

A Critical Review of Submergence Tolerance Breeding beyond *Sub 1* Gene to Mega Varieties in the Context of Climate Change

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ABSTRACT

Flash flood, stagnant flooding and anaerobic germination are various forms of submergence that occurs at different stages of rice growth causing significant damage to crop production for more than 15 millions poor farmers in the rainfed lowland region of South and Southeast. Before the discovery of *Sub 1* gene farmers had to rely on low yielding, submergence-intolerant varieties that incurred yield drastic reduction to complete failure of the crop every year. With the discovery of major quantitative trait loci (QTL) submergence 1 (*Sub 1*) which is responsible for this trait, submergence tolerance breeding has achieved its milestone to overcome the burning issue. Cloning and isolation of *SUB1* locus in popular submergence tolerance parent *FR13A* that led to the identification of ethylene-responsive factor (ERF) gene *SUB1A-1* that is responsible for submergence tolerance. The *Sub 1* QTL is a single gene that has the LOD score of 36 which explained phenotypic variance of 69% conferring tolerance to complete submergence of two weeks. The submergence -1 (*Sub-1*) locus representing a cluster of three ethylene responsive factor (ERF) genes: *sub1A*, *sub1B* and *sub1C*. Identification of the *SUB1* gene was the entry point for enabled marker assisted selection (MAS) for submergence tolerance. Popular varieties also are known as mega varieties which possessed high yielding and good grain quality was used as the recurrent parent in marker assisted backcrossing (MABC). Beyond the *Sub 1* varieties, the present day rice breeders paid attention to tolerance to stagnant flooding and tolerance to anaerobic germination under deep water and flash flood during seedling germination respectively. Quantitative Trait Loci (QTLs) have been identified for anaerobic germination also referred to as AG (*AG1* and *AG2*) and stagnant flooding for deepwater rice (*SNORKEL1* and *SNORKEL2*). Furthermore, the combination of both traits conferring tolerance to stagnant flooding with *Sub 1* or *Sub 1* with anaerobic germination (AG) is the future direction of rice breeding through gene pyramiding approach to develop the rice varieties tolerant to multiple stresses to enhance yield and quality to combat the future challenge of food and nutritional insecurity.

Keywords: Submergence, Flash Flood, Stagnant Flooding, Anaerobic Germination, Marker Assisted Backcrossing.

INTRODUCTION

Flooding is a major abiotic stress and leading constraint limiting the crop production for the rice growers basically in the rainfed lowland areas and irrigated areas where flash flood and deepwater conditions prevails for few weeks to longer [20], [30]. Around 40 millions hectares of rice growing areas are affected by drought and flooding stress at different forms and intensities at various growing stages [9]. Both of these major stresses are common phenomenon in the rainfed lowland ecosystem almost in same growing areas within same season. Submergence stress is mainly caused by flash flood during the monsoon season with varying intensity and periods. The flash flood inundates the rice fields completely at the early stage before or immediately after the crop establishment for few days to weeks. When rice is submerged for more than five days the crop dies and cannot recover from the stress after water is receded [30]. The farmers who experience flash floods every year cultivate tall and submergence tolerant local varieties that are mostly low yielding in nature. In some cases farmers even have high yielding but submergence-intolerant landraces usually suffer from crop failure or massive yield reduction due to occurrence of flash flood. In the lowland areas the

flood water remains in the rice field for more than two weeks to several months. In such a circumstances, water level reaches 20-50 cm in depth. Such a phenomenon is commonly known as stagnant flooding [28], [20], [35], [30]. In such a situation, rice production also reduced due to poor tillering and highly prone to lodging [37], [35], [30]. In some areas, stagnant flooding is immediately followed by flash flood where farmers have only option to grow their locally adapted landraces that are capable of avoiding the stress eventhough they are poor yielding. Similarly, in low lying areas where water level remain for longer period and reached upto height of the crop, farmers cultivate floating rice or deepwater rice that have capacity of internode elongation that facilitates the crop to expose leaves and panicles above the water surface [3]. Such deepwater rice have low yield due to excessive vegetative growth. Stagnant flooding is also a kind of submergence stress during which water remains in the field at depths of 50 to 60 cm for up to several months [21], [5]. Stagnant flooding is particular to rainfed lowland areas in Eastern India, Bangladesh, Nepal, Cambodia, Thailand and some parts of Africa.

Submergence is the widespread constraint to more than 15 millions poor farmers of rice growing areas in rainfed lowland region of South and Southeast. In these areas farmers cultivate their own local landrace that can withstand submergence but are basically poor yielders. Likewise, high yielding and submergence intolerant rice varieties that are grown by farmers in large scale have been affected by flash floods frequently. The occurrence of flash floods are triggered by unpredicted and variability in weather factors causing irratic rainfall during the monsoon season. To overcome such situations, some sustainable solutions are required which could be development of high yielding and submergence tolerant varieties that would be widely accepted by farmers in a affected areas [28].

In deepwater and floating rice areas, water stagnates for longer than two weeks and varieties overcome the situation by shoot elongation to cope with innundation [26], [7], [16], [18]. The studies estimated that flood affected area is in increasing trend within this one decade affecting 19 million hactare to 40 million hactare of cultivated land. This type of scenario have be arised due to vulnearable climatic conditions [39], [18]. Flash floods are also causing extensive crop loss at the germination and early seedling stage that leads to poor plant stand i.e. population in aerobic rice fields. It is common in unlevelled land of irrigated system and flood prone rainfed ecosystem in condition where rainfall occures soon after the seeding rice [14], [1], [30]. Existing rice varieties with farmers donot germinate, grow and survive under innundation and as a result of that seedling establishment is resulted into poor seedling [18]. The occurrence of submergence at the seed germination period impart difficulty to the farmers in adopting the direct seeded rice technology. In such a areas farmers fail to timely cultivate the crop due to lack of such varieties. When crop is damaged by floods immediately after seeding they have no ther alternative either reseed or transplant . It becomes very expensive and costly due to increased labor that affecting the subsequent crop as well [17], [18].

Many efforts were made in the past to develop direct seeded rice varieties with tolerance to flooding during germination however success has not been achieved due to lack of tolerant donor parents and complex trait itself [42], [43], [2], [30]. With rigorous screening of thousands of rice accessions and landraces tolerant donors of submergence during germination were identified and regarded as anaerobic germination [1], [30].

Continued work to develop submergence tolerant varieties, it became essential to combine stagnant flooding tolerance with submergence (based on *SUB1*). This work was initiated with mega variety Swarna *Sub 1* in which both submergence and stagnant flooding were combined with conventional breeding through phenotypic selection. The new variety IRRI 119 was developed by IRRI and released by Philippines as PSB Rc68 [28], [20], [30]. Later on breeding lines with high yielding and stagnant flooding tolerance have also been developed through conventional breeding [20] [30].

2. HISTORY OF SUBMERGENCE BREEDING AND SUB1 BEYOND

A milestone achievement in submergence tolerance breeding was begun with the discovery of major quantitative trait loci (QTL) submergence 1 (*Sub 1*) which is responsible for this trait [40], [5], [30]. With continued efforts of the plant breeders and biotechnologist cloning and isolation of *SUB1* locus in popular submergence tolerance parent FR13A that led to identification of ethylene-responsive factor (ERF) gene SUB1A-1 that is responsible for submergence tolerance [41], [30].

The donor parent of *SUB1* gene FR13A, a pure line variety derived from landrace after selection in Dhalputtia a native landrace from Orissa of India was found as highly tolerant cultivar and later on subsequently used in breeding as tolerant donor. Thereafter many breeding lines have been developed for submergence tolerance, new high-yielding and adapted breeding lines derived from FR13A including IR49830 and IR40931 were developed at IRRI which is highly tolerant and also good yielding [19], [5], [30]. Although this variety is not popular among the farmers for cultivation due to lack of some special traits that present in their preferred variety [24], [30].

After investigation and isolation of *Sub 1*, through introgression of *Sub 1* into farmers preferred widely adopted submergence tolerant mega rice varieties like IR64-*Sub 1*, Samba Mahsuri-*Sub 1*, Thadokkamq-*Sub 1*, BR11-*Sub 1* and Swarna *Sub 1* are released in Asia and Africa [27], [34], [6], [18].

2.1 Genetics of submergence tolerance in rice

According to [22], submergence tolerance was found to be governed by a single dominant gene. The findings of recent studies on genetic basis of submergence tolerance is controlled by major quantitative trait locus (QTL) on chromosome 9, designated as *Sub* (T), is responsible for this trait which is derived from the parent FR13A [40], [23], [25], [37], [28]. *Sub* (T) locus had a remarkable high LOD score of about 36 and accounted for approximately 69% of the phenotypic variation for the trait [40], [25], [28] that confers tolerance to complete submergence for two weeks. According to [13] the rice submergence tolerance level is largely controlled by a single gene designated *Sub1* which is accounted for 69% of the phenotypic variance of the trait. The submergence tolerance in rice is induced by submergence -1 (*Sub*-1) locus representing a cluster of three ethylene responsive factor (ERF) genes: *sub1A*, *sub1B* and *sub1C* (Fig. 1) where *sub1* genes are member of group of group VII of ERF gene family associated than other rice ERF genes. The two *sub1B* and *sub1C* are found in both *indica* and *japonica* accessions while *sub1A* occurs in only *indica* has been discovered that submergence tolerant rice varieties contain *sub1A*-1 allele but the susceptible accessions only possess *sub1A*-2 alleles. Thus it has been believed that *sub1A*-1 allele is responsible for regulation of submergence tolerance in rice [41], [12], [28].

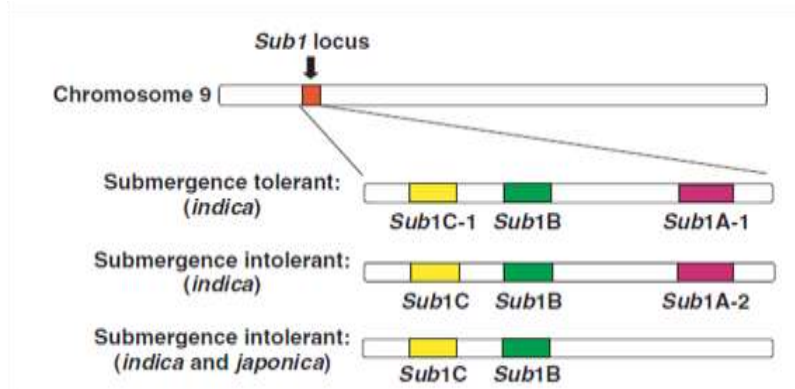


Figure 1: *Sub1* locus organisation in rice (*Oryza sativa* L.) (Fukao et al. 2009)

The *Sub 1* locus has cluster of three genes, encoding ethylene response factors. Two of these genes, *Sub1B* and *Sub1C*, are invariably present in the *Sub 1* region. In contrast, the presence of *Sub1A* is variable. A survey identified two alleles within those *indica* varieties that possess this gene: a tolerance-specific allele named *Sub1A*-1 and an intolerance-specific allele named *Sub1A*-2. Overexpression of *Sub1A*-1 in a submergence-intolerant *O. sativa* ssp. *japonica* conferred enhanced tolerance to the plants, downregulation of *Sub1C* and upregulation of Alcohol dehydrogenase 1 (*Adh1*), indicating that *Sub1A*-1 is a primary determinant of submergence tolerance. The FR13A *Sub1* locus was introgressed into a widely grown Asian rice cultivar using marker-assisted selection (MAS). The variety developed from this approach possesses high yield and all the traits similar to recurrent parent and have very good submergence tolerance [41].

Mechanism of submergence response of deepwater rice and lowland rice are contrasting; ‘submergence-escape’ and ‘submergence-tolerance’ (Fig. 2). The ‘submergence escape’ response of deepwater rice is to promote internode elongation under submerged conditions to outgrow submergence. The rapid underwater growth is an energy-consuming process which requires the consumption of stored carbohydrates and other energy resources. The submergence escape response is only successful if photosynthetic organs emerge prior to exhaustion of carbohydrate reserves and other energy resources. Indeed, even deepwater rice dies within 14 days of complete submergence [41], [31], [10]. In contrast, the ‘submergence-tolerance’ of lowland rice is mediated by the promotion of shoot elongation and the consumption of carbohydrate reserves during submergence [32] [11] [41] [10]. The tolerant cultivars can survive complete submergence for up to 14 days and can resume the initiation of plant growth upon desubmergence. In the tolerant lowland rice under submergence conditions, ethylene production is low and carbohydrate consumption is also low resulting into reduced elongation of internodes that were mediated by *Sub1A* which inhibits ethylene response. Plants undergo complete tolerance of submergence for two weeks and after desubmergence plants resume growth by utilizing the reserved carbohydrate [10].

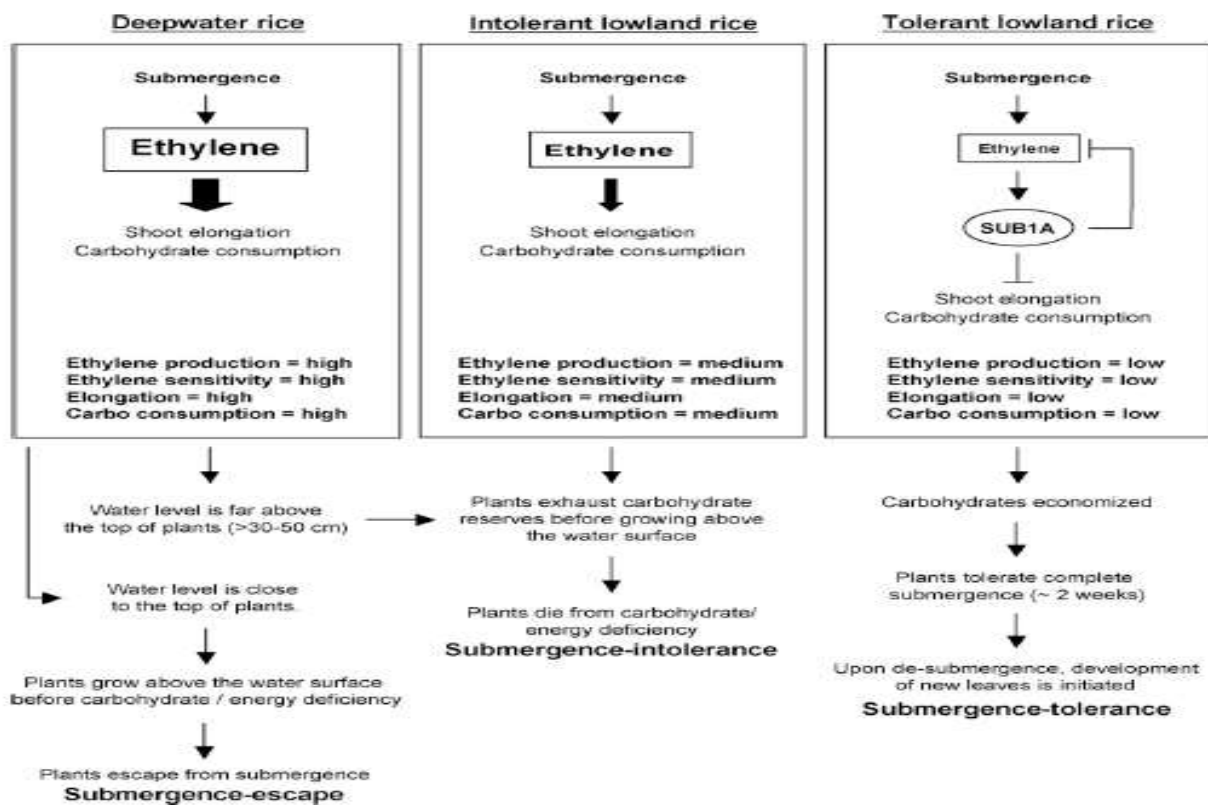


Figure 2. Ethylene-mediated regulation of distinct submergence responses in rice [10]

3. BREEDING RICE FOR TOLERANCE TO SUBMERGENCE

3.1 Flash flood tolerance during vegetative stage

After identification of submergence tolerant donor FR13A, many breeders extensively used this variety and unravelled major QTL *Sub1* responsible for submergence tolerance. Subsequently, *Sub 1* gene was fine mapped using F2 segregating population and underlying genes cloned as a cluster of three ethylene responsive factor (ERF) genes, SUB1A, SUB1B and SUB1C [41]. Furthermore, it was obvious that *SUB1A* was the mainly responsible for tolerance [41]; later on this finding has been confirmed through a progeny test of recombinants identified within the *SUB1* cluster in several thousand individuals in segregating populations [28], [30]. Based on allelic survey of *Sub1A* it was further revealed that *Sub1A-1* is tolerant and *Sub1A-2* is intolerant [33], [29]. Identification of the *SUB1* gene was the entry point for enabled marker assisted selection (MAS) for submergence tolerance. Popular varieties also known as mega varieties which possessed high yielding and good grain quality were used as recurrent parent in marker assisted backcrossing (MABC). FR13A derived two improved lines were used as donors IR49830-7-1-2-2 (IR49830-7) and IR40931-33-1-3-2 (IR40931-33) [19], [28]. By using precision MABC, the high yield and desirable grain and eating qualities of these mega-varieties were retained [28] [34] [30].

To shorten the breeding period and high throughput three steps of MABC were used : (1) foreground selection, in which markers tightly linked to *SUB1* are used to select for the locus; (2) recombinant selection, in which closely linked flanking markers are used to minimize the donor chromosomal segment containing *SUB1*; and (3) background selection, in which DNA markers are used to accelerate the recovery of the recurrent parent genome (Figure 4), [4].

The method of selecting recombinants on both sides of the target locus in at least two backcross (BC) generations during MABC was first proposed by [44] and applied in rice by Chen and colleagues (2000). Some *Sub 1* varieties developed through MABC have been given in the Table 1 and commonly used markers used in development of *Sub 1* gene is presented in Table 2.

Table1. Some popular Sub1 varieties developed through MABC

Recurrent parent	Country of origin	Donor parent	Generation	Introgression size (Mb)	IRRI designation	Country where released	Year of release
Swarna	India	IR49830	BC ₃ F ₂	2.3–3.4	IR05F102	India & Indonesia, Bangladesh, Nepal & Myanmar	2009 2010 2011
IR64	Philippines	IR40931	BC ₂ F ₂	6.5–7.8	IR07F102	Philippines & Indonesia	2009
Samba Mahsuri	India	IR49830	BC ₂ F ₂	6.5–9.2	IR07F101	Nepal, India	2011 2013
TDK1	Laos	IR40931	BC ₃ F ₂	1.5–2.5	IR07F289	N/A ^a	
BR11	Bangladesh	IR40931	BC ₂ F ₂	0.3–2.6	IR07F290	Bangladesh	2010
CR1009	India	IR40931	BC ₂ F ₃	2.7–6.4	IR07F291	N/A ^b	
Ciherang	Indonesia	IR64-Sub1	BC ₁ F ₂	6.5–7.8	IR09F436	Indonesia, Bangladesh	2012 2013
PSB Rc18	Philippines	IR64-Sub1	BC ₁ F ₂	6.5–7.8	IR09F437	N/A ^c	

Table 2. Commonly used markers used in development of *Sub 1* gene is presented here for reference [30]

Primer	Sequence	Tm (°C)	Position	Type of marker	Accession	Expected size (bp)
RM8300 (SC3)F	GCTAGTGCAGGGTTGACACA	60	~300 kb upstream of <i>SUB1A</i>	SSR	NB ^a	200
RM8300 (SC3)R	CTCTGGCCGTTTCATGGTAT	60				
GnS2F	CTTCTTGCTCAACGACAACG	60	exon of <i>SUB1A</i>	CAPS (<i>AluI/PvuII</i>)	Teqing	242
GnS2R	TCGATGGGGTCTTGATCTCT	60			26D17 ^b NB ^a	132 & 110 No product
AEX1F	AGGCGGAGCTACGAGTACCA	62	non-synonymous SNP for <i>SUB1A</i>	mismatch	26D17 ^b	231
AEX1R	GCAGAGCGGCTGCGA	62		specific for tolerance	Teqing NB ^a	No product No product
ART5F	CAGGGAAAGAGATGGTGGA	60	<i>SUB1C</i> promoter	15 bp insertion in NB/93-11	NB ^a /93-11	217
ART5R	TTGGCCCTAGGTTGTTTCAG	60			118k20 ^b	202
Sub1C173F	AACGCCAAGACCAACTTCC	60	exon of <i>SUB1C</i>	9 bp deletion in NB/93-11	NB ^a /93-11	164
Sub1C173R	AGGAGGCTGTCCATCAGGT	60			118k20 ^b	173

^aNipponbare^bderived from IR40931-26

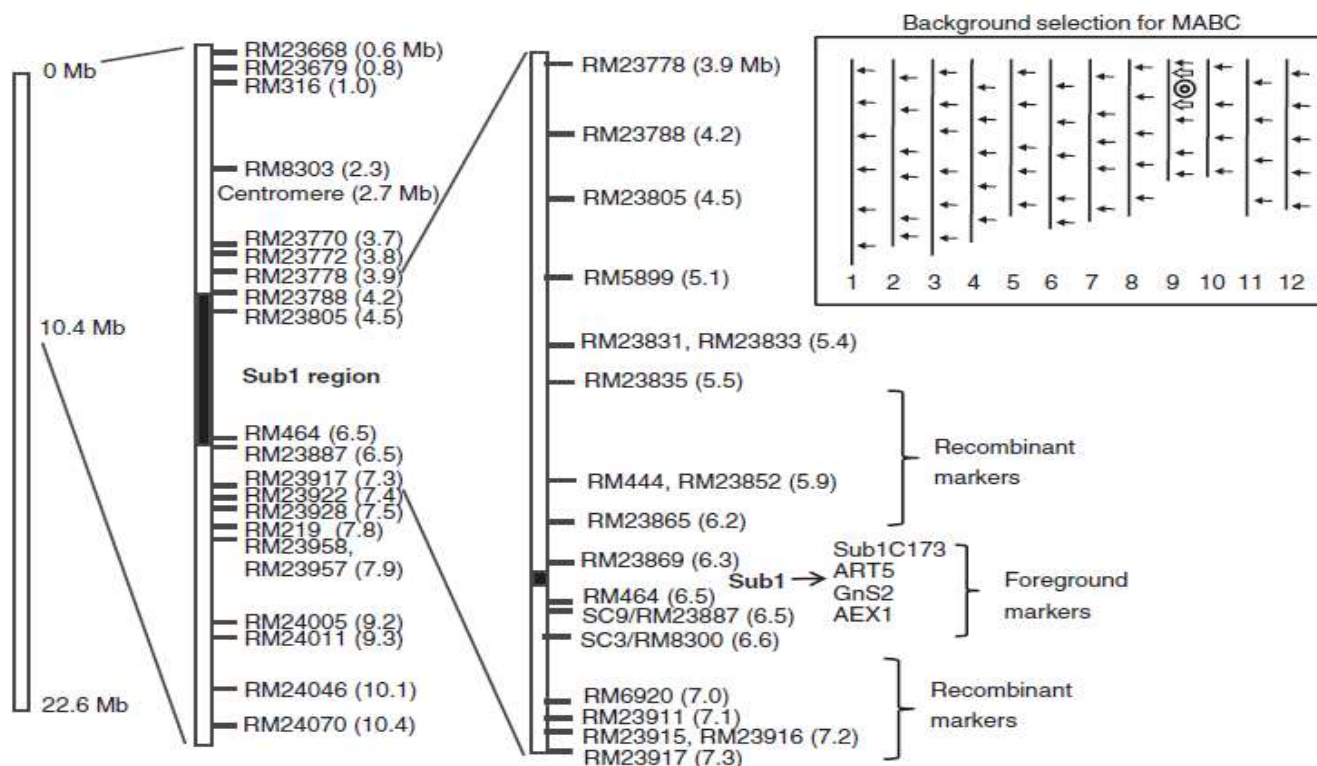


Figure 3. Marker-assisted backcrossing (MABC) for *SUB1* [4].

3.2 Breeding rice for anaerobic germination

The phenomenon of tolerance to flooding during seed germination, referred to as anaerobic germination (AG), is one of the indispensable character needed to ensure optimum plant population in direct seeded rice in both rain-fed flood-prone and irrigated ecosystems. QTLs for AG tolerance were found on chromosomes 1, 2, 5, and 7 [15], [30]. The study also revealed promising QTLs in Khao Hlan On, a tolerant donor from Myanmar [1], [30]. The QTL with the largest effect was detected on the long arm of chromosome 9 (*qAG-9-2* or *AG1*), having a logarithm of odds (LOD) score of 20.3 and explaining 33.5% of the variation for this trait. Similarly, on the short arm of chromosome 7 a major QTL was detected (*qAG7.1* or *AG2*), derived from the tolerant donor variety Ma-Zhan Red [30]. The combination of these two big effects QTLs to provide higher tolerance, assuming that the effect of the two QTLs proved to be additive or synergistic.

3.3 Breeding rice for deepwater or stagnant flooding

The major characteristics feature for the survival of deepwater rice is internode elongation under stagnant flooding condition which facilitates the uppermost leaves come protruding the water level and ensures photosynthesis [38], [3], [30]. Several studies have identified QTLs for deepwater traits, such as internode elongation and number of elongated internodes. It was reported that the QTL on chromosome 12 contributed the most rapid internode elongation in deepwater stress conditions [30]. By positional cloning, QTL within the genes: *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) were identified.

4. FUTURE PRIORITY FOR RICE BREEDING

4.1 Breeding rice for Anaerobic germination tolerance

The major QTL *AG1* has been successfully introgressed derived from Khao Hlan On (KHO) variety into IR64 and IR64-*Sub1* and effort are being made to introgression of this QTL into other genetic backgrounds such as Ciherang-*Sub1*, PSB Rc18-*Sub1*, and PSB Rc82 and other mega varieties in the subsequent years. Likewise introgression of othe major QTL *AG2* derived from Ma-Zhan Red into many popular varieties are on prime focus and work has been underway. The major QTLs controlling tolerance of anaerobic germination are scattered throughout the rice genome [1] [30] (Angaji et al. 2010; Septiningsih et al. 2013; unpublished data). QTL pyramiding is an very viable strategy assuming that the effects of the QTSs are additive in nature. Ultimately, these pyramided lines could be used directly as improved varieties.

4.2 Breeding rice for tolerance to stagnant flooding

The genetic control mechanism and physiology of stagnant flooding is still less known. All the efforts are concentrated towards unravelling the genetics of stagnant flooding in the current breeding programs. To achieve the progress, mapping population

development (F2 derived bulks) and selective genotyping to identify QTLs using classical QTL mapping approach to shorten the breeding cycle. A RIL population has been already prepared from IRRI 154 source, a identified variety of stagnant flooding tolerance for QTL mapping. Backcross derived mapping population and association mapping is a most viable tool to combine QTL mapping for development of new breeding lines. So far, new breeding lines have been developed and evaluated by IRRI, Philippines among its partners in India, Bangladesh and Nepal. The achievement of the breeding program to develop tolerance to stagnant flooding are quite encouraging.

4.3 Sub 1 and Beyond

Pyramiding Sub 1 genes with other biotic and abiotic stress tolerance genes is also research focus of the rice breeding program. Alongwith this considerable works are being done to develop more efficient *Sub 1* varieties and *Sub 1^{plus}* rice varieties to confer additional tolerance to transient flash flooding.

5. CONCLUSION

Tremendous efforts and resources were invested on breeding for submergence tolerance and significant achievements were harvested in the past. Series of submergence tolerance varieties were developed in many countries and contributed to the food security and many popular varieties at respective areas that were susceptible to submergence were improved by introgression of *Sub 1* gene. Besides this, several efforts were being done to overcome the other effects of submergence like stagnant flooding and flooding tolerance during germination particularly in the direct-seeded rice in rainfed and irrigated ecosystems. The progress towards the breeding for both of these submergence effects are in desired level and few anaerobic germination regarded as AG lines have been developed introgression with *Sub 1* varieties . This will provide the added advantages for the rice varieties. Similarly, *Sub 1* varieties are also further improved by providing stagnant flooding tolerance after identification of suitable donors for this environments. To cope with the adverse effect of climate change the submergence tolerant varieties are also strived to tolerate other biotic and abiotic stresses by pyramiding the resistance genes of interest to provide added tolerance to every kind of stresses.

REFERENCES

- [1] Angaji SA, Septiningsih EM, Mackill DJ and Ismail AM, "QTLs associated with tolerance of flooding during germination in rice (*Oryza sativa* L.)", *Euphytica*, 2010, vol. 172, pp.159–168
- [2] Biswas JK and Yamauchi M., "Mechanism of seedling establishment of direct-seeded rice (*Oryza sativa* L.) under lowland conditions", *Botanical Bulletin of Academia Sinica*, 1997, vol. 38, pp.29–32
- [3] Catling D. "Rice in deep water", International Rice Research Institute, 1992, Manila, Philippines.
- [4] Collard BCY and Mackill DJ, "Marker-assisted selection: An approach for precision plant breeding in the twentyfirst century", *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 2008, vol.363, pp.557–572.
- [5] Collard BCY, Septiningsih EM, Das SR, Carandang JJ, Pamplona AM, Sanchez D, Kato Y, Ye G, Reddy JN, Singh US, Itekharruddaula KM, Venuprasad R, Vera-Cruz CN, Mackill DJ and Ismail AM, "**Developing New Flood-Tolerant Varieties At The International Rice Research Institute (Irri)**", *SABRAO Journal of Breeding and Genetics*, 2013, vol. 45 (1), pp.42-56
- [6] Dar M H, Janvry A D, Emerick K, Raitzer D and Sadoulet E., " Flood-tolerant rice reduces yield variability and raises expected yield, differentially benefitting socially disadvantaged groups", 2013, *Sci Rep*, vol. 3: pp.1–8
- [7] Das KK, Panda D, Sarkar RK, Reddy JN and Ismail AM, "Submergence tolerance in relation to variable floodwater conditions in rice", *Environ Exp Bot*, 2009, vol. 66(3), pp.425–434
- [8] Das KK, Sarkar RK and Ismail AM, "Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice", *Plant Sci.*, 2005, vol. 168, pp.131–136
- [9] Dixit, Shalabh, Anshuman Singh, Nitika Sandhu, Aditi Bhandari, Prashant Vikram and Arvind Kumar (2017). "Combining drought and submergence tolerance in rice: marker-assisted breeding and QTL combination effects", *Mol Breeding*, 2017, vol. 37(143), pp.1-12, <https://doi.org/10.1007/s11032-017-0737-2>
- [10] Fukao T and Bailey-Serres J., "Submergence tolerance conferred by *Sub1A* is mediated by *SLR1* and *SLR11* restriction of gibberellin responses in rice", *Proceedings of the National Academy of Sciences of the United States of America*, 2008, vol.105, pp.16814–16819

- [11] Fukao T, Xu K, Ronald PC and Bailey-Serres J., "A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice", *Plant Cell*, 2006, vol. 18, pp.2021–2034
- [12] Fukao T., Harris T and Serres B., "Evolutionary analysis of the Sub1 gene cluster that confers submergence tolerance to domesticated rice", *Annals of Botany*, 2009, vol. 103, pp.143-150
- [13] Hua CY, C. SZ, Quan YQ, Sheng LY, Rong WX and Ying XG, 2007. "Tolerance of submergence in rice: gene studies using differential display technique", *Chinese Journal of Agricultural Biotechnology*, 2007, vol. 4, pp.139-144
- [14] Ismail AM, Ella ES, Vergara GV and Mackill DJ, "Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*)", *Annals of Botany*, 2009, vol. 103, pp197–209.
- [15] Jiang L, Ming-yu HW, Ming C and Jian-min W., "Quantitative trait loci and epistatic analysis of seed anoxia germinability in rice (*Oryza sativa* L.)", *Rice Science*, 2004, vol. 11, pp.238–244
- [16] Kato Y, Collard BCY, Septiningsih EM and Ismail AM, "Physiological analyses of traits associated with tolerance of long-term partial submergence in rice", *AoB PLANTS*, 2014, vol. 6, pp.
- [17] Konchan S and Kono Y., "Spread of direct seeded lowland rice in Northeast Thailand: Farmers' adaptation to economic growth", *Southeast Asian Studies*, 1996, vol. 33, pp.523–546
- [18] KUANAR SR, RAY A, SETHI SK, CHATTOPADHYAY K and SARKAR RK, "Physiological Basis of Stagnant Flooding Tolerance in Rice", *Rice Science*, 2017, vol. 24(2), pp. 73–84
- [19] Mackill DJ, Amante MM, Vergara BS and Sarkarung S., "Improved semidwarf rice lines with tolerance to submergence of seedlings", *Crop Science*, 1993, vol. 33, pp.749–753
- [20] Mackill DJ, Ismail AM, Pamplona AM, Sanchez DL, Carandang JJ and Septiningsih EM, "Stress tolerant rice varieties for adaptation to a changing climate", *Crop, Environment and Bioinformatics*, 2010, vol. 7, pp.250–259
- [21] Mallik S, Kundu C, Banerji C, Nayak DK, Chatterjee SD, Nanda PK, Ingram KT and Setter TL, "Rice germplasm evaluation and improvement for stagnant flooding", In: Ingram KT (ed) *Rainfed lowland rice: agricultural high-risk environments*, 1995, International Rice Research Institute.
- [22] Mishra SB, Senadhira D and Manigbas NL, "Genetics of submergence tolerance in rice (*Oryza sativa* L.)", *Field Crops Research*, 1996, vol. 46, pp.177-181
- [23] Nandi S, Subudhi PK, Senadhira D, Manigbas NL, Sen-Mandi S, Huang N., "Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping" *Molecular and General Genetics*, 1997, vol. 255, pp.1–8
- [24] Neeraja CN, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard BCY, Septiningsih EM, Vergara G, Sanchez D, Xu K, Ismail AM and Mackill DJ, "A marker-assisted backcross approach for developing submergence-tolerant rice cultivars", *Theoretical and Applied Genetics*, 2007, vol. 115, pp.767–776
- [25] Ram PC, Singh BB, Singh AK, Ram P, Singh PN, Singh HP, Boamfa I, Harren F, Santosa E, Jackson MB, Setter TL, Reuss J, Wade LJ, Singh VP and Singh RK, "Submergence tolerance in rainfed lowland rice: physiological basis and prospects for cultivated improvement through marker-aided breeding", *Field Crops Research*, 2002, vol. 76, pp.131-152.
- [26] Sarkar R K, De R N, Reddy J N, and Ramakrishnayya G., "Studies on the submergence tolerance mechanism in relation to carbohydrate, chlorophyll and specific leaf weight in rice (*Oryza sativa* L.)", *J Plant Physiol*, 1996, vol. 149(5), pp.623–625
- [27] Sarkar R K and Panda D., "Distinction and characterisation of submergence tolerant and sensitive rice cultivars, probed by the fluorescence OJIP rise kinetics", *Funct Plant Biol*, 2009, vol.36(3), pp.222–233
- [28] Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, Ismail AM and Mackill DJ, "Development of submergence-tolerant rice cultivars: The *Sub1* locus and beyond", *Annals of Botany*, 2009, vol. 103, pp.151–160
- [29] Septiningsih EM, Sanchez DL, Singh N, Sendon PMD, Pamplona AM, Heuer S and Mackill DJ, "Identifying novel QTLs for submergence tolerance in rice cultivars IR72 and Madabaru", *Theoretical and Applied Genetics*, 2012, vol. 124, pp.867–874

- [30] Septiningsih EM., Collard B. C. Y., Heuer Sigrid, Bailey-Serres Julia, Ismail AM., and Mackill DJ, "Applying Genomics Tools for Breeding Submergence Tolerance in Rice", *Translational Genomics for Crop Breeding, Volume II: Abiotic Stress, Yield and Quality, 2013*, Edited by Rajeev K. Varshney and Roberto Tuberosa. 2013 John Wiley & Sons, Inc. Published 2013 by John Wiley & Sons, Inc.
- [31] Setter TL and Laureles EV, "The beneficial effect of reduced elongation growth on submergence tolerance of rice", *J. Exp. Bot.* 1996, vol. 47, pp.1551–1559
- [32] Singh HP, Singh BB and Ram PC, "Submergence tolerance of rainfed lowland rice: search for physiological marker traits", *J. Plant Physiol.*, 2001, vol. 158, pp.883–889
- [33] Singh N, Dang TTM, Vergara GV, Pandey DM, Sanchez D, Neeraja CN, Septiningsih EM, Mendioro M, Tecson-Mendoza EM, Ismail AM, Mackill DJ and Heuer S., "Molecular marker survey and expression analyses of the rice submergence-tolerance gene *SUB1A*", *Theoretical and Applied Genetics*, 2010, vol. 121, pp. 1441–1453.
- [34] Singh S, Mackill DJ and Ismail AM., "Responses of *SUB1* rice introgression lines to submergence in the field: Yield and grain quality", *Field Crops Res*, 2009 vol. 113(1), pp.12–23
- [35] Singh S, Mackill DJ and Ismail AM, "Tolerance of longer term partial stagnant flooding is independent of the *SUB1* locus in rice", *Field Crops Research*, 2011, vol. 121 pp.311–323
- [36] Toojinda T, Siangliw M, Tragoonrung S and Vanavichit A., "Molecular genetics of submergence tolerance in rice: QTL analysis of key traits", *Annals of Botany*, 2003, vol. 91, pp.243–253
- [37] Tuong TP, Pablico PP, Yamauchi M, Confesor R, Moody K., "Increasing water productivity and weed suppression of wet seeded rice: Effect of water management and rice genotypes", *Experimental Agriculture*, 2000, vol. 36, pp.71–89
- [38] Vergara BS, Jackson MB and De Datta SK, "Deepwater rice and its response to deep-water stress", In: *Climate and Rice, 1976*, International Rice Research Institute, Los Baños, Philippines, pp. 301.
- [39] Wasmann R, Jagadish S V K, Heuer S, Ismail A, Redona E, Serraj R, Singh R K, Howell G, Pathak H and Sumfleth K., "Climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies" *Adv Agron*, 2009, vol. 101, pp.59-122
- [40] Xu K and Mackill DJ, "A major locus for submergence tolerance mapped on rice chromosome 9". *Molecular Breeding*, 1996, vol. 2, pp.219–224
- [41] Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC and Mackill DJ, "Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice", *Nature*, 2006, vol.442, pp.705–708
- [42] Yamauchi M, Aguilar AM, Vaughan DA and Seshu DV, "Rice (*Oryza sativa* L.) germplasm suitable for direct sowing under flooded soil surface", *Euphytica*, 1993, vol. 67, pp.177–184
- [43] Yamauchi M, Winn T, "Rice seed vigor and seedling establishment in anaerobic soil", *Crop Science*, 1996, vol. 36, pp.680–686
- [44] Young ND and Tanksley SD, "RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding", *Theoretical and Applied Genetics*, 1989, vol. 77, pp.353–359