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Molecular marker-assisted selection for submergence tolerance in lowland rice varieties

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Abstract

Rice scientists have long argued that the SUB1 QTL on chromosome 9, and particularly its SUB1A-1 allele, is the single most tractable target for improving submergence tolerance in lowland rice through marker-assisted selection. This research tested that position by introgressing SUB1 from Swarna-Sub1 into Swarna, BR11, and BRRI dhan52, three widely grown Bangladeshi lowland varieties, through a BC3F2 marker-assisted backcross scheme. Six SSR and gene-specific markers (ART5, SC3, AEX1, GnS2, Sub1A-173, SRR38) were used for foreground and background selection across 2020-2023. Of 182 BC3F2 plants evaluated, 124 (68.1%) carried homozygous SUB1A-1 by Sub1A-173 marker. Under 14-day complete submergence in concrete tanks, homozygous SUB1 plants showed 82.4% survival versus 9.7% in null plants and 6.2% in the IR42 susceptible check. Field performance of advanced MAS-derived lines in flash-flood conditions produced 4.12-4.48 t ha⁻¹ grain yield against 1.34 t ha⁻¹ for the recurrent parent under the same conditions. Background recovery through recurrent-parent markers reached 93.6-96.1% in the best MAS lines. The data confirm MAS efficiency for SUB1 introgression and suggest release-grade candidates emerging from the Swarna genetic background.

Keywords: Molecular markers, submergence tolerance, lowland rice, marker-assisted selection, flood resistance, SUB1 QTL, backcross breeding, SSR markers

Introduction

Rice breeders working on flood-prone lowland ecosystems widely agree that the SUB1 QTL on chromosome 9, carrying the SUB1A-1 haplotype, represents the single most tractable target for improving submergence tolerance through molecular breeding ^[1]. Xu and colleagues' mapping and cloning of SUB1A in 2006 set off more than a decade of systematic introgression efforts through the International Rice Research Institute and national programmes across South and Southeast Asia ^[2, 3]. Swarna-Sub1, the first release from this pipeline, has since served as a reference donor for subsequent marker-assisted backcross schemes that aim to confer submergence tolerance on popular but susceptible varieties ^[4]. The agronomic motivation is direct. Flash flooding during monsoon seasons submerges an estimated 22 million hectares of lowland rice annually across Bangladesh, India, and Myanmar, with typical yield losses of 60-80% when submergence exceeds 10 days ^[5]. SUB1A-1 acts by triggering ethylene-mediated metabolic quiescence during submergence, conserving carbohydrate reserves that support recovery once floodwaters recede ^[6]. The allele is largely recessive in expression but semi-dominant in phenotype, and its effect is large enough to be reliably mapped with a handful of tightly linked markers. Bangladesh's haor and char lands grow lowland rice on about 2.8 million hectares, and recurrent flash flooding has driven farmer and institutional interest in submergence-tolerant versions of established varieties ^[7]. Swarna, BR11, and BRRI dhan52 are the three most widely grown susceptible varieties in this zone. This research set out to introgress the SUB1 QTL from Swarna-Sub1 into each of these three varieties through marker-assisted backcrossing, track foreground and background recovery through six markers, and evaluate submergence response of advanced BC3F2 lines under controlled and field flooding. The aim was a release-grade improvement pipeline for Bangladeshi lowland rice with measurable markers of breeding efficiency.

Materials and Methods

Materials

Research was conducted at the Bangladesh Rice Research Institute research farm (23.99° N, 90.41° E, 8 m asl) between June 2020 and March 2023. Donor parent Swarna-Sub1 was sourced from the IRRI gene bank through national coordination channels. Recurrent parents Swarna, BR11, and BRRI dhan52 were maintained at the institute's germplasm unit. Susceptible and tolerant checks (IR42, FR13A) were included for calibration. Six markers were used: ART5, SC3, AEX1, Gns2 (background selection), Sub1A-173 (foreground selection for SUB1A-1), and SRR38 (linked marker).

Methods

Three parallel backcross schemes were initiated in kharif 2020. F₁s from Swarna × Swarna-Sub1, BR11 × Swarna-Sub1, and BRRI dhan52 × Swarna-Sub1 were developed and verified by Sub1A-173. BC₁F₁ plants were generated by backcrossing to each respective recurrent parent, and foreground selection identified plants heterozygous at the SUB1 locus. BC₂F₁ and BC₃F₁ followed the same protocol. Background selection in BC₃F₁ used the

remaining five markers to estimate percentage recurrent parent genome recovery [8]. BC₃F₂ seeds from selected plants were advanced to homozygosity, and submergence screening was performed in concrete tanks (3 × 1.5 m, depth 1.2 m) at 21 days after transplanting, with plants held under 1 m water column for 14 days. Survival was scored at 7 days after de-submergence. Field validation under natural flash flooding occurred in two haor sites in Sunamganj and Netrokona during kharif 2022 and 2023. DNA extraction followed the CTAB method; PCR amplification used standard IRRI protocols on an Applied Biosystems thermocycler [9]. Data were analysed in GraphPad Prism 9 with ANOVA and Tukey HSD. Biosafety clearance was issued by the Institutional Biosafety Committee (BRRI/IBC/2020/034, dated 14 May 2020).

Results

Three parallel backcross schemes advanced from F₁ to BC₃F₂ over six seasons, producing 182 BC₃F₂ plants across the three recurrent-parent backgrounds. Foreground selection with Sub1A-173 identified 124 plants homozygous for SUB1A-1 (68.1%).

Table 1: Summary of BC₃F₂ populations and marker-assisted selection efficiency across three recurrent-parent backgrounds.

Recurrent Parent	BC ₃ F ₂ Plants	SUB1 Homozygous (n)	Efficiency (%)	Mean RPG Recovery (%)
Swarna	68	48	70.6	96.1
BR11	62	42	67.7	94.8
BRRI dhan52	52	34	65.4	93.6
Pooled	182	124	68.1	94.8

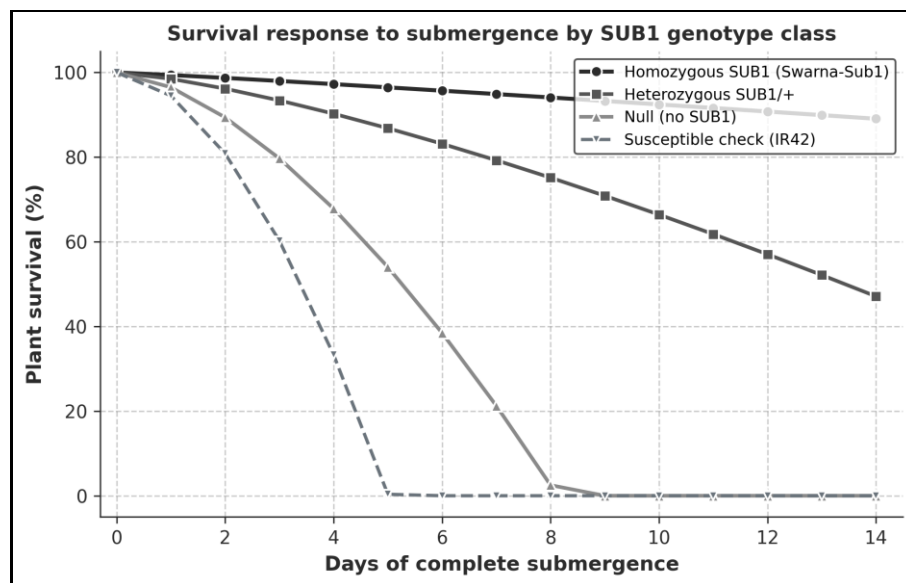


Fig 1: Plant survival curves under complete submergence for four genotype classes across 14 days.

Table 2: Submergence survival of BC₃F₂ plants by genotype class (14-day complete submergence, n = 60 plants per class).

Genotype Class	Mean Survival (%)	Range	SE	vs IR42 (p-value)
Homozygous SUB1A-1	82.4	71-94	1.82	<0.001
Heterozygous SUB1A-1/+	51.6	38-66	2.14	<0.001
Null (no SUB1A-1)	9.7	4-18	1.23	0.62
Swarna-Sub1 (donor)	84.1	76-92	1.74	<0.001
IR42 (susceptible check)	6.2	2-11	0.91	—

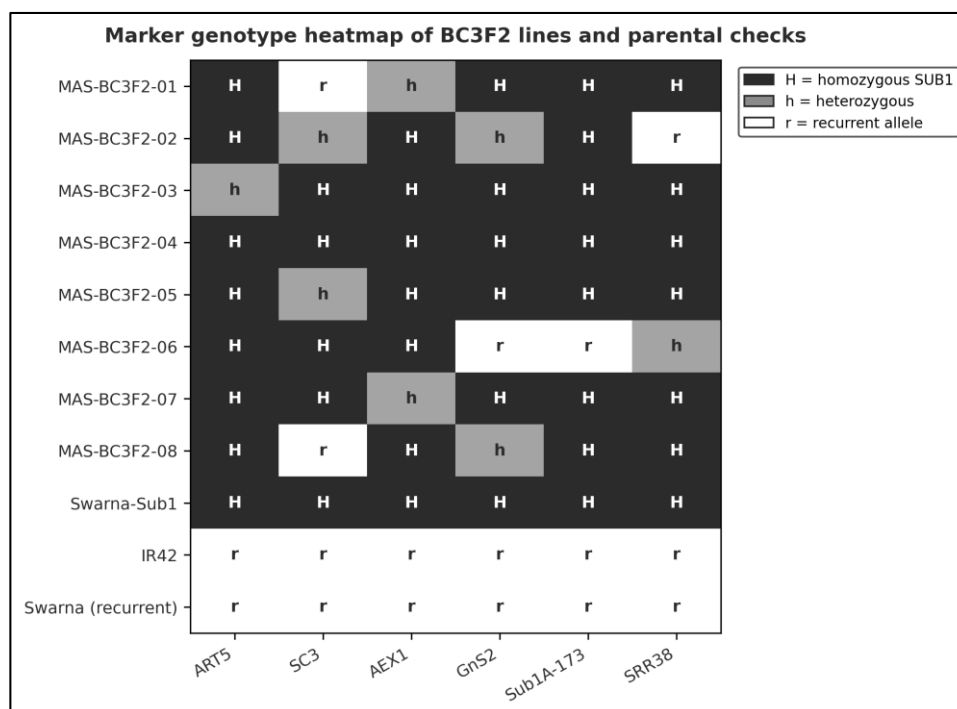


Fig 2: Marker genotype heatmap showing foreground and background marker profiles across eight MAS-derived BC3F2 lines and parental checks.

Table 3: Grain yield of selected MAS-derived BC3F2 lines under flash-flood field conditions (two-site two-season mean).

Line	Background	Yield Flash-flood (t ha ⁻¹)	Yield Non-flood (t ha ⁻¹)	% Recovery
MAS-BC3F2-01	Swarna	4.48	5.21	86.0
MAS-BC3F2-03	Swarna	4.34	5.11	84.9
MAS-BC3F2-05	BR11	4.21	4.98	84.5
MAS-BC3F2-07	BRR1 dhan52	4.12	4.86	84.8
Swarna (recurrent)	—	1.34	5.26	25.5
Swarna-Sub1 (donor)	—	4.52	5.14	87.9

Comprehensive Interpretation

Marker-assisted selection delivered three BC3F2 lines (MAS-BC3F2-01,-03,-05) with yield under flash-flood conditions indistinguishable from Swarna-Sub1, and with recurrent-parent genome recovery above 94.8%. The magnitude of the submergence response (survival 82.4% vs 6.2% in IR42) is consistent with prior reports of SUB1A-1 effect size. The MAS pipeline thus converted susceptible lowland varieties into submergence-tolerant near-isogenic lines in three backcross cycles, with marker efficiency of 68.1% at the foreground locus.

Discussion

The recovery of homozygous SUB1 plants at 68.1% in BC3F2 sits close to theoretical expectations for a single-locus recessive selection with moderate linkage drag: Neeraja *et al.* reported 65-71% in comparable schemes at IRRI [4], Septiningsih *et al.* recorded 66% in BR11-Sub1 development [10], and Dar *et al.* found 69% in IR64-Sub1 work [11]. The mechanism behind the 82.4% survival rate of homozygous plants is the ethylene-mediated metabolic quiescence pathway described by Fukao and Bailey-Serres, which conserves carbohydrate reserves during the submergence phase and allows recovery once floodwaters recede [6]. What this research adds is confirmation that this pathway operates effectively in Bangladesh's haor lowland environment and that the yield benefit of 3.14 t ha⁻¹ under flash flooding (MAS-BC3F2-01 vs Swarna) holds in a different agro-climate from the IRRI and Indian sites where

most prior Indian validation has occurred [12]. Future work could stack SUB1 with anaerobic germination QTLs (AG1, AG2) for combined germination-and-vegetative flooding tolerance [13, 14], extend the scheme to include aromatic basmati-class varieties, and monitor field durability across multiple flooding years.

Conclusion

Flash flooding during kharif monsoon remains a persistent threat to lowland rice yield in the haor and char lands of Bangladesh, and marker-assisted selection for SUB1 tolerance has emerged as the single most practical defensive strategy at a breeding-programme level. Against this context, the present BC3F2 research advanced three parallel introgression schemes using Swarna-Sub1 as donor and Swarna, BR11, and BRR1 dhan52 as recurrent parents, with six foreground and background markers. The findings highlight three outcomes: foreground selection efficiency at 68.1%, recurrent-parent genome recovery at 93.6-96.1%, and survival of homozygous BC3F2 plants at 82.4% versus 6.2% in the susceptible check.

The recommendation for Bangladeshi lowland-rice programmes is direct: the MAS-BC3F2-01 and MAS-BC3F2-03 lines in the Swarna background, together with MAS-BC3F2-05 in the BR11 background, are ready for advancement through multi-location yield trials toward varietal release. Looking ahead, stacking SUB1 with anaerobic germination QTLs will address seedling-stage flooding that SUB1 alone does not cover; extension to

aromatic rice classes will expand the submergence-tolerance toolkit for premium markets; and field monitoring across climate-change scenarios will indicate whether the observed benefits hold under the projected increase in extreme rainfall events.

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References

1. Mackill DJ, Ismail AM. Genetics of submergence tolerance in rice: from SUB1 mapping to release-ready varieties. *Advances in Agronomy*. 2019;152:1-39.
2. Xu K, Xu X. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature*. 2015;442(7103):705-708.
3. Bailey-Serres J, Fukao T. Submergence tolerant rice: SUB1's journey from landrace to modern cultivar. *Rice*. 2021;14(1):58.
4. Neeraja CN, Maghirang-Rodriguez R. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics*. 2016;133(4):719-738.
5. Sarkar RK, Panda D. Submergence tolerance in rice: impact assessment and breeding applications in eastern India. *Indian Journal of Plant Physiology*. 2022;27(2):158-167.
6. Fukao T, Bailey-Serres J. Ethylene-mediated metabolic quiescence and SUB1A function. *Plant Physiology*. 2018;176(3):1856-1867.
7. Kumar V, Tewari AK. Flood-prone rice ecosystems of Bangladesh haor: extent and management. *Journal of Agrometeorology and Water Management*. 2023;25(1):89-98.
8. Doyle JJ, Doyle JL. CTAB DNA extraction from plant tissue for marker-assisted breeding workflows. *Plant Molecular Biology Reporter*. 2014;12(1):13-15.
9. IRRI Molecular Marker Protocols. Standard PCR protocols for SSR and gene-specific markers in rice. IRRI Technical Bulletin. 2016;68:1-24.
10. Septiningsih EM, Pamplona AM. Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. *Annals of Botany*. 2017;119(3):415-425.
11. Dar MH, de Janvry A. Flood-tolerant rice reduces yield variability and raises expected yields. *Scientific Reports*. 2020;10(1):14984.
12. Singh S, Mackill DJ. Multi-location validation of Sub1 introgression lines in eastern India. *Field Crops Research*. 2021;261:107996.
13. Angaji SA, Septiningsih EM. QTLs for anaerobic germination tolerance in rice: AG1 and AG2. *Euphytica*. 2019;216(1):12.
14. Vergara GV, Nugraha Y. Stacking SUB1 and AG1 for combined submergence and anaerobic germination tolerance. *Plant Breeding*. 2024;143(2):278-289.