

For submergence tolerance, rice has developed quiescent strategy for flash flood situation and escape strategy through internode elongation for stagnant or deepwater situation with differential regulation of key hormones viz, ethylene, gibberellins (Fig. 1).

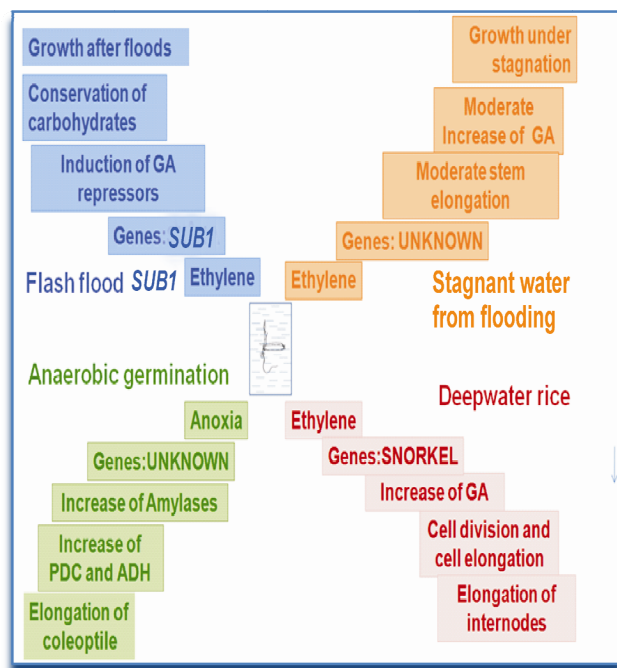


Figure 1. Differential response of rice to flooding stress based on the stage of the plant.

With genome analysis, the genes and alleles responsible for the submergence tolerance at different stages of rice plant have been elucidated. The knowledge gained is rightly being applied across the world to develop varieties using marker assisted selection. The study of the *SUB1* locus and its application is the first illustration of introgression of a major QTL in abiotic stress through molecular markers in rice with direct impact on farmers. The systematic approach and integration of the applied and basic research led to the development of the products *SUB1* introgressed lines proving the concept of validation of two/three backcrosses for the transfer of a gene/QTL and MABC using mega varieties. Five *SUB1* varieties have been officially approved reaching around 1,000,000 farmers a million hectares of rice and three varieties are under advanced stage of evaluation (Mackill et al 2012). Especially the MABC approach of introgression into the popular varieties has been rewarding considering their ready adoption by the farmers and the replacement of original variety in the flood-prone areas. With the available information of major and minor QTLs for important abiotic stresses and the candidate genes identified for most of the QTLs, precision molecular breeding as demonstrated in the case of the development of *SUB1* varieties will be an important strategy in future rice improvement programs.

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Citation: Neeraja CN, Septiningsih EM, Collard BCY, Reddy JN and Mackill DJ. 2013. Molecular breeding for submergence tolerance in rice (*Oryza sativa* L). In: Muralidharan K and Siddiq EA, eds. 2013. *International Dialogue on Perception and Prospects of Designer Rice*. Society for Advancement of Rice Research, Directorate of Rice Research, Hyderabad 500030, India, pp 238-244.