

## Introgression of Submergence Tolerance into CO 43, a Popular Rice Variety of India, through Marker-assisted Backcross Breeding

HIFZUR RAHMAN, VIJAYALAKSHMI DAKSHINAMURTHI, SASIKALA RAMASAMY, SUDHA MANICKAM, ASHOK KUMAR KALIYAPERUMAL, SUCHISMITA RAHA, NARESH PANNEERSELVAM, VALARMATHI RAMANATHAN, JAGADEESHSELVAM NALLATHAMBI, ROBIN SABARIAPPAN and MUTHURAJAN RAVEENDRAN\*

Centre for Plant Molecular Biology and Biotechnology,  
Tamil Nadu Agricultural University, Coimbatore, India

\*Corresponding author: [raveendrantnau@gmail.com](mailto:raveendrantnau@gmail.com)

### Abstract

Rahman H., Dakshinamurthi V., Ramasamy S., Manickam S., Kaliyaperumal A.K., Raha S., Panneerselvam N., Ramanaathan V., Nallathambi J., Sabariappan R., Raveendran M. (2018): Introgression of submergence tolerance into CO 43, a popular rice variety of India, through marker-assisted backcross breeding. Czech J. Genet. Plant Breed., 54: 101–108.

Submergence is a major threat in rice growing areas of South and South-East Asia. Identification of a major QTL, *Sub1* and advancements in marker-assisted backcrossing strategy enabled breeders to develop submergence tolerant versions of popular rice cultivars. In the present study, a marker-assisted backcross breeding (MABB) approach was used to introgress the *Sub1* locus from the tolerant FR13A variety into CO 43, one of the popular rice varieties of southern India. Evaluation of early generation segregating progenies derived from the cross of CO 43 and FR13A revealed the introgression of *Sub1*, leading to increased tolerance to submergence and better revival ability after it. Genotyping and phenotyping of BC<sub>3</sub>F<sub>3</sub> generation resulted in elite NILs of CO 43 harbouring *Sub1* the locus and possessing 94.37% and 95.78% of the recurrent parental CO 43 genome. Selected NILs performed equal as the recurrent parent CO 43 under normal conditions, but were more tolerant to submergence and revived better, afterwards. Under flooding NILs yielded about 25–30% higher than the recurrent parent CO 43.

**Keywords:** flooding tolerance; molecular breeding; rice

Rice (*Oryza sativa* L.) is one of the major cereal food crops in Asia and Africa. Indian rice production should reach 120 million tons by 2030 and 160 million tons by 2050 from current production of about 100 million tons. Current yield trends are not sufficient to meet the projected growth in production by 2050 (RAY *et al.* 2013). Abiotic stresses such as drought, flooding, temperature extremes are also affecting increased rice productivity (SARKAR *et al.* 2006). Submergence is a major constraint in India and in Tamil Nadu affecting 0.3 million ha of rice

area during the northeast monsoon season (HOSAIN & LABORTE 1996). Developing submergence/flooding tolerant versions of these popular varieties will be readily accepted by the local farmers and it will be useful in sustaining rice productivity in the state. In the last 15 years, rapid advancements have been made in understanding the molecular genetic basis of submergence tolerance in rice. A major QTL *Sub1* on chromosome 9 was mapped in a tolerant *indica* cultivar FR13A (XU & MACKILL 1996; XU *et al.* 2000) and efforts aimed at fine mapping result-

ed in the identification of ethylene response factors (ERFs)/ethylene-responsive element binding proteins/APETALA2-like proteins underlying tolerance (XU *et al.* 2006). Recent advancements in the molecular marker technology accelerated the development of submergence tolerant versions of mega rice varieties through a marker-assisted backcross breeding (MABB) approach (NEERJA *et al.* 2007; SEPTININGSIH *et al.* 2015) which increases the speed of development of improved versions of mega varieties. MABB has been successfully employed in rice breeding to incorporate the bacterial blight resistance gene *Xa21* (CHEN *et al.* 2000, 2001), and *waxy* gene (ZHOU *et al.* 2003) into elite varieties. Following this, submergence tolerant versions of mega varieties of India, Swarna, Samba Mahsuri, CR1009 and IR 64, have been developed and co-ordinated by IRRI, Philippines. The aim of this study was to develop a submergence tolerant version of CO 43, a ruling “mega rice variety” of South India, through marker-assisted introgression of *Sub1* locus from the tolerant FR13A.

## MATERIAL AND METHODS

**Material.** CO 43, a popular rice variety of Tamil Nadu known for high yield and salinity tolerance was used as a recurrent parent. A submergence tolerant photosensitive rice genotype FR13A was used as a donor parent.

**Evaluation of parents for their responses to submergence.** Fifty seedlings of CO 43 and FR13A were raised in three replications each in pots of 15 cm in diameter and 20 cm height filled with 2 kg of field soil mixed with required amount of fertilizer [1.25 g of  $(\text{NH}_4)_2\text{SO}_4$ , 0.08 g muriate of potash (KCl), and 0.08 g single superphosphate (SSP)]. Twenty days old seedlings were subjected to flooding as described by XU and MACKILL (1996) with minor modifications. Seedlings were submerged in 1.5 m height plastic tanks filled with water for 13 days (August 10–22, 2010). On day 14 after submergence, pots with seedlings were taken out from the tanks and evaluated for their level of tolerance. Recovery ability of genotypes was assessed after 10 days of de-submergence.

**Validation of the *Sub1* locus introgression in the genetic background of CO 43.** Breeding scheme for developing a submergence tolerance version of CO 43 is drawn in Figure 1. Plants of  $F_1$  generations derived from CO 43 and FR13A were selfed and used for  $F_2$  generation development. Leaf samples were collected from all the  $F_2$  plants and used for genomic DNA extraction using the modified CTAB protocol as described by AUSUBEL *et al.* (1994) and genotyped using an *InDel* marker ART5 tightly linked to *Sub1* locus (SEPTININGSIH *et al.* 2009).  $F_2$  plants homozygous and heterozygous for CO 43 and FR13A alleles of ART5 were identified.

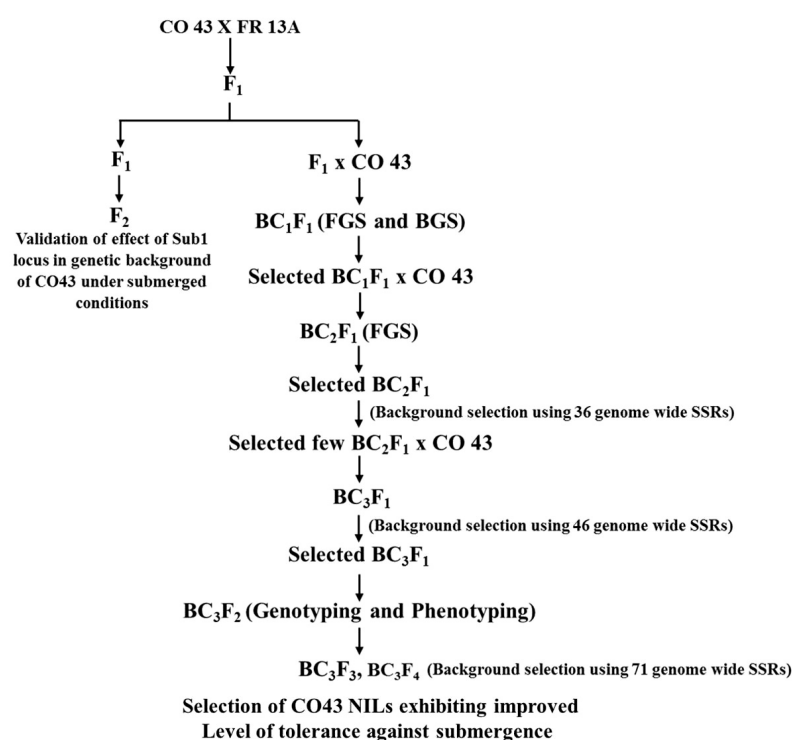


Figure 1. Breeding scheme for developing a submergence tolerant version of CO 43 rice variety

<https://doi.org/10.17221/149/2017-CJGPB>

Progenies of 5 independent  $F_2$  plants harbouring CO 43 allele of ART5 and 5 independent  $F_2$  plants harbouring FR13A allele of ART5 were raised in pots under greenhouse conditions and evaluated for submergence tolerance. Fifty seedlings of each progeny were raised in pots and subjected to submergence stress from 20<sup>th</sup> October 2011 to 4<sup>th</sup> November 2011 as described earlier by XU and MACKILL (1996).

**Development of CO 43 NILs harbouring *Sub1* locus from FR13A.** An *InDel* marker ART5 (located within the gene *Sub1C*), two SSRs RM219 and RM23877 were used for foreground selection (NEERAJA *et al.* 2007; SEPTININGSIH *et al.* 2009). One SSR marker RM3769 (11.75 Mb on chromosome 9) located downstream of *Sub1* locus was used for recombinant selection. Evenly spaced microsatellite markers (at least 4–6 markers per chromosome) were selected and used for background selection.

$F_1$  plants derived from CO 43 and FR13A were backcrossed with CO 43.  $BC_1F_1$  plants were subjected to foreground selection (FGS) using ART5 and recombinant selection (RS) using RM3769. Recombinants were subjected to background selection (BGS) using a set of 26 polymorphic SSR markers covering all the 12 chromosomes. In  $BC_2F_1$ , the same strategy of FGS, RS and BGS (36 markers) was followed. In  $BC_3F_1$ , BGS was done using a set of 46 SSR markers covering all the 12 chromosomes. In  $BC_3F_2$ , about 26 plants per individual  $BC_3F_1$  were raised and subjected to FGS using ART5. Identified positive  $BC_3F_2$  plants were forwarded to  $BC_3F_3$  generation and evaluated for agronomic traits during October–February 2013, i.e., plant height, number of tillers, panicle length etc.

**Evaluation of NILs under artificial screening facility.**  $BC_3F_3$  plants were evaluated under arti-

cial screening facility (tanks) for their submergence tolerance ability.  $BC_3F_3$  progenies were raised in the 8'× 8' tanks along with the parents and grown up to 45 days. On day 46, submergence stress was imposed by filling the tanks with water and submergence stress was continued for 13 days. De-submergence was done on day 14 and tolerance was assessed based on the survival percentage and recovery ability. Submergence tolerant families were identified and superior single plants (agronomically similar to CO 43) were selected and multiplied. Shortlisted NILs were subjected to BGS using 71 SSR markers evenly distributed along all 12 chromosomes.

**Evaluation of NILs ( $BC_3F_4$ ) for field performance.** Based on the genotyping and phenotyping data, two superior  $BC_3F_3$  progenies were selected for further multiplication and evaluation. Identified NIL was evaluated under field conditions along with its recurrent parent for yield, agronomic performance and submergence tolerance through All India Coordinated Rice Improvement Project (AICRIP) trials. Data on all the morphological characters (DUS characters), days to flowering, yield component traits, single plant yield and plot yield was recorded.

## RESULTS

**CO 43 exhibited susceptibility against submergence.** Upon de-submergence, all seedlings of FR13A were found to remain green when compared to CO 43 in which more than 75% of the leaves showed yellowing/rotting/decaying symptoms (Figure 2A). Seedlings of FR13A were found to recover better and showed 100% survival and produced fresh leaves within 10 days of de-submergence whereas the re-

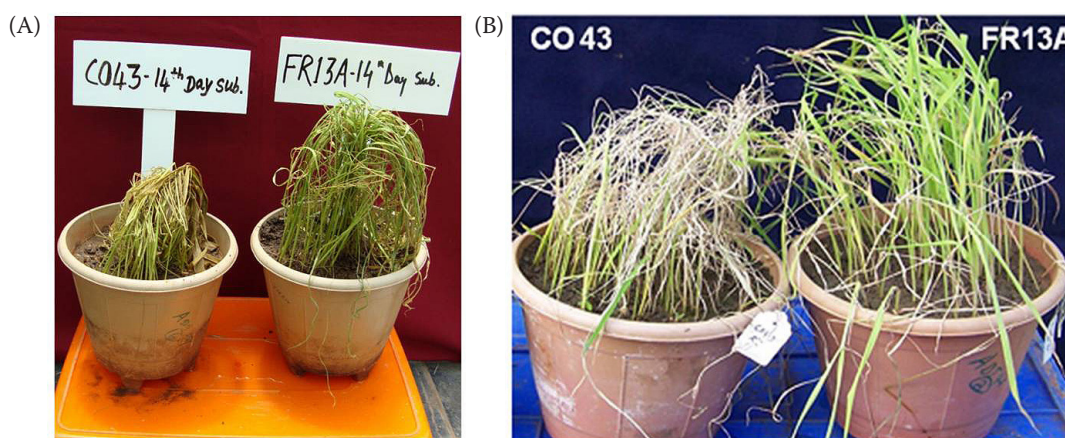


Figure 2. Differential responsiveness of rice genotypes (CO 43 and FR13A) to 13 days of submergence stress: immediately after de-submergence (A), 10 days after de-submergence (B)



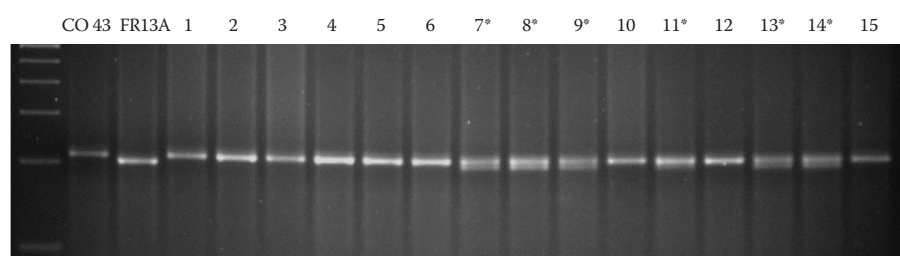


Figure 3. Identification of rice  $F_1$  hybrids CO 43  $\times$  FR13A (\*) using an *InDel* marker ART5 tightly linked to *Sub1* locus

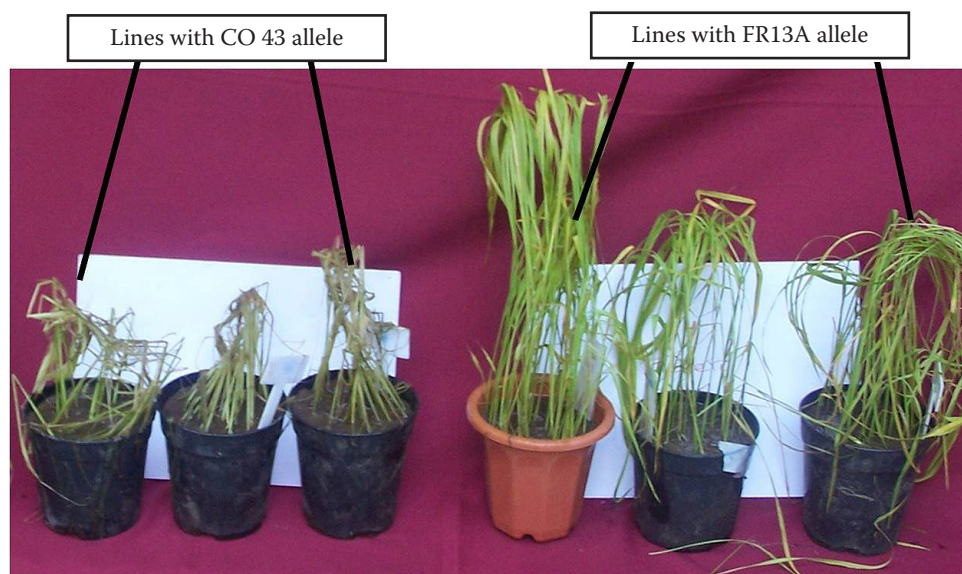


Figure 4. Differential responsiveness of rice  $F_3$  progenies (CO 43  $\times$  FR13A) segregating for *Sub1* locus to 13 days of submergence stress

covery ability of CO 43 was found to be only 8% (Figure 2B).

**Validation of effect of *Sub1* locus in the genetic background of CO 43.** Evaluation of  $F_3$  progenies against submergence revealed that leaves in the progenies harbouring the FR13A allele of ART5 were found to remain green and healthy and showed 95% survival at 15 days after de-submergence when compared to the progenies harbouring the CO 43 allele of ART5 which showed decay of more than 75%

of the leaves and only 9% of the seedlings survived after de-submergence (Figures 3 and 4).

**Development of submergence tolerant version of CO 43 through MABB.** SSR marker RM23877 and *InDel* marker ART5 (in the *Sub1C* gene) were used for foreground selection (Table 1). RM3769 (11.75 Mb) was used for recombinant selection downstream of *Sub1* locus (Table 1). We did not employ recombinant selection at the telomeric end due to its closeness to telomere. A parental polymorphism survey using

Table 1. Details of markers used for foreground and recombinant selection in rice

Marker	Position (Mb)	Forward primer	Reverse primer	Motif	No. of repeats
ART5	<i>Sub1C</i> promoter	CAGGGAAAGAGATGGTGGGA	TTGGCCCTAGGTTGTTTCAG	— <sup>a</sup>	
RM23877	6.4	TGCCACATGTTGAGAGTGATGC	TACGCAAGCCATGACAATTCTG	CA	30
RM219	7.8	CGTCGGATGATGTAAAGCCT	CATATCGGCATTTCGCCTG	CT	17
RM3769	11.75	TGCATGCTTCGTTTCAGCTAG	GTCTCCGAGCTCCTCAGGTC	GA	18

<sup>a</sup>InDel

<https://doi.org/10.17221/149/2017-CJGPB>

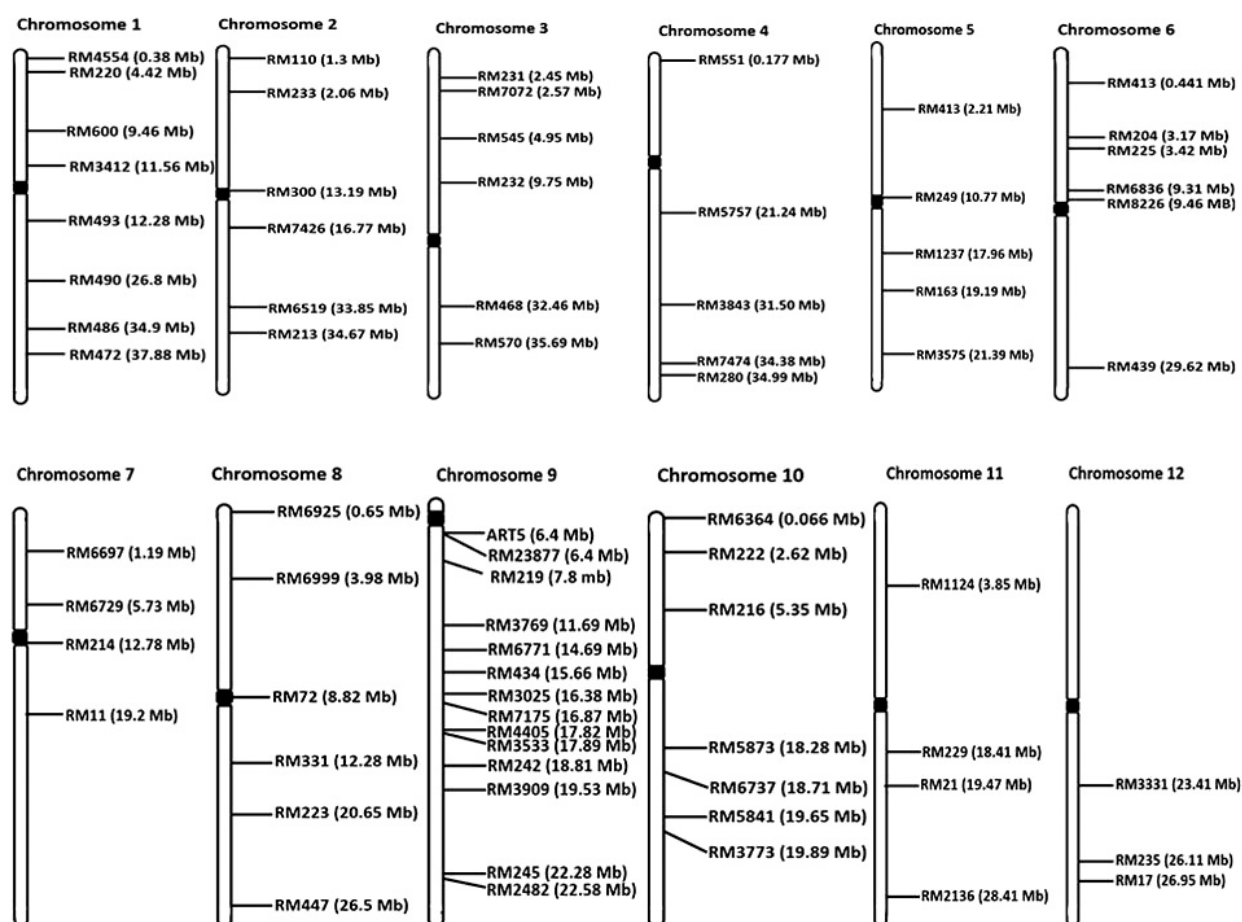


Figure 5. Markers used for foreground, recombinant and background selection in the study with rice

350 SSR markers resulted in the identification of 71 polymorphic SSRs covering all 12 chromosomes (Figure 5). In  $BC_1F_1$ ,  $BC_2F_1$  and  $BC_3F_1$  generation, background selection was done using 26, 36 and 46 genome-wide SSR markers, respectively (Figure 5).

Foreground selection of  $BC_1F_1$  plants using ART5 resulted in the identification of 26  $BC_1F_1$  plants posi-

tive for the *Sub 1* locus and background selection using 26 genome-wide polymorphic SSR markers revealed that the  $BC_1F_1$  plants were found to possess 23.08% to 65.40% of the recurrent parent genome (Table 2). Background analysis among  $BC_2F_1$  progenies using 36 polymorphic SSR markers showed that the recurrent parent genome recovery ranged

Table 2. Details on the number of backcross and selfed progenies of rice generated and genotyped during different cycles of backcross breeding

Generations	Total No. of plants evaluated	No. of positive plants identified through FGS <sup>a</sup>	No of markers used for BGS <sup>b</sup>	Recovery of recipient genome (%)
$F_1$	127	23	–	–
$BC_1F_1$	180	26	26	23.08–65.40
$BC_2F_1$	130	21	36	58.33–83.33
$BC_3F_1$	167	25	46	76.08–91.30
$BC_3F_2$	130	30 + 26	–	–
$BC_3F_3$	56	39 + 2	71	94.37–95.78

<sup>a</sup>represents foreground selection; <sup>b</sup>represents background selection



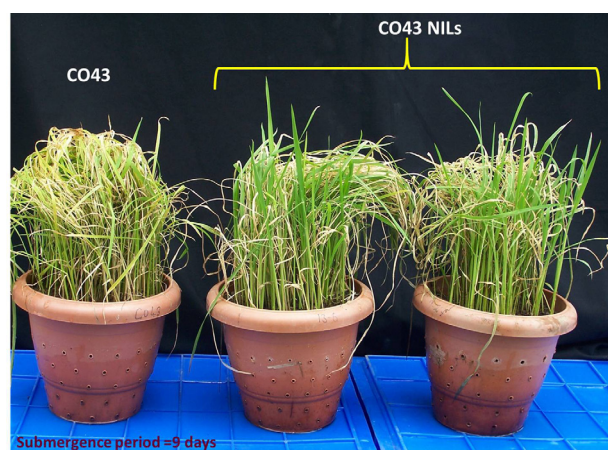


Figure 6. Evaluation of rice CO 43 NILs for submergence tolerance at seedling stage

from 58.33% to 83.33%. Genotyping of  $BC_3F_1$  plants resulted in the identification of 25 positive plants having the recurrent parent genome recovery between 76.08 and 91.30%. Five  $BC_3F_1$  plants possessing > 88% CO 43 genome were selfed and their  $BC_3F_2$  progenies were evaluated under field conditions. Foreground selection of  $BC_3F_2$  plants resulted in the identifica-

tion of 30 plants homozygous for ART5 (*Sub1* locus) and 26 plants heterozygous for ART5 (Table 2). Background selection of superior  $BC_3F_3$  progenies using 71 SSR markers covering all 12 chromosomes revealed the background genome recovery ranging between 94.37% and 95.78% (Table 2).

**Evaluation of CO 43 *Sub1* NILs for yield and submergence tolerance.** CO 43 NILs were found to exhibit an enhanced level of tolerance to submergence over its recurrent parent CO 43 at both seedling stage (Figure 6) and vegetative stage (Figure 7A–D). CO 43 NILs harbouring *Sub1* locus were able to tolerate submergence stress and showed 90.9% survival after submergence (Figure 7D). During the vegetative stage submergence, CO 43 showed only 8% recovery after 13 days of submergence stress whereas NILs harbouring *Sub1* locus showed 90–100% recovery. Field evaluation of two superior NILs revealed their genetic relatedness with their recurrent parent CO 43 in terms of agronomic performance and grain quality traits (Table 3). Field evaluation under national trials revealed that the submergence tolerant version of CO 43 was found to be on par with its recurrent parent CO 43 under normal conditions and found to

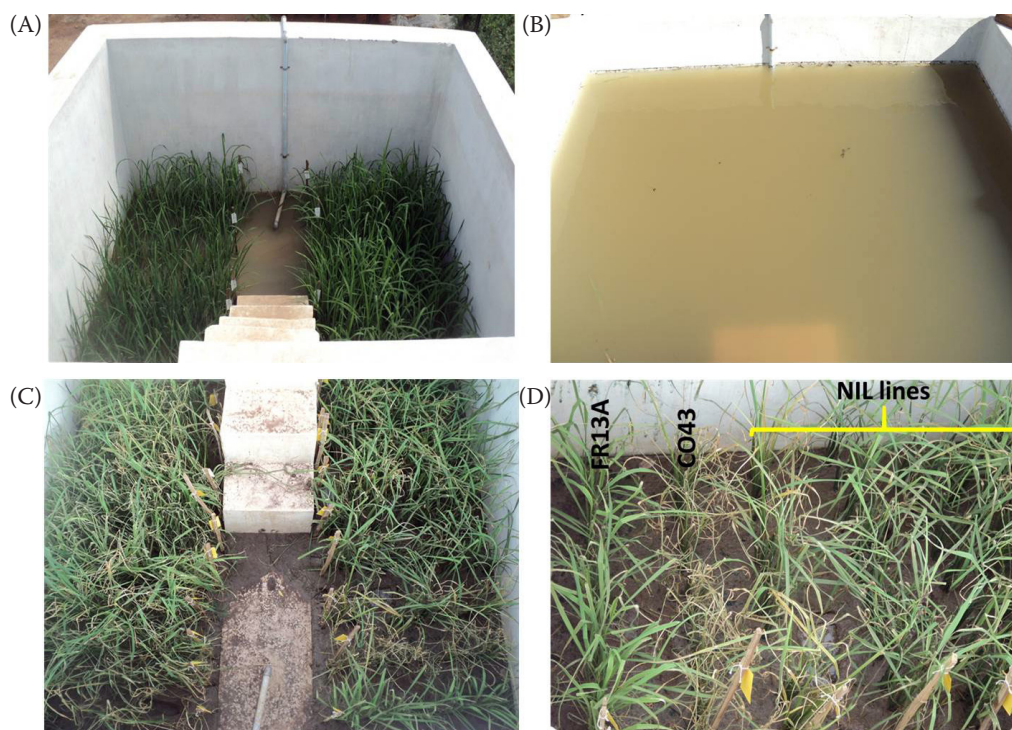


Figure 7. Evaluation of advanced backcross progenies of rice CO 43 harbouring *Sub1* locus for their submergence tolerance during vegetative stage: forty-five days old seedlings of advanced backcross progenies and their parents grown in submergence tanks (A), seedlings under submerged conditions (B), differential responses of rice genotypes upon de-submergence (C) and recovery of backcross progenies and donor FR13A after de-submergence (D)

<https://doi.org/10.17221/149/2017-CJGPB>

Table 3. Agronomical traits observed in parent CO 43 and two superior NILs of rice in terms of grain quality, yield and other yield attributing traits

	CO 43 <sup>a</sup>	NILs <sup>a</sup>	
		NIL 1	NIL 2
Plant height (cm)	116.17 ± 2.62	112.67 ± 1.44	116.00 ± 2.62
Total No. of tillers	19.33 ± 0.27	20.00 ± 2.49	20 ± 1.25
Total No. of productive tillers	19.33 ± 0.27	20.00 ± 2.49	18.67 ± 0.72
Panicle length (cm)	23.73 ± 1.06	23.73 ± 0.40	22.67 ± 0.49
No. of spikelets/panicle	263 ± 22.90	281.00 ± 26.03	250.33 ± 10.25
1000 grain weight (g)	20.12 ± 0.19	19.96 ± 0.19	20.64 ± 0.88
Grain length (mm)	7.93 ± 0.04	8.03 ± 0.04	8.06 ± 0.04
Grain width (mm)	2.63 ± 0.05	2.73 ± 0.09	2.83 ± 0.05

<sup>a</sup>Mean of three replications recorded from three independent plants

possess 20–25% yield advantage over CO 43 under submergence conditions (Table 4).

## DISCUSSION

Flash flood affects around 15 Mha of rice growing areas in South and Southeast Asia and 3 Mha in India (ISMAIL *et al.* 2010). Developing submergence tolerant rice genotypes has been an important breeding objective for more than three decades (MOHANTY & CHAUDHARY 1986) by exploiting tolerant donors like FR13A, Godaheenati *etc.* but not popular among farmers. Discovery of a major QTL *Sub 1* controlling submergence tolerance in FR13A and its deployment into breeding applications enabled improving mega varieties for submergence tolerance (NEERAJA *et al.* 2007).

Popular variety CO 43 exhibited susceptibility against submergence. Tolerant FR13A exhibited more rapid recovery than susceptible CO 43. The effect of introgression of *Sub1* locus from FR13A into CO 43 was confirmed by screening the F<sub>2:3</sub> progenies harbouring CO 43 and FR13A alleles of *Sub1* locus linked markers against submergence. Quick

regeneration following submergence is a desirable trait under prolonged flooding, as it can ensure early recovery and production of sufficient biomass for optimum productivity. Initiation of new leaves and their subsequent growth require availability of a sufficient amount of unutilized non-structural carbohydrates (SARKAR *et al.* 2006).

The use of molecular markers for background selection accelerated identification of backcross progenies possessing maximum recovery of the recurrent parent genome (VISSCHER *et al.* 1996; SERVİN & HOSPITAL 2002). Background selection enabled identification of progenies possessing 65.40% of recurrent parent genome at BC<sub>1</sub>F<sub>1</sub>, 83.33% of recurrent parent genome at BC<sub>2</sub>F<sub>1</sub> and 95.78% of recurrent parent genome at BC<sub>3</sub>F<sub>3</sub> generation. This demonstrated that the use of SSR markers at an average distance of 20 cM and a minimum of four to six markers per chromosome as recommended in earlier studies (SERVIN *et al.* 2004) appears to be sufficient for achieving accelerated recovery of the recipient parent genome.

In this study, it is demonstrated that applying both phenotypic and genotypic selection at early generations helped in developing CO 43 NILs exhibiting an

Table 4. Overall yield performance of CO 43 NIL of rice harbouring *Sub1* locus from FR13A under normal and submergence conditions

Trials	Yield (kg/ha)			Increase (%)	
	CO 43 NIL	CO 43	Best Check Swarna <i>Sub-1</i>	CO 43 (RP)	Best Check Swarna <i>Sub-1</i>
Submergence (6)	3250	2850	2046	14.0	58.8
Control (17)	4818	4641	–	4	–

Values in parentheses indicate the number of test locations (AICRIP 2015, 2016); RP – recurrent parent

<https://doi.org/10.17221/149/2017-CJGPB>

improved level of submergence tolerance in an accelerated manner. CO 43 NILs exhibited a significantly higher level of survival rate upon submergence when compared to CO 43. This demonstrated that *Sub 1* confers tolerance to submergence in different genetic backgrounds as already demonstrated in various other genetic backgrounds like Swarna, Samba Mahsuri, TDK *Sub 1*, BR 11 *Sub 1*, CR 1009 *Sub1* etc. (NEERAJA *et al.* 2007; SEPTININGSIH *et al.* 2009). Field evaluation of improved NILs along with recurrent parent and Swarna *Sub1* (positive check) across several locations at a national level indicated that the NILs were similar to CO 43 in all agronomic values under normal conditions. NILs exhibited 20–25% yield advantage over its recurrent parent CO 43 under submergence which clearly indicated that this improved version can be cultivated in places wherever this mega variety is cultivated (AICRIP 2015, 2016).

## References

- AICRIP (2015): All India Coordinated Rice Improvement Project. Progress Report 2015. Varietal Improvement. Vol. 1. Hyderabad, ICAR-Indian Institute of Rice Research.
- AICRIP (2016): All India Coordinated Rice Improvement Project. Progress Report 2016. Varietal Improvement. Vol. 1. Hyderabad, ICAR-Indian Institute of Rice Research.
- Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J., Smith J.A., Struhl K. (1994): Current Protocols in Molecular Biology. Vol. I. New York, John Wiley & Sons.
- Chen S., Lin X., Xu C., Zhang Q. (2000): Improvement of bacterial blight resistance of Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Crop Science*, 40: 239–244.
- Chen S., Xu C., Lin X., Zhang Q. (2001): Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Plant Breeding*, 120: 133–137.
- Hossain M., Laborte A. (1996): Differential Growth in Rice Production in Eastern India: Agroecological and Socio-economic Constraints. *Physiology of Stress Tolerance of Rice*. Los Banos, NDUAT, IRRI: 221–239.
- Ismail A., Thomson M., Vergara G., Rahman M., Singh R., Gregorio G., Mackill D. (2010): Designing resilient rice varieties for coastal deltas using modern breeding tools. In: Hoanh C.T., Szuster B., Kam S., Ismail A., Noble A. (eds.): *Tropical Deltas and Coastal Zones: Food Production, Communities and Environment at the Land and Water Interface*. CAB International: 154–165.
- Mohanty H., Chaudhary R. (1986): Breeding for submergence tolerance in rice in India. *Progress in Rainfed Lowland Rice*. Los Banos, International Rice Research Institute: 191–200.
- Neeraja C., Maghirang-Rodriguez R., Pamplona A., Heuer S., Collard B., Septiningsih E., Ismail A. (2007): A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics*, 115: 767–776.
- Ray D.K., Mueller N.D., West P.C., Foley J.A. (2013): Yield trends are insufficient to double global crop production by 2050. *PLoS ONE*, 8: e66428.
- Sarkar R., Reddy J., Sharma S., Ismail A.M. (2006): Physiological basis of submergence tolerance in rice and implications for crop improvement. *Current Science*, 91: 899–906.
- Septiningsih E.M., Pamplona A.M., Sanchez D.L., Neeraja C.N., Vergara G.V., Heuer S., Mackill D.J. (2009): Development of submergence-tolerant rice cultivars: the *Sub1* locus and beyond. *Annals of Botany*, 103: 151–160.
- Septiningsih E.M., Hidayatun N., Sanchez D.L., Nugraha Y., Carandang J., Pamplona A.M., Collard B.C.Y., Ismail A.M., Mackill D.J. (2015): Accelerating the development of new submergence tolerant rice varieties: the case of Ciherang-Sub1 and PSB Rc18-Sub1. *Euphytica*, 202: 259–268.
- Servin B., Hospital F. (2002): Optimal positioning of markers to control genetic background in marker-assisted backcrossing. *Journal of Heredity*, 93: 214–217.
- Servin B., Martin O.C., Mézard M. (2004): Toward a theory of marker-assisted gene pyramiding. *Genetics*, 168: 513–523.
- Visscher P.M., Haley C.S., Thompson R. (1996): Marker-assisted introgression in backcross breeding programs. *Genetics*, 144: 1923–1932.
- Xu K., Mackill D.J. (1996): A major locus for submergence tolerance mapped on rice chromosome 9. *Molecular Breeding*, 2: 219–224.
- Xu K., Xu X., Ronald P., Mackill D. (2000): A high-resolution linkage map of the vicinity of the rice submergence tolerance locus Sub1. *Molecular Genetics and Genomics*, 263: 681–689.
- Xu K., Xu X., Fukao T., Canlas P., Maghirang-Rodriguez R., Heuer S., Mackill D. J. (2006): Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature*, 442: 705.
- Zhou P., Tan Y., He Y., Xu C., Zhang Q. (2003): Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. *Theoretical and Applied Genetics*, 106: 326–331.

Received for publication September 11, 2017

Accepted after corrections February 9, 2018

Published online April 23, 2018