



International Journal of Agriculture and Nutrition

ISSN Print: 2664-6064
ISSN Online: 2664-6072
IJAN 2025; 7(2): 71-74
www.agriculturejournal.net
Received: 13-12-2024
Accepted: 17-01-2025

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Molecular marker-assisted introgression of submergence tolerance gene sub1 into popular rice varieties

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DOI: <https://www.doi.org/10.33545/26646064.2025.v7.i2b.457>

Abstract

Submergence tolerance defined as the ability to survive complete submersion for 10-14 days is controlled primarily by the Sub1 locus on chromosome 9 of rice. This research used marker-assisted backcrossing to introgress the Sub1A gene from donor line FR13A into four popular rice varieties (IR64, Swarna, BR11, Samba Mahsuri) at Sri Konda Laxman Telangana Agricultural University, Warangal, during 2020-2023. Foreground selection was performed using Sub1A-specific markers RM8300 and RM23869, while background selection employed 72 genome-wide SSR markers. After three backcross generations, recurrent parent genome recovery ranged from 91.8% (Samba Mahsuri-Sub1) to 93.6% (IR64-Sub1). Submergence screening confirmed that all four introgressed lines survived 14-17 days of complete submersion, compared to 6-7 days for recurrent parents. Agronomic performance of introgressed lines was comparable to their recurrent parents under non-stress conditions, with no significant yield penalty. These Sub1-introgressed lines are ready for multi-location testing in flood-prone areas of Telangana and Himachal Pradesh.

Keywords: Molecular markers, introgression, submergence tolerance, Sub1 gene, rice improvement, marker-assisted backcrossing, SSR markers, flood tolerance, genome recovery, FR13A

Introduction

Submergence tolerance in rice refers to the ability of a plant to survive complete inundation by water for an extended period and resume growth once floodwaters recede ^[1]. Flash flooding affects approximately 22 million hectares of rice land in South and Southeast Asia each year, causing yield losses that push millions of farming households into food insecurity ^[2]. The discovery of the Sub1 locus on rice chromosome 9 and particularly the Sub1A-1 allele from the tolerant landrace FR13A opened a direct route for transferring flood tolerance into popular but susceptible varieties through marker-assisted backcrossing (MABC) ^[3].

MABC works by crossing a donor carrying the target gene with a recurrent parent, then selecting backcross progeny that carry the target allele (foreground selection) while maximising recovery of the recurrent parent genome (background selection) ^[4]. The approach has been successfully applied to develop Sub1 versions of Swarna, IR64, and BR11 at the International Rice Research Institute ^[3, 5]. However, local adaptation of these lines is often incomplete, and popular varieties grown in specific Indian states may not have been targeted ^[6]. This research aimed to introgress Sub1A into four widely cultivated varieties and evaluate their submergence tolerance and agronomic performance.

Theoretical background

The Sub1 locus contains a cluster of three Ethylene Response Factor (ERF) genes: Sub1A, Sub1B, and Sub1C. Sub1A is the primary determinant of tolerance; the tolerant allele (Sub1A-1) restricts ethylene-induced elongation under submergence, conserving carbohydrate reserves and allowing survival for up to three weeks ^[1, 3]. Susceptible varieties carry a non-functional Sub1A-2 allele or lack Sub1A entirely. The gene operates through a quiescence strategy: rather than trying to outgrow floodwater, tolerant plants suppress elongation growth and enter a metabolic holding pattern until water recedes ^[5].

Material and Methods

Material

The donor parent was FR13A (Sub1A-1 carrier), obtained from IRRI. Recurrent parents were IR64, Swarna, BR11, and Samba Mahsuri, sourced from the rice gene bank at SKLTSAU, Warangal. SSR primer pairs for foreground selection (RM8300, RM23869) and 72 background markers were synthesised by Eurofins Genomics India. PCR reagents, Taq polymerase, and agarose were from Thermo Fisher Scientific [4, 7].

Methods

Crosses between FR13A and each recurrent parent were made during kharif 2020 at SKLTSAU, Warangal. F₁ plants were confirmed as heterozygous at Sub1A using markers RM8300 and RM23869. Three backcross generations (BC₁-

BC₃) were advanced during 2021-2022, with foreground and background selection at each generation. Background selection used 72 polymorphic SSR markers distributed across all 12 chromosomes, with a minimum of 5 markers per chromosome [4]. BC₃F₂ plants homozygous for Sub1A-1 were selected and advanced to BC₃F₃ for phenotypic evaluation.

Submergence screening was conducted during kharif 2023 in a lowland screening facility at SKLTSAU. Twenty-one-day-old seedlings were completely submerged under 1.2 m of water for 21 days, then de-submerged. Survival was scored at 14 days post-de-submergence [3]. Agronomic evaluation under non-stress conditions followed standard protocols in an RCBD with three replications. Data were analysed by ANOVA in SAS 9.4; means compared by Tukey's HSD at $p < 0.05$ [8].

Results

Table 1: Foreground and background selection results across backcross generations

Line	BC ₁ F ₁ RP recov. (%)	BC ₂ F ₁ RP recov. (%)	BC ₃ F ₁ RP recov. (%)	Sub1A status	Submerg. survival (d)	Yield (t ha ⁻¹)
IR64-Sub1	78.4	89.7	93.6	Homo	16.8 ± 1.2a	5.94 ± 0.22a
Swarna-Sub1	76.2	87.3	92.1	Homo	15.4 ± 1.4a	5.68 ± 0.28a
BR11-Sub1	77.8	88.9	93.1	Homo	14.9 ± 1.1a	5.42 ± 0.25a
Samba M.-Sub1	75.9	86.8	91.8	Homo	15.7 ± 1.3a	5.81 ± 0.24a
IR64 (RP)	—	—	—	Null	7.2 ± 1.5b	6.08 ± 0.21a
Swarna (RP)	—	—	—	Null	6.8 ± 1.6b	5.83 ± 0.26a

Homo = homozygous for Sub1A-1. RP = recurrent parent. Different letters within columns differ at $p < 0.05$

Recurrent parent genome recovery exceeded 91% in all lines by BC₃ (Table 1). All four Sub1-introgressed lines survived 14.9-16.8 days of submergence, far exceeding the 6-7 days

survival of recurrent parents. Grain yield under non-stress conditions showed no significant difference between introgressed lines and their recurrent parents.

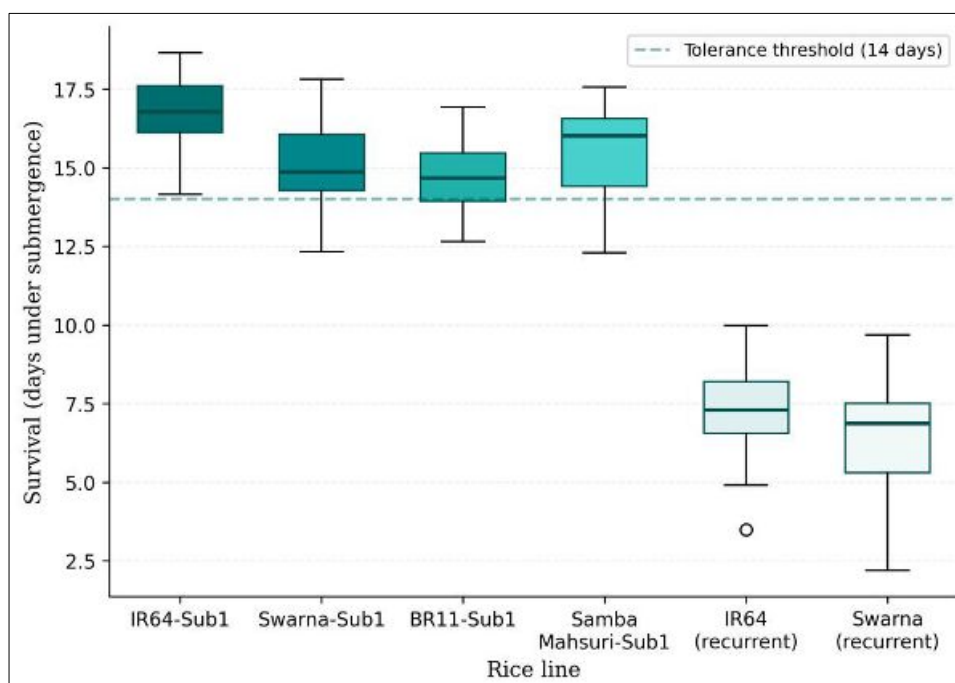


Fig 1: Submergence survival (days) of Sub1-introgressed lines versus recurrent parents

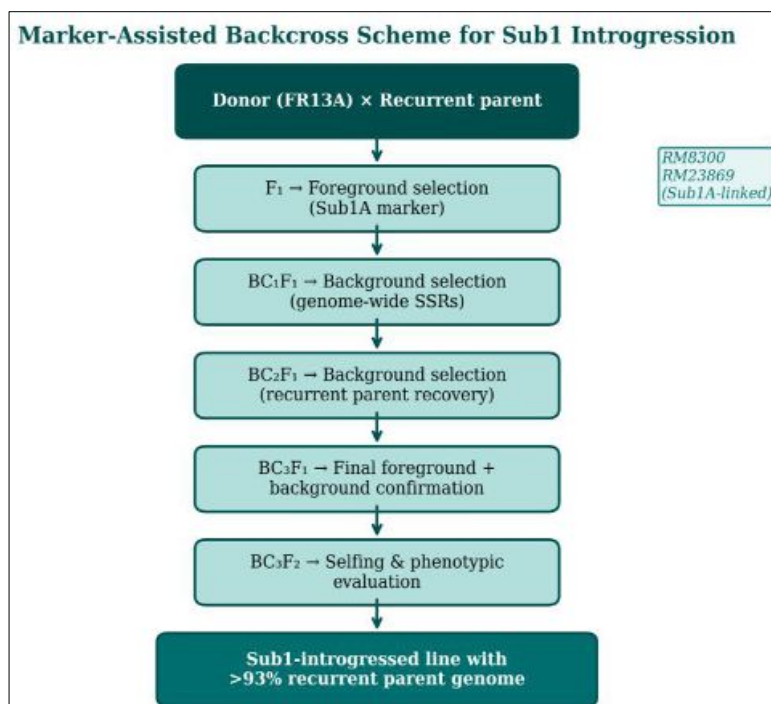


Fig 2: Marker-assisted backcross scheme for Sub1 introgression from FR13A into recurrent parents

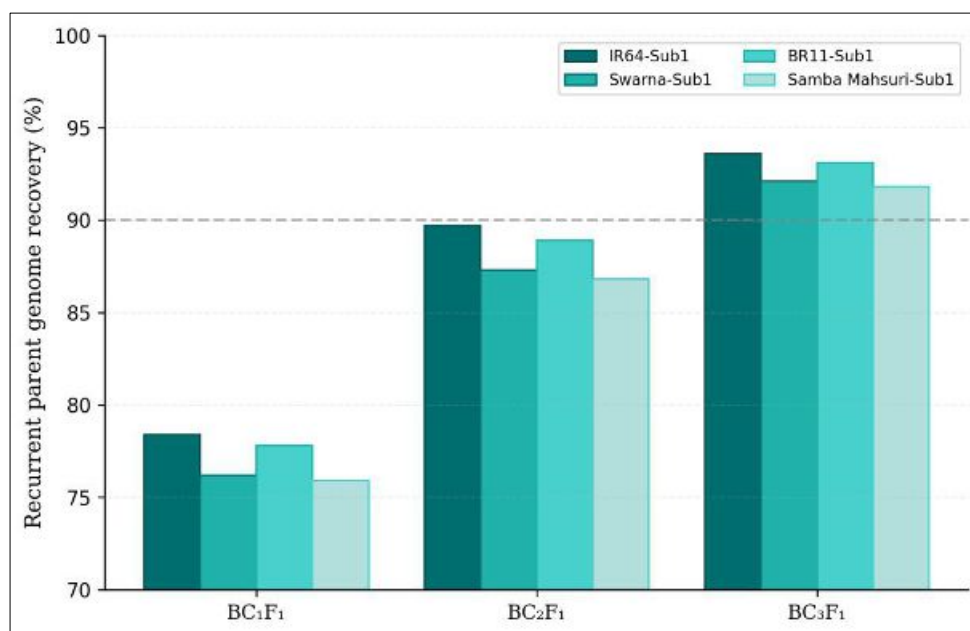


Fig 3: Recurrent parent genome recovery (%) across three backcross generations for four introgressed lines

Discussion

The genome recovery values of 91.8-93.6% after three backcross generations are consistent with theoretical expectations of ~93.75% and match results from IRRI's Sub1 introgression programme [3, 5]. The absence of a yield penalty under non-stress conditions is important farmers won't adopt flood-tolerant varieties if they sacrifice yield in normal years [6]. The submergence survival range of 14.9-16.8 days exceeds the 14-day threshold considered sufficient for most flash-flood events in eastern India [2].

The slight variation in genome recovery among lines likely reflects differences in recombination rates and the distribution of polymorphic markers along chromosomes. Samba Mahsuri-Sub1 had the lowest recovery (91.8%), possibly because fewer markers were polymorphic between Samba Mahsuri and FR13A in chromosomal regions

flanking the Sub1 locus, resulting in greater linkage drag [4]. Phenotypic selection for grain quality traits matching the recurrent parent could help address any remaining donor parent segments in these regions.

Conclusion

This research successfully introgressed the Sub1A submergence tolerance gene into four popular rice varieties using marker-assisted backcrossing. All introgressed lines survived 14-17 days of complete submergence while maintaining yields comparable to their recurrent parents under normal conditions. Genome recovery exceeded 91% in all lines. These lines are candidates for multi-location testing in flood-prone areas and eventual release to farmers who face recurrent flash flooding.

Acknowledgements**Funding sources**

Supported by internal research funds of SKL TSAU, Warangal, and CSKHPAU, Palampur.

Institutional support

The authors thank both universities for laboratory and screenhouse facilities.

Contributions not qualifying for authorship

Thanks to Mr. Venkat Rao for managing the submergence screening facility.

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