Indian J. Genet. Plant Breed., (2025); 85(2): 208-214-
https://doi.org/10.31742/ISGPB.85.2.4

ISSN: 0975-6906

www.isgpb.org

RESEARCH ARTICLE

Mapping of QTL for anaerobic germination using the donor AC39416A in the genetic background of variety Swarna Sub-1 of rice (*Oryza sativa* L.)

B. Saimohan*#, N. Chamundeswari[1#], T. Haritha[2] and N. Veronica[1]

Abstract

Climate change is causing a decline in rice productivity and sustainability in coastal irrigated areas due to abiotic stresses. Flooding during germination, triggered by unexpected and heavy rainfall, poses a significant threat to the growth and survival of rice plants. Anaerobic germination (AG) is an important trait in particular circumstances for cultivation under the direct seeding method in *kharif* season. To identify QTLs responsible for AG, phenotypic screening was carried out using the 188 $F_{2:3}$ population of Swarna Sub1/AC39416A in the present investigation. The mean anaerobic germination percent recorded after the 2 weeks of submergence ranged from 0 to 95%, with an overall mean of 47.51%, whereas, for 3 weeks of submergence, the mean anaerobic germination percent recorded between 0 and 95%, with an overall mean of 37.66%. Parental polymorphism studies revealed that 134 out of 687 SSR markers were polymorphic. Linkage analysis with 83 polymorphic SSR markers was done using the QTL IciMapping software version 4.1.1. The length of the linkage map constructed across the whole genome was 3600.8 cM. Four QTLs were identified *viz*., *qAG7-1, qAG7-2, qAG10* and *qAG12* with the ICIM method. All these 4 QTLs explained a phenotypic variance of about 18.81% collectively for AG trait, with their individual contributions ranging from 2.99 to 8.67% of phenotypic variation and LOD scores of 5.05 to 5.86. The LOD score and phenotypic variance is 5.86 and 8.67% respectively for *qAG10* a novel QTL identified in the present study using ICIM method.

Keywords: Anaerobic germination, Phenotypic screening, SSRs, Genotyping, QTL mapping.

Introduction

Rice (*Oryza sativa* L.) production is subjective by many of the biotic and abiotic stresses throughout the world, where abiotic stresses alone contribute to nearly half of the total losses in yield. Under coastal irrigated ecosystems, major abiotic stresses, *viz*., floods, cyclones (causes lodging of the crop) and salinity, resulting in a decline in the productivity of rice. Severe and unexpected heavy rains leave no time to leach excess water into to ground, which leads to flooding, a major climate change challenge severely affecting productivity and often places a major limitation on the cultivation. Three types of floods, *viz*., submergence during germination (germination under anaerobic conditions), flash floods (complete submergence up to 2 weeks) and stagnant flooding (30-50cm water depth), are the most prevailing types of floods in coastal Andhra Pradesh (Reddy *et al*., 2015).

Flooding during seed germination might be a consequence of unevenly leveled fields, early and unforeseen rains, or even when rice fields are purposely flooded after sowing to combat weeds. Among all abiotic stress, tolerance to flooding during the process of seed germination, *i.e.,* anaerobic germination (AG), is the rarest

Department of Genetics and Plant Breeding, Agricultural College, Bapatla 522 101, Acharya N G Ranga Agricultural University (ANGRAU), Andhra Pradesh, India

[1]Regional Agricultural Research Station (RARS), Maruteru 534 122, West Godavari, Andhra Pradesh, India

[2]Agricultural College, ANGRAU, Bapatla 522 601, Andhra Pradesh, India

[#]Both authors contributed equally

***Corresponding Author:** B. Saimohan, Department of Genetics and Plant Breeding, Agricultural College, Bapatla 522 101, Acharya N G Ranga Agricultural University (ANGRAU), Andhra Pradesh, India, E-mail: saimohanbollineni@gmail.com.

How to cite this article: Saimohan B., Chamundeswari N., Haritha T. and Veronica N. 2025. Mapping of Qtl for anaerobic germination using the donor AC39416A in the genetic background of variety Swarna Sub-1 of rice (*Oryza sativa* L.). Indian J. Genet. Plant Breed., **85**(2): 208-214.

Source of support: ANGRAU, Lam, Guntur, Govt. of Andhra Pradesh

Conflict of interest: None.

Received: Sept. 2024 **Revised:** April 2025 **Accepted:** April 2025

phenomenon (Zhang et al. 2018). Rice is the only chief cereal that exhibits a degree of tolerance to anaerobic conditions during germination, which is limited to coleoptile emergence and partial growth but not adequate to triumph over the stress (Miro et al. 2017). The semi-aquatic nature of rice made it survive a few days of submergence and broad genetic diversity among the rice landraces and traditional varieties has enabled its cultivation in different agroecological zones and water regimes. Although rice can tolerate flooding, its germination is limited to coleoptile elongation as in susceptible genotypes; however, root and primary leaves fall short of developing normally (Kumar et al. 2018). Tolerance of rice crops to flooding stress through enhanced germination and early growth of the seedlings is a prerequisite for successful cultivation in regions where floods are a recurrent problem.

Anaerobic germination is characterized by rapid elongation of coleoptile under submergence, with concomitant delay in the development of radicals (Kretzschmar et al. 2015). A series of biochemical properties, such as changes in the enzymatic activities of α-amylase, peroxidase and alcohol dehydrogenase, influences anaerobic stress tolerance in rice. The positive influence of α-amylase in improving the germination of the seed is by converting starch into sugars (Perata et al. 1993).The tolerance mechanism that enables rice to germinate in the absence of O_2 is based mainly on the fact that rice seeds are able to degrade their starchy reserves under anoxia also (Nghi et al. 2019). The ability of the rice coleoptile to elongate under anoxia represents an unveiled enigma, whereas the mechanisms and genes involved in the adaptation of rice flora to submergence have been newly discovered.

The identification of a QTL for submergence tolerance SUBMERGENCE 1 (SUB1) gene from a landrace, tremendous progress has been achieved in the last decade in the development of flood-tolerant varieties through DNA marker technology such as marker-assisted backcrossing (Khalil et al. 2024; Phukan et al. 2024). With the available germplasm adapted to the submergence environment can be assured that several genetic stocks provide useful genetic reserves for tolerance to submergence. Although several mechanisms of submergence tolerance have been identified in the germplasms, only SUB1 has been extensively used for genetic transfer into varieties. Identification of QTLs for specific traits in rice, such as anaerobic germination of deepwater rice earlier (Rohila et al. 2020), may further enhance the probability of developing suitable varieties for submergence tolerance.

Identification of the molecular markers linked to QTLs or genes controlling tolerance to submergence during germination would assist selection for this character, which has low heritability (Angaji et al. 2010). Screening of markers for polymorphism between the parents forms the basis for tagging the desired gene, fine mapping of the gene in the rice chromosome and in the subsequent marker-assisted selection (MAS) programs (Reddy *e*t al. 2018). Marker-assisted breeding offers unprecedented opportunities for contemporary plant breeders to enable them to breed futuristic crop varieties (Singh et al. 2019). Of the various types of DNA markers, PCR-based markers called simple sequence repeats (SSRs) or microsatellites are used widely due to their high degree of polymorphism, technically simple method of finding and efficient (Gonzaga et al. 2015). The QTL analysis and other molecular methods are employed in order to find the genetic locus that underlies the trait of interest. If a genetic locus has been discovered and characterized has a major effect on the trait, it can be transferred subsequently into modern high-yielding cultivars, but are stress-sensitive using marker-assisted breeding technology to achieve stress-tolerant cultivars efficiently (Mustroph 2018). QTL mapping for AG in rice has begun to identify the promising loci that promote increased germinability under flooding in experiments carried out earlier by other researchers (Jiang et al. 2006; Angaji et al. 2010; Baltazar et al. 2014).

Identifying new germplasm resources with tolerance to high and low extremes of precipitation is required to meet the impelling demand of climate-resilient varieties (Roy et al. 2023). Breeding for flooding tolerance during germination (AG) has been attempted previously by many workers, but the progress is little due to the limitation of donors with AG i.e. genetic diversity, limited knowledge on the genetics and complex mechanisms of tolerance and methods used for screening or measurement of tolerance (Jiang et al. 2004). Keeping in view the importance of anaerobic germination, the present investigation was planned and executed using the parents Swarna Sub1 and AC39416A for generating a mapping population to identify the QTLs responsible for AG in order to develop varieties with improved tolerance to anaerobic conditions, particularly in flooded or waterlogged areas.

Materials and methods

Plant material and mapping population

The experimental plant material consisting of 188 $F_{2:3}$ mapping population was developed by crossing Swarna Sub1 and AC39416A at RARS Maruteru (formerly APRRI), West Godavari district of Andhra Pradesh. Swarna sub1 is a variety developed by IRRI, has submergence tolerance during the vegetative stage for 7 to 10 days, but lacks tolerance to submergence during germination. After screening of large material, the cultures AC39416A and AC39397 were identified as donors for the AG trait. Hence, in the present study, the parent Swarna sub1 is crossed with AC39416A to create a population with diverse genetic backgrounds, *i.e.,* mapping population. The 188 $F_{2:3}$ lines, along with their corresponding contrast parents, were screened

phenotypically and genotypically to develop reliable data in an attempt to unravel the tolerance of submergence during germination.

Screening for tolerance to anaerobic conditions during germination

Phenotypic screening of 188 $F_{2:3}$ population of Swarna Sub1/AC39416A along with parents was conducted using a complete randomized design with 2 replications in a concrete tank as per Septiningsih et al. (2013b). Initially, anaerobic stress is created and then the level of their tolerance is recorded. The pre-germinated seeds (25 per population) were sown in pro-trays filled with well-puddled soil. Pro-trays were arranged randomly inside the concrete tank and submergence (stress) was imposed by filling water up to 15 cm above the trays. Constant depth of water is maintained throughout the submergence treatment for 2 weeks and 3 weeks in separate experiments. After submergence treatment, pro-trays were kept outside of the concrete tank for about 1 week of de-submergence treatment and finally, the data from surviving lines was recorded.

Genotyping of mapping population

Genomic DNA isolation of young leaf samples collected from all 188 $F_{2:3}$ lines and their respective parents was done using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Zheng et al. (1995). Parental polymorphism survey was conducted using a total of 687 SSR (Microsatellite) markers. The SSR primer sequences and other information were obtained from the Gramene markers database (http://www.gramene.org.in). Genotyping of the entire population was done using the polymorphic SSR markers. 7.5 µL of the master mix was added to each well of the PCR plate having 2.5 µL of template DNA to make the final volume 10 µL per cell. Then PCR plate was kept in a thermal cycler for the reaction to take place. PCR amplified products of DNA samples (10 µL) were loaded into wells of 3% agarose gels and run for 2 hours at constant mode with 110 volts steady electric field. The pores in the gel separate the linear fragments of DNA according to their size. The DNA fragments were then visualized under a UV-trans-illuminator as bands and documented using a gel documentation system (SYNGENE Gene flash U.K.).

Linkage map construction

Integrated software called QTL IciMapping software version 4.1.1 (Wang et al. 2015) was used for linkage analysis using 83 polymorphic SSR markers. Grouping of all 83 markers across 12 linkage groups (chromosomes) was done based on anchor information. Unanchored markers were removed and anchor order for ordering the markers for fitting on the best positions. Then output is done, after which the map show tool is selected to draw a linkage map of SSRs. Linkage maps were constructed using the linkage map construction tool in biparental populations (MAP) of ICIM software by following the Kosambi mapping function.

QTL analysis

QTL analysis was performed with 83 SSR markers that are polymorphic between the contrasting parents to study the association of genotypic and phenotypic data of the entire population screened using integrated software QTL IciMapping software 4.1.1 (Wang et al. 2015). QTLs were detected by single marker analysis (SMA) and Inclusive Composite Interval Mapping for the additive QTL (ICIM-ADD) methods in the present study. QTL IciMapping software version is integrated software for linkage analysis and genetic mapping in biparental populations.

Results and discussion

Phenotypic screening of mapping population

Early generation biparental mapping population consisting of 188 $F_{2:3}$ lines developed with contrasting parents Swarna Sub1/AC39416A was screened for AG. Phenotypic screening revealed significant differences among the population for the traits studied. The mean AG percent recorded after the 2 weeks of submergence ranged from 0 to 95% with an overall mean of 47.51%, whereas the mean AG percent of the contrasting parents was 40 and 85%, respectively. For 3 weeks of submergence treatment, the mean AG percent recorded between 0 and 95%, with an overall mean of 37.66% and the mean AG percent of the two contrasting parents was 27 and 75.6%, respectively, indicating that there was wider variation in the mapping population for anaerobic germination. (Fig. 1). Among 188 $F_{2:3}$ population studied 12 lines for two weeks of treatment and five lines for three weeks of treatment have shown AG percent on par with the donor parent AC39416A. A similar trend of variation in AG percent was reported by Barik et al. (2019).

The average survival rate calculated by counting the seedlings that survived after one week of de-submergence of SwarnaSub1 and AC39416A was 35 and 80%, respectively, after 2 weeks of submergence, whereas for the $F_{2:3}$ mapping

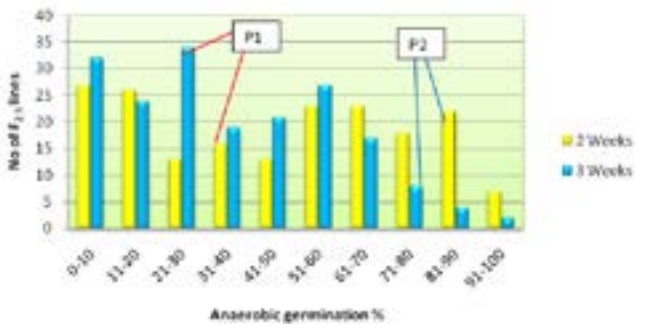


Fig. 1. Frequency distribution for Anaerobic Germination (%) in $F_{2:3}$ mapping population of Swarna Sub1 (P1)/AC39416A (P2).

population, it ranged from 0 to 95% with overall mean of 36.74%. The plant survival rate after 3 weeks of submergence for Swarna Sub1 was 17.6%, whereas 72% for the donor AC39416A. The average survival rate of the population was 15.5%, which indicated clear-cut variation. (Fig. 2). Doley *et al*. (2018) noticed that survival percent was correlated positively with coleoptile elongation, which helps in obtaining oxygen from surroundings. Variation in AG among germplasms has been reported earlier by many workers. A similar pattern of variation in survival percent of population was described earlier by Septiningsih et al. (2013b) and Baltazar *et al*. (2014) in $F_{2:3}$ populations derived from IR 64/Ma-Zhan Red and IR 64/Nanhi, respectively in the screening for tolerance to anaerobic conditions during germination, whereas reports of Angaji (2008) showed less variation in the BC_2F_2 population of IR64/KHAIYAN, screened for tolerance during germination stage submergence.

In general, rice seeds contain the complete set of enzymes needed for the degradation and use of starch for the growth and maintenance of the growing embryo; however, the activities of these enzymes are affected by anaerobic conditions due to the low availability of oxygen (Ismail et al. 2012). Some of these enzymes, especially alcohol dehydrogenase 1 (ADH1), rice alpha-amylase (RAmy3D), and sucrose synthase, are more active in anoxia-tolerant rice genotypes under low-oxygen conditions during germination but are inhibited in sensitive genotypes, RAmy3D encoding starch-degrading enzymes, up-regulated during germination under anaerobic conditions. This increased gene expression under anaerobic conditions leads to higher amylase activity for starch hydrolysis, which in turn enhances the activity of ADH1, a key enzyme involved in alcohol fermentation that is crucial for rice seed germination under anaerobic conditions. Upon germination, ethylene produced by the growing embryo may further promote cell expansion and starch hydrolysis, along with reduced abscisic acid (ABA) biosynthesis and increased gibberellic acid (GA) biosynthesis (Rauf et al. 2019). Hence, tolerance of anaerobic conditions during germination is an essential trait for direct-seeded rice cultivation in both rainfed and irrigated ecosystems (Septiningsih et a*l*. 2013b).

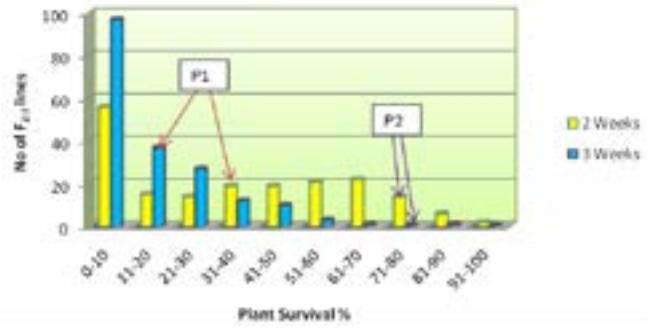


Fig. 2. Frequency distribution for Plant Survival (%) in $F_{2:3}$ mapping population of Swarna Sub1 (P_1) / AC39416A (P_2)

SSR marker-based linkage map construction

The contrasting parents, Swarna Sub1 and AC39416A, selected for the development of the mapping population, were initially surveyed for polymorphism using SSR markers. Only 134 (19.42%) SSR markers were found to be polymorphic amongst 687 SSRs screened. However, only 83 SSR markers are used to generate genotypic data for the construction of the linkage map and QTL analysis. The level of polymorphism ranged from 7.50 to 27.59%, with an average of 13.25% for all the chromosomes. (Table 1). Among the 12 chromosomes surveyed, chromosome 3 recorded the maximum number of polymorphic markers (eleven), followed by chromosome 12 (nine) and chromosomes 2 and 7 (eight). The polymorphism percentage was reported to be highest in chromosome 2 (27.59%) and lowest in chromosome 9 (7.58%).

The whole genome length of the linkage map constructed was 3600.8 cM (Figure 3). The map length of each chromosome varied with the number of markers used in each linkage group. The map lengths of all linkage groups are 219.09, 384.8, 482.98, 224.4, 254.08, 220.23, 357.68, 296.02, 127.63, 220.24, 280.71, 532.94 cM, respectively. Earlier studies of parental polymorphism using SSR markers in rice done by Jiang et al. (2006), Angaji (2008), Angaji et al. (2010), Septiningsih et al. (2013b) and Waghmare *et al*. (2018) revealed that 121 (32%), 170 (27.8%), 192 (28%), 115 (10.5%), 118 (11.1%) and 41 (20.82%) primers were polymorphic from a total of 197, 1066, 1074, 680, 610 and 653 SSR's surveyed. The extent of polymorphism recorded in the present investigation, 19.42% is comparable with earlier reports. Reports are also available on performed linkage mapping with 60 SSR primers using QTL ICIM software version 4.0 Software as well as QTL ICIMapping V3.2 software for

Table 1. Chromosome-wise list of markers screened, number of polymorphic markers along with percent of polymorphism

Chromosome number	Number of markers			Polymorphism (%)
		Polymorphic	Anchored	
1	37	10	7	18.92
2	29	9	8	27.59
3	64	14	11	17.19
4	54	12	6	11.11
5	80	10	6	7.50
6	61	16	5	8.20
7	62	12	8	12.90
8	68	8	7	10.29
9	64	8	5	7.81
10	57	13	6	10.53
11	49	11	5	12.24
12	62	11	9	14.52
Total	687	134	83	13.25

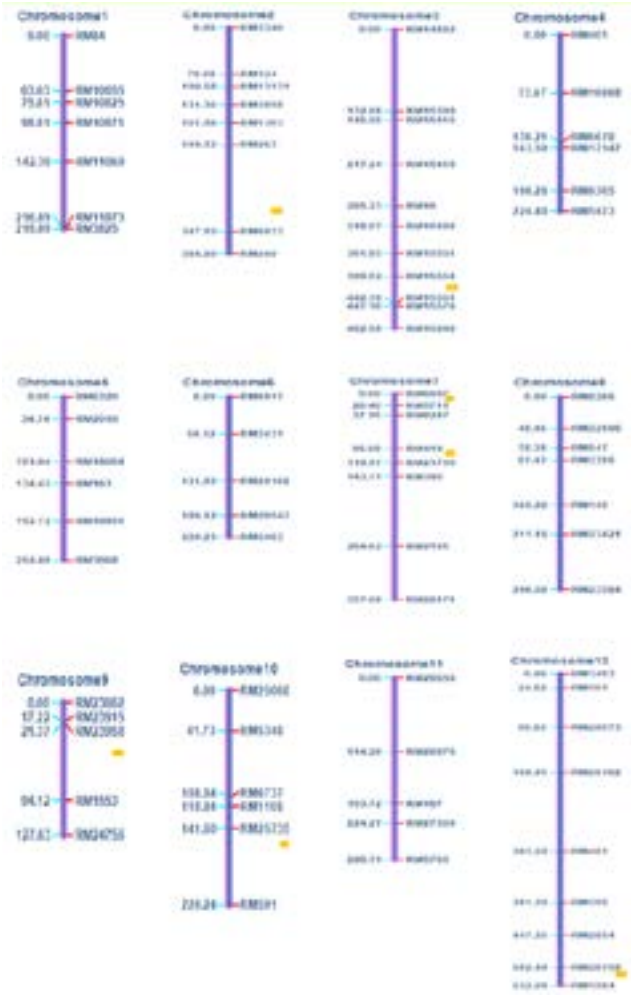


Fig. 3. Linkage map of 83 SSR markers showing the positions of the QTLs. Yellow-colored rectangle indicates the QTLs detected in QTL IciMapping V.4.1 software

construction of linkage map using 97 SNP and 7 SSR markers (Pramudyawardani *et al*. 2018).

QTL mapping analysis

QTL analysis performed with 83 SSRs revealed that, in the single marker analysis (SMA) method, five markers *viz*., RM 401, RM 5711, RM 21700, RM 28073 and RM 1584, were found to be linked with AG trait, located on chromosome 4, 7, 7, 12 and 12, respectively. LOD score of 3.31 and phenotypic variance of 4.71% has been recorded with RM 401on chromosome 4, whereas RM 5711, RM 21700, RM 28073 and RM 1584 have varying LOD scores of 3.85, 3.88, 4.28 and 7.39 and phenotypic variance of 5.45, 5.48, 6.03 and 10.01%, respectively. All these QTLs individually accounted for a total of 4.72 to 10.02% phenotypic variance (R^2). The highest phenotypic variance (10.02%) was recorded on chromosome 12 by *qAG12-1* with peak marker RM 1584 (Table 2).

A total of four putative QTLs *viz*., *qAG7-1, qAG7-2, qAG10* and *qAG12* were identified and mapped using the inclusive composite interval mapping (ICIM) method (Table 3). Out of 4 QTLs, 2 QTLs were located on chromosome 7, 1 QTL on each of the chromosomes 10 and 12. All these 4 QTLs explained a phenotypic variance of about 18.81% collectively for the AG trait, with their individual contributions ranging from 2.99 to 8.67% of phenotypic variation and LOD scores of 5.05 to 5.86. The QTLs *qAG7-1 & qAG7-2* on chromosome 7 were flanked between RM 6697 & RM 5711, RM 418 & RM 21700, have LOD scores of 5.05 & 5.85 and phenotypic variation of 3.52 & 3.62%, respectively. The QTLs *qAG10* on chromosome 10, *qAG12* on chromosome 12 were flanked between RM 25735 & RM 591, RM 28759 and RM 1584 with 5.86, 5.47 and 8.67, 2.99% of LOD scores and phenotypic variation, respectively. The phenotypic variance explained by *qAG10* was highest (8.67%) was mapped on chromosome 10, whereas, the

Table 2. Peak markers linked to anaerobic germination identified in $F_{2:3}$ population of Swarna Sub1/AC39416A using single marker analysis

S.No	QTL	Chromosome	Peak marker	LOD	PVE(%)	Add	Dom
1	*qAG-4* (novel QTL)	4	RM401	3.31	4.72	0.24	-16.04
2	*qAG-7-1*	7	RM5711	3.86	5.46	-1.36	14.48
3	*qAG-7-2*	7	RM21700	3.88	5.49	2.17	-15.29
4	*qAG-12-1*	12	RM1584	7.39	10.02	-7.03	-18.73
5	*qAG-12-2*	12	RM28073	4.29	6.03	6.23	13.47

Table 3. QTLs for tolerance to submergence during germination identified in $F_{2:3}$ population of Swarna Sub1/AC39416A in Inclusive Composite Interval Mapping method

S. No	QTL	Chromosome	Flanking Markers	LOD	PVE(%)	Add	Dom
1	*qAG-7-1*	7	RM6697 - RM5711	5.05	3.53	-0.64	15.91
2	*qAG-7-2*	7	RM418 - RM21700	5.86	3.62	1.08	-16.27
3	*qAG-10* (novelQTL)	10	RM25735 - RM591	5.86	8.67	-2.39	23.76
4	*qAG-12*	12	RM28759 - RM1584	5.47	2.99	-4.52	-14.20

highest LOD score (5.86) was shown by *qAG10* and *qAG-7-2*. Two novel QTLs were mapped in this study are *qAG-4* (SMA method) with 3.31 and 4.72% and *qAG10* (ICIM method) with 5.86 and 8.67% LOD score and phenotypic variation, respectively. Among the QTLs identified for AG in the present investigation *qAG4, qAG7-1, qAG7-2, qAG10, qAG12-1* and *qAG12-2* using SMA and ICIM methods, the QTLs *qAG7-1, qAG7-2, qAG12-1* and *qAG12-2* were also reported in other populations in the earlier studies. In both the SMA and ICIM methods, the QTLs viz. *qAG7-1, qAG7-2* and *qAG12* are commonly identified, indicating the presence of genomic regions responsible for tolerance to anaerobic germination on chromosomes 7 and 12. Among the QTLs identified, the QTL *qAG12-1*, has shown the highest LOD score (7.39) and phenotypic variance (10.02%) and considered as major QTL for AG in the $F_{2:3}$ population of Swarna Sub1/AC39416A.

A similar trend of results of QTL analysis using QTL cartographer was reported by Angaji (2008) where QTL *qAG12* located on chromosome 12 with LOD score of 5.71 and phenotypic variation of 29.24% by IM method was also found to be linked with peak marker RM 28759 in the present investigation. Among QTLs reported for tolerance of flooding conditions during germination, the highest LOD and phenotypic variation of 15.32 and 20.59, respectively, were noted on chromosome 9 for QTL *qAG9-2* by Angaji *et al*. (2010) was in line with the identified QTLs of the present investigation. Similar QTLs were identified by Septiningsih et al. (2013b) on chromosomes 7 and 12 for submergence tolerance during germination. Baltazar *et al*. (2014) also identified similar QTLs, *qAG7* on chromosome 7 with LOD score and phenotypic variation of 13.93 and 14.06%, respectively. Similar QTLs on chromosome 7 were also identified and mapped by Baltazar et al. (2019), governing tolerance to submergence during germination. In conclusion, the QTLs identified in the study, majorly *qAG12-1* may be considered for introgression into popular elite rice varieties. Otherwise, susceptible to AG after characterization of the mechanism underlying AG and fine mapping.

Supplementary material

Supplementary Figs. S1 and S2 are provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualization of research (NC); Designing of the experiments (BSM, NC); Contribution of experimental materials (BSM, NC); Execution of field/lab experiments and data collection (BSM, NC); Analysis of data and interpretation (BSM, NC, TH); Preparation of the manuscript (BSM, NC, TH, NY).

Acknowledgment

The first author expresses gratitude to Acharya N G Ranga Agricultural University, Lam, Guntur, Andhra Pradesh, India, for providing financial support for the successful completion of a research project.

References

Angaji S.A. 2008. Mapping qtls for submergence tolerance during germination in rice. African J. Biotechnol., 7 (15): 2551-2558.

Angaji S.A., Septiningsih E.M., Mackill D.J. and Ismail A.M. 2010. Qtls associated with tolerance of flooding during germination in rice (Oryza sativa L). Euphytica, **172**: 159-168.

Baltazar M.D., Ignacio J.C.I., Thomson M.J., Ismail A.M. and Septiningsih E.M. 2014. QTL mapping for tolerance of anaerobic germination from IR64 and the aus landrace Nanhi using SNP genotyping. Euphytica, **197**: 251-260.

Baltazar M.D., Ignacio J.C.I., Thomson M.J., Ismail A.M., Mendioro M.S. and Septiningsih E.M. 2019. QTL mapping for tolerance to anaerobic germination in rice from IR64 and the aus landrace Kharsu 80A. Breed. Sci., **18159**: 1-7.

Barik J., Kumar V., Lenka S.K. and Panda D. 2019. Genetic potentiality of lowland indigenous indica rice (*Oryza sativa* L.) landraces to anaerobic germination potential. Indian J. Plant Physiol., **1**-13.

Doley D., Barua M., Sarma D. and Barua P.K. 2018. Screening and enhancement of anaerobic germination of rice genotypes by pre-sowing seed treatments. Curr. Sci., **115**(6): 1185-1190.

Gonzaga Z.J., Aslam K., Septiningsih E.M. and Collard B.C. 2015. Evaluation of SSR and SNP markers for molecular breeding in rice. Plant Breed. Biotechnol., **3** (2): 139-152.

Ismail A.M., Johnson D.E., Ella E.S., Vergara G.V. and Baltazar A.M. 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. AoB Plants, **2012**: 1-18.

Jiang L., Liu S., Hou M., Tang J., Chen L., Zhai H. and Wan J. 2006. Analysis of qtls for seed low temperature germinability and anoxia germinability in rice (Oryza sativa L.). Field Crops Res., **98** (1): 68-75.

Khalil M.I., Hassan M.M., Samanta S.C., Chowdhury A.K., Hassan M.Z., Ahmed N.U., Somaddar U., Ghosal S., Robin A.H.K., Nath U.K., Mostofa M.G., Burritt D.J., Ha C.V., Gupta A., Tran L.P. and Saha G. 2024. Unraveling the genetic enigma of rice submergence tolerance: Shedding light on the role of ethylene response factor-encoding gene SUB1A-1. Plant Physiol. Biochem., **206**: 108224.

Kretzschmar T., Pelayo M.A.F., Trijatmiko K.R., Gabunada L.F.M., Alam R., Jimenez R., Mendioro M.S., Slamet-Loedin I.H., Sreenivasulu N., Bailey-Serres J., Ismail A.M., Mackill D.J. and Septiningsih E.M. 2015. A trehalose-6-phosphatephosphatase enhances anaerobic germination tolerance in rice. Nature Plants, **1**: 1-5.

Kumar D., Das P.K. and Sarmah, B.K. 2018.Reference gene validation for normalization of RT-q PCR assay associated with germination and survival of rice under hypoxic condition. J. Appl. Genet., **59**: 419–430.

McCouch S.R. 2008. CGSNL (Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative). Gene nomenclature system for rice. Rice, **1**(1): 72-84.

Miro B., Longkumer T., Entila F.D., Kohli A. and Ismail A.M. 2017. Rice seed germination underwater: Morpho-physiological responses and the bases of differential expression of alcoholic fermentation enzymes. Front. Plant Sci., **8**: 1-17.

Mustroph A. 2018. Improving flooding tolerance of crop plants. Agronomy, **8**(9): 1-25.

Nghi K.N., Tondelli A., Vale G., Tagliani A., Mare C., Perata P. and Pucciariello C. 2019. Dissection of coleoptile elongation

in *japonica* rice under submergence through integrated genome wide association mapping and transcriptional analyses. Plant, Cell Env., **42** (6): 1832-1846.

Perata P., Geshi N., Yamaguchi J. and Akazawa I. 1993. Effect of anoxia on the induction of -amylase in cereal seeds. Planta, **191**: 402-408.

Phukon M., Das J., Sruthi R., Verma R.K., Modi M.K., Bhattacharyya A. and Chetia S.K. 2024. Study on submergence tolerance of rice in a core panel of North-East India using GWAS. Indian J. Genet. Plant Breed., **84**(2): 193-201. https://doi.org/10.31742/ISGPB.84.2.6

Pramudyawardani E.F., Aswidinnoor H., Purwoko B.S., Suwarno W.B., Islam M.R., Verdeprado H. and Collard B.C. 2018. Genetic Analysis and QTL Mapping for Agronomic and Yield-Related Traits in Ciherang-Sub1 Rice Backcross Populations. Plant Breed. Biotechnol., **6** (3): 177-192.

Rauf M., Choi Y.M., Lee S., Lee M.C. and Oh S. 2019. Evaluation of anaerobic germinability in various rice subpopulations: identifying genotypes suitable for direct-seeded rice cultivation. Euphytica, **215**(2): 1-15.

Reddy A.V., Girija Rani M., Satyanarayana P.V., Suryanarayana Y., Chamundeswari N., Ravikumar B.N.V.S.R., Ramana R.P.V. and Vishnuvardhan K.M. 2015. Physiological and molecular response of rice genotypes for different types of flooding. Curr. Biotica, **8**(4): 345-350.

Reddy B.S. and Rani M.G. 2018. Evaluation of Near Isogenic Lines (NILs) of rice for major abiotic stresses of coastal areas. Elect. J. Plant Breed., **9**(3): 808-814.

Rohilla M., Singh N., Mazumder A., Sen P., Roy P., Chowdhury D., Singh N.K. and Mondal T.K. 2020. Genome-wide association studies using 50 K rice genic SNP chip unveil genetic architecture for anaerobic germination of deepwater rice population of Assam, India. Mol. Genet Genom., **295**(5): 1211-1226.

Roy S., Chakraborty K., Banerjee A., Kumar J., Sar P., Verma B.C., Priyamedha. and Mandal N.P. 2023. Evaluation of rice germplasm for tolerance to multiple abiotic stresses using multivariate techniques and molecular screening. Indian J. Genet. Plant Breed., **83**(1): 15-23.

Singh A. K., Krishnan S. G., Vinod K. K., Ellur R. K., Bollinedi H., Bhowmick P. K. and Nagarajan, M. 2019. Precision breeding with genomic tools: A decade long journey of molecular breeding in rice. Indian J. Genet. Plant Breed., **79**(Sup-01): 181-191.

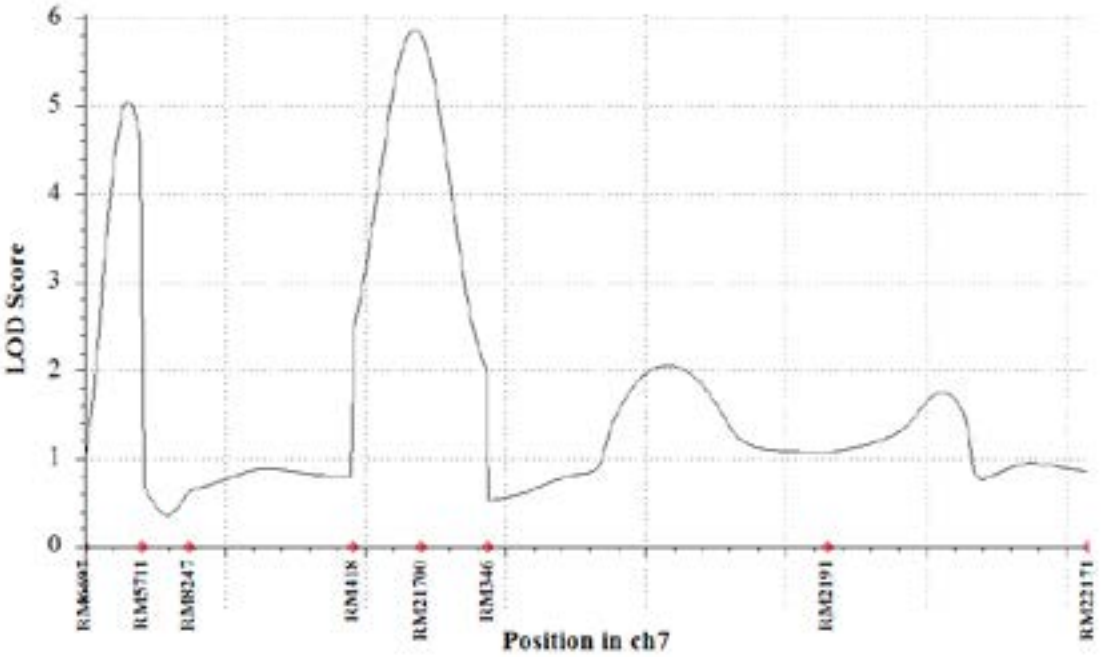
Septiningsih E.M., Ignacio J.C.I., Sendon P.M.D., Sanchez D.L., Ismail A.M. and Mackill D.J. 2013b. QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. Theor. Appl. Genet., **126**: 1357–1366.

Waghmare S.G., Sindhumole P., Shylaja M.R., Mathew D., Francies R.M., Abida P.S. and Sajini S. 2018. Research Article Analysis of simple sequence repeat (SSR) polymorphism between N22 and Uma rice varieties for marker assisted selection. Elect. J. Plant Breed., **9**(2): 511-517.

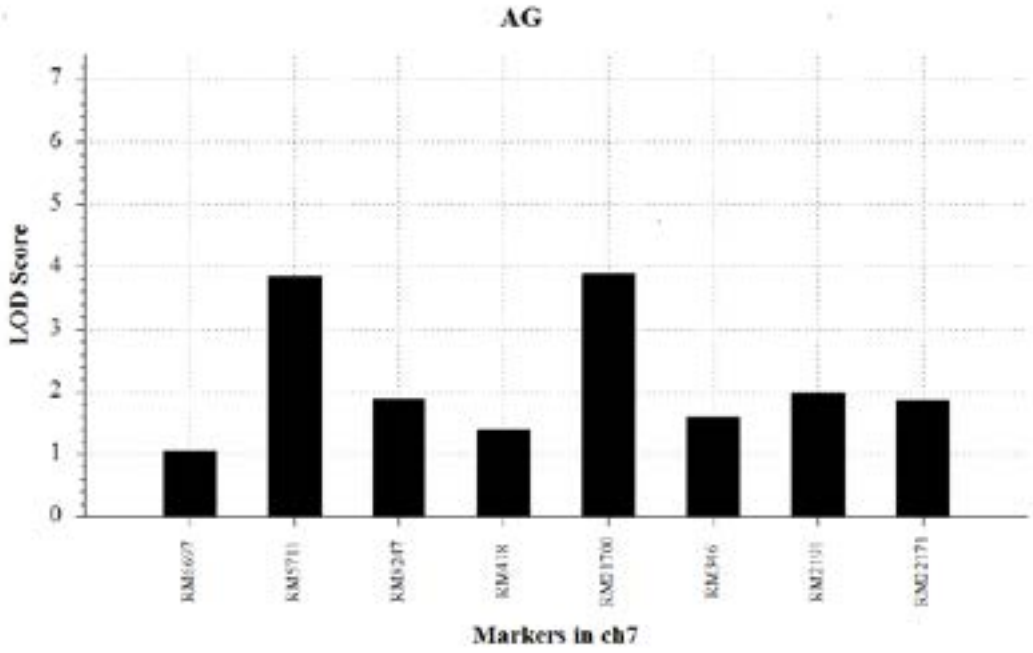
Wang J., Meng L., Li H. and Zhang L. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop J., **3**(3): 269-283.

Zhang J. and Rahman A.R.B. 2018. Preferential geographic distribution pattern of abiotic stress tolerant rice. Rice, **11** (1): 1-16.

Zheng K., Subudhi P.K., Domingo J., Magantay G. and Huang N. 1995. Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genet. News Letter, **12**: 255-258.



Supplementary Fig. 1. Graph showing LOD scores (peak) for AG QTLsmapped on Chromosome 7 (ICIM method)



Supplementary Fig. 2. Depiction of LOD score for AG QTLs mapped on Chromosome 7 (SMA)