

Research Article

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Mapping of QTLs for flood tolerance in rice using recombinant inbred lines of Indra and a new plant genetic resource AC 39416 A

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Abstract

Rice crop is affected by different types of floods at different stages of the crop cycle. Constant efforts of researchers resulted in the development of rice varieties for anaerobic germination, flash floods and stagnant flooding by both conventional and molecular breeding approaches. Detection of QTLs for different types of floods in new genetic source (AC39416A) is needed to combat adverse effects of climate change. Present investigation was carried out to identify QTLs for flood tolerance using recombinant inbred lines derived from Indra and AC39416A. QTL mapping resulted in identification of QTLs, *qAG3.1* on chromosome 3 for anaerobic germination and *qSF10.1* on chromosome 10 for plant survival % under stagnant flooding. These QTLs explain 59.08 and 13.21% of phenotypic variance respectively. Two candidate genes were identified in *qAG3.1* region, LOC_Os03g42130 gibberellin 20 oxidase2 and LOC_Os03g44170 glutathione S-transferase. The underlying mechanism might be the inhibition of gibberellic acid synthesis and thereby protecting seedlings from oxidative stress under anoxia condition. Genomic region of *qSF10.1* revealed LOC_Os10g35020 glycosyltransferase and LOC_Os10g35050 aquaporin protein loci, which might be responsible for adaptive mechanism for plant survival % under stagnant flooding. This indicates that the new genetic resource AC39416A has an ability to adopt to different types of flood tolerance in response to environmental stress. Unveiling physiological and molecular mechanisms for flood tolerance in AC39416A using advanced omics studies would help in precise genomic selections for sustained production in flood-prone areas.

Introduction

Rice is an important staple food crop for more than half of the world's population. Rice productivity has to be improved enormously to meet the demands of growing population. Rice farmers in flood-prone ecosystem are more vulnerable to changing climatic conditions and constitute about 7% of global rice area (Yang *et al.*, 2017). Enhancing rice productivity in marginal environments is essential to improve the livelihood of the farming community (Panda and Barik, 2021). Rice crop suffers four major types of floods from seed germination to harvesting stage: (a) anaerobic germination, where submergence happens during germination, (b) flash floods where plants are completely submerged for 2 weeks, (c) stagnant flooding (SF) with up to 30–50 cm deep water due to prolonged floods and (d) deep water ecology with water depth more than 50 cm for most of time (Mackill *et al.*, 2010). Submergence up to 50% plant height at any growth stage leads to reduction of rice yield by at least 25% (Swain *et al.*, 2005) and yield loss up to 47% under SF (Kato *et al.*, 2014). Two major adaptive mechanisms for flood tolerance are quiescence and escape.

Anaerobic germination is prerequisite not only for rice cultivation under direct seeded condition but also required for survival of crop at nursery stage in flood-prone lowland areas during monsoon. Multiple QTLs for anaerobic germination *qAG-1*, *qAG-2*, *qAG-7*, *qAG-5a* and *qAG-5b* (Ling *et al.*, 2004), *qAG-1*, *qAG-2-1*, *qAG-11* and *qAG-12* from KHAIYAN (Angaji, 2008), *qAG-1-2*, *qAG-3-1*, *qAG-7-2*, *qAG-9-1*, *qAG-9-2* using Khao Hlan (Angaji *et al.*, 2010), a large QTL on chromosome 7 from Mazhan Red (Septiningsih *et al.*, 2013) and *qAG7* from Nanhi (Baltazar *et al.*, 2014) were identified. One major QTL on *AG1* was incorporated in Chierangsub1 (Toledo *et al.*, 2015).

Flash flood tolerance conferring *Sub1A*, an ethylene-responsive factor gene, was identified from FR 13 A (Xu *et al.*, 2006). This *Sub1A* gene was widely exploited by incorporating it into popular rice varieties globally (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009, 2014; Khanh *et al.*, 2013; Nawarathna *et al.*, 2014; Ara *et al.*, 2015; Girijarani *et al.*, 2015; Iftekharrudaula *et al.*, 2015, 2016; Singh *et al.*, 2016; Ahmed *et al.*, 2016; Korinsak *et al.*, 2016; Aditi *et al.*, 2019). Three non-*Sub1* QTLs were identified from IR 72 (Septiningsih *et al.*, 2012), three from FR



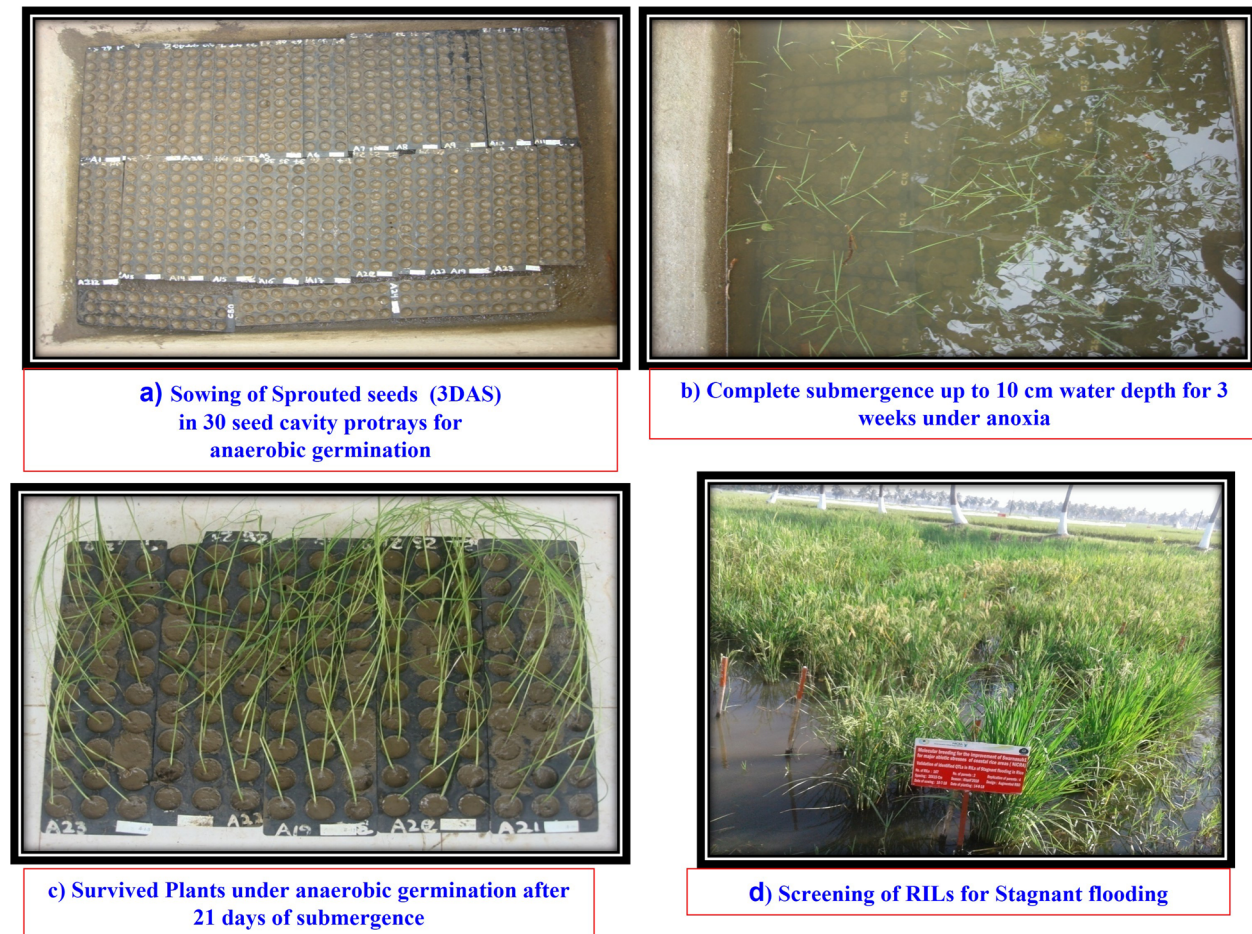


Fig. 1. Phenotypic screening of RILs for anaerobic germination and SF.

13A on chromosomes 1, 8 and 10 (Gonzaga *et al.*, 2016) and a major QTL for submergence *qSUB8.1* from Ciherang Sub1 (Gonzaga *et al.*, 2017).

Most of the *Sub1* incorporated lines are vulnerable to survive under SF (Sarkar and Bhattacharjee, 2011; Sandhya Rani *et al.*, 2019). Survival per cent and yield under SF are dependent on moderate elongation, high tillering, lesser carbohydrate depletion and higher fertility (Vergara *et al.*, 2014). QTLs for grain yield under SF, along with days to flowering, flag leaf length and leaf sheath length, were detected by Singh *et al.* (2017a, 2017b). Existence of compensatory mechanisms between tiller growth and shoot elongation under SF results in poor yields in addition to lodging risk (Zhu *et al.*, 2018). Land races tolerating flash floods and SF were assessed for genetic diversity (Barik *et al.*, 2020). Genetic resources like AC37887 and AC39416A that can tolerate anaerobic germination and SF were identified by Sandhya *et al.* (2017).

Submergence-tolerant varieties with the *Sub1* gene do not usually possess traits for anaerobic germination and SF indicating that the genes governing these traits are independent to *Sub1*. QTL mapping and candidate gene discovery from new genetic resources that have excellent adaptation to different kinds of flooding is very important for breeding climate-resilient flood-tolerant rice varieties (Singh *et al.*, 2017a, 2017b).

The present study is designed to identify QTLs for flood tolerance using 184 recombinant inbred lines (RILs) developed using Indra (MTU 1061) as female parent and new genetic resource AC 39416A for anaerobic germination and SF.

Materials and methods

Development of RILs

Indra (MTU 1061), a high yielding popular rice variety, was developed by crossing PLA 1100 and MTU 1010 and was released in 2006 by Regional Agricultural Research Station (RARS), Maruteru of Acharya NG Ranga Agricultural University (ANGRAU). Indra variety that is tolerant to flash floods for 1 week and susceptible to anaerobic germination and SF (Girijarani *et al.*, 2013; Reddy *et al.*, 2015) was used as female parent. New genetic resource AC39416A collected from National Rice Research Institute (NRRI), Cuttack was used as donor. AC39416A can tolerate 3 weeks of anaerobic germination and SF (Sandhya *et al.*, 2017). Cross was initiated during wet season of 2013 and 4000 plants were obtained in F_2 generation. One hundred eighty-four single plants from F_2 population were randomly selected and advanced up to F_6 generation by single seed descent method at RARS, Maruteru during 2014–2016.

Genotyping of RILs

Genomic DNA was isolated using the method of Zheng *et al.* (1995). Quality and quantity were estimated using eight channel vis spectrophotometer (Thermo scientific, USA). Polymerase chain reaction mixture of 10 μ l comprising of 10 \times Taq buffer A 1 μ l, forward and reverse primer each 1 μ l (Sigma aldrich), 2.5 mm dntp 0.5 μ l (Genei), one unit of Taq DNA polymerase

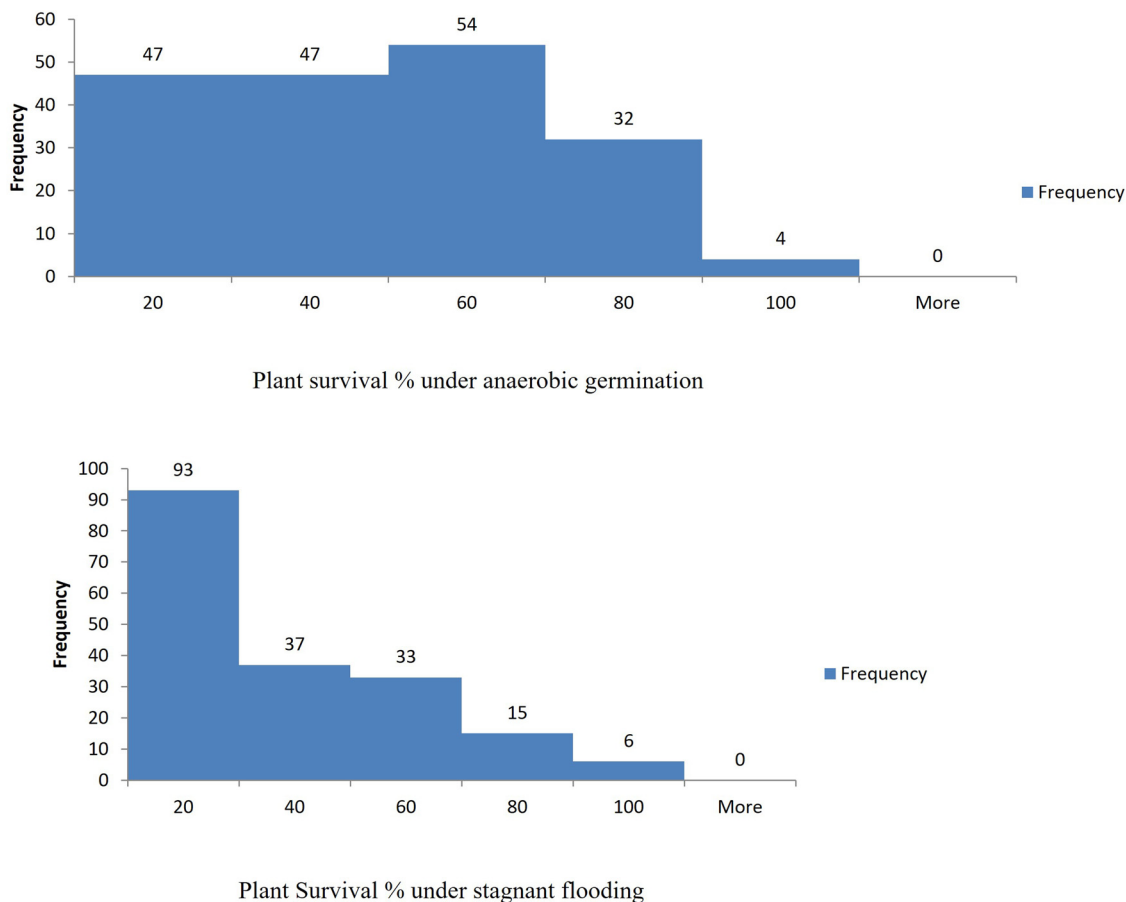


Fig. 2. Frequency distribution of 184 RILs for plant survival % under anaerobic germination and SF.

Table 1. Summary of anaerobic germination and plant survival % under stagnant flooding among RILs

Particulars	Plant survival %	
	Anaerobic germination	Stagnant flooding
Mean	43.32	26.93
Standard error	1.63	1.86
Standard deviation	22.13	25.19
Sample variance	489.57	634.68
Kurtosis	-0.85	0.02
Skewness	0.01	0.83
Minimum	0.00	0.00
Maximum	90.00	100.00
Indra (female parent)	10.00	16.67
AC39416 A (male parent)	88.70	78.69
Swarnasub1 (check)	34.30	20.20

1 μ l (Genei), 25 ng of genomic DNA 3 μ l and sterile distilled water 2.5 μ l was used for amplification. Thermo profile of initial denaturation at 94°C for 5 min followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 0.5 min, extension at

72°C for 1.0 min and ending up with 7 min at 72°C for the final extension was adopted using Pro S master cycler (Eppendorf). Electrophoresis was carried out on 3% agarose gels and images were visualized using Syngene gel documentation system.

Out of 624 markers screened for parental polymorphism between Indra and AC 39416 A at RARS, Maruteru, 104 polymorphic simple sequence repeats markers were used to genotype 184 RILs. Saturated fine mapping was performed using more markers within the identified QTL regions. Five polymorphic markers were identified between RM15848 and RM15561 for *qAG3.1* and six for *qSF10.1* between RM 304 and RM 6100 for fine mapping. Gel images were scored as A for Indra allele, B for AC 39416A allele and H for heterozygote. QTL mapping was performed using QCIM software with 1000 permutations as per Wang *et al.* (2016).

Phenotyping of RILs

Anaerobic germination (AG)

For each RIL, 30 pre germinated seeds were sowed on third day in pro trays. These trays were submerged in a concrete tank by maintaining 10 cm deep for 3 weeks during 2016 and 2017 (Fig. 1(a)–(c)). Survived plants after 21 days were counted for anaerobic germination.

Stagnant flooding (SF)

Thirty-day-old seedlings of RILs were transplanted in submergence pond with a spacing of 20 cm between rows and 15 cm between

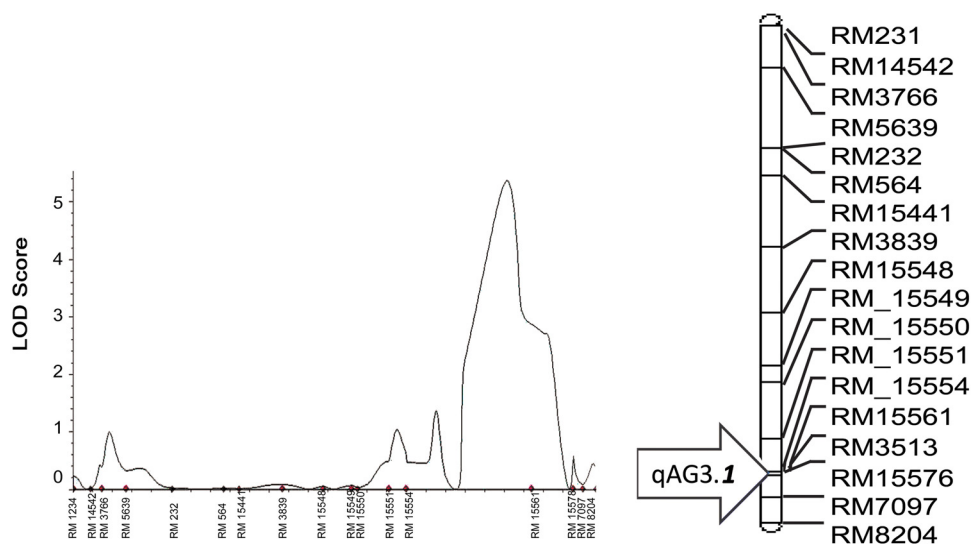


Fig. 3. QTL for Anaerobic germination *qAG 3.1* on Chromosome 3.

plants with 25 hills per row. Water depth of 30–50 cm was maintained from 1 week after transplanting to reproductive phase during 2017 and 2018 (Fig. 1(d)). Survived plants were counted at 30 days after transplanting. Plant survival % was calculated as number of (plants survived/total number of plants) \times 100.

Results

Plant survival % for anaerobic germination shows a wide variation (0–90%) even under SF (0–100%) (Table 1). Majority of RILs have an anaerobic germination per cent ranging between 40 and 60% with a mean of 43.32. Similarly, plant survival under SF is on the lower end of distribution with a mean of 26.93% (Fig. 2). Only four RILs for anaerobic germination and six RILs under SF show maximum plant survival %, with a range between 81 and 100%. Only one RIL has a maximum performance with about 70% plant survival rate under AG and 100% under SF. Parent AC 39416 A has a higher plant survival rate of 88.80% under anaerobic condition and 78.69% under SF than the check Swarnasub1 (34.30% AG, 20.20% SF) and female parent Indra (10% AG, 16.67% SF).

Results of QTL mapping revealed that QTLs for anaerobic germination *qAG3.1* were found on chromosome 3 between RM 15848 (24.68 Mbp) and RM 15561 (24.82 Mbp) with a LOD score of 2.89. The phenotypic variation explained is about 7.16% with an additive effect of 4.48 (Table 2). Fine mapping of *qAG 3.1* resulted in identification of a major QTL with LOD score of 5.36 that explains a phenotypic variance of 59.08%. This QTL is between RM 15554 (24.72 Mbp) and RM 15561 (24.82 Mbp) (Fig. 3). The identified QTL *qAG3.1* was also validated in another population consisting of BC₁F₁ lines of Swarnasub1 and AC 39416 A that was developed under NICRA project during 2018.

QTL for plant survival % under SF *qSF10.1* was detected on chromosome 10 with a LOD score of 5.66, a phenotypic variance of 13.21% and an additive effect of 10.79. The identified QTL for SF *qSF10.1* was validated in the year 2018 by screening RILs under SF with a LOD score of 3.10, phenotypic variance of 7.56 and an additive effect of 7.71 between RM 304 (18.65 Mbp) and RM 6737 (18.71 Mbp) represented in Fig. 4 and Table 2.

Rice gene annotation (<http://rice.plantbiology.msu.edu/>) revealed LOC_Os03g42130 gibberellin 20 oxidase2 and LOC_Os03g44170 glutathione S-transferase as putative candidate gene loci that might be responsible for anaerobic germination in our identified QTL *qAG 3.1* genomic region on chromosome 3. The genomic region of QTL for plant survival %, *qSF10.1* revealed LOC_Os10g35020 glycosyltransferase and LOC_Os10g35050 aquaporin proteins as putative candidate genes that play a role for plant survival % under SF.

Discussion

Variation in RILs for anaerobic germination and plant survival % under SF indicated that expression of alleles for different types of floods is different and it depends on plant adaptive mechanism in response to stress signalling. Rice plant coleoptile has to grow faster under anoxia for germination and show moderate elongation under SF. In the present study too, only one RIL was detected as tolerant for both situations and AC39416A has significantly higher plant survival % than Swarnasub1 and Indra. Rumanti *et al.* (2022) and Agbeleye *et al.* (2019) also found significant variation in plant survival % for both AG and SF and identified different tolerant accessions for each.

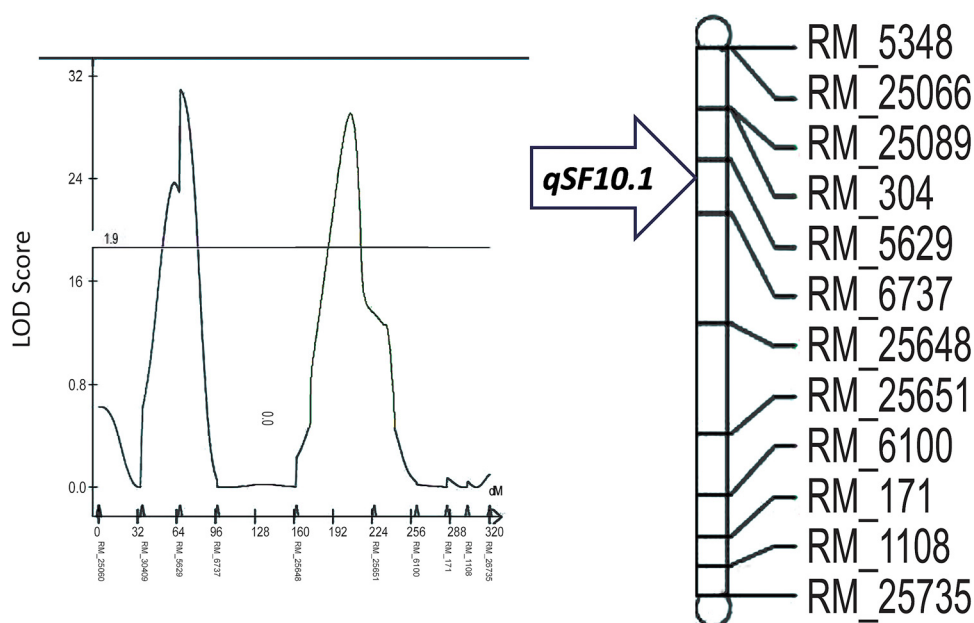
QTLs for anaerobic germination *qAG 3.1* on chromosome 3 and *qSF10.1* for plant survival % under SF on chromosome 10 were detected using RILs of Indra and AC 39416A. This indicated that alleles contributing to different types of floods are present in AC39416A.

Angaji *et al.* (2010) also reported QTL for anaerobic germination *qAG 3* between RM 7094 (26.87 Mbp) and RM 520 (30.91 Mbp) on chromosome 3 using Khao Hlan On as donor, RILs of Nampyeong/PBR cross (Jeong *et al.*, 2020), F_{2:3} population of Nanhi/IR64 (Baltazar *et al.*, 2014) and IR64/Kharsu 80A (Baltazar *et al.*, 2019). The above results support the idea that QTL *qAG3.1* possesses genes that trigger signals for anaerobic germination from AC39416A.

Identified QTL for plant survival % under SF is in the vicinity of a reported QTL for plant survival on chromosome 10 between RM 222 (20.70 Mbp) and *qSUB10.1* at RM25835 (21.31 Mb; Gonzaga *et al.*, 2016). QTLs for plant survival % (Toojinda *et al.*,

Table 2. Identified QTLs for flood tolerance using RILs of AC39416A

Year	Trait name	Chromo some	QTL	Marker interval	LOD	Phenotypic variance (%)	Additive effect
2016	Anaerobic germination	3	<i>qAG 3.1</i>	RM15848-RM15561	2.89	7.16	4.48
2017				RM15554-RM15561	5.36	59.08	8.96
2017	Stagnant flooding	10	<i>qSF10.1</i>	RM304-RM6100	5.66	13.21	10.79
2018				RM 304-RM6737	3.10	7.56	7.71

**Fig. 4.** QTL *qSF10.1* for plant survival % under stagnant flooding.

2003) in F_2 derived population of Jao Him Nin/KDML 105, grain weight and days to 50% flowering under SF were also identified on chromosome 10 in RILs of Ciharang-Sub1/IR10F365 (Singh *et al.*, 2017a, 2017b) and Swarna/Rashpanjor (Chattopadhyay *et al.*, 2021).

LOC_Os03g42130 gibberellin 20 oxidase2, a putative gene locus, inhibits gibberellic acid biosynthesis under anoxia conditions. Production of α -amylase does not require gibberellic acid for germination under anaerobic conditions (Loreti *et al.*, 2003) and the amylase activity remained unchanged under anaerobic germination in AC39416A and FR 13A (Sweetaleena *et al.*, 2019). LOC_Os03g44170 glutathione S-transferase, a putative candidate gene locus, might also play a role in crosstalk between submergence tolerance during germination (Thapa *et al.*, 2022) and hormone response pathways (Jain *et al.*, 2010), and also protects the plants from oxidative stress under anoxia conditions (Kumar and Trivedi, 2018).

Results of gene prediction between RM 304 (18.650 Mbp) and RM 6737 (18.71 Mbp) revealed putative candidate gene LOC_Os10g35020 glycosyltransferase and LOC_Os10g35050 aquaporin protein that might be responsible for plant survival % under SF. Glycosyltransferase plays a role in antioxidant defence mechanism under flooding (Sanhezz-Bermudez *et al.*, 2022) and submergence tolerance on chromosome 10 (Qi *et al.*, 2005) by expression of genes with response to ethylene and gibberellin. LOC_Os10g35050 aquaporin protein putative candidate genes also play a role in adaptive mechanism for plant survival

% under SF. Partial to prolonged SF might have triggered protein accumulation of aquaporins (Tyerman *et al.*, 2002). Plant aquaporin not only play a role to facilitate osmotic water transport across membranes but also transports nutrients like urea (Gaspar *et al.*, 2003), ammonia (Loque *et al.*, 2005) and CO_2 (Hanba *et al.*, 2004). The presence of glycosyltransferase loci might trigger hormone response pathways for plant survival % and aquaporin proteins loci might manifest the plant for nutrient uptake and gas diffusion for adaptation under SF.

Conclusion

In this study, identified QTLs *qAG 3.1* for anaerobic germination for 21 days and *qSF10.1* for plant survival % under SF from RILs generated by Indra/AC39416A can be further exploited for marker-assisted gene pyramiding using AC 39416A as donor for both anaerobic germination and SF. Studies on gene prediction revealed that AC39416 A adapts to anaerobic germination and SF by constitutive protein production in response to particular environmental signalling which has to be further traced out by advanced physiological and molecular studies.

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Conflict of interest. None.

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