Mapping of Qtl for Anaerobic Germination Using the Donor Ac39416a in the Genetic Background of Swarna Sub-1 (Oryza Sativa L.)

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Research Article

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Abstract

Background: Anaerobic germination is an important trait in particular for cultivation under direct seeding method in *kharif* season, as well as during nursery rising for transplant rice, as sometimes unexpected rains immediately after sowing will drastically reduce the plant population.

Methods and Results: In the present investigation phenotypic screening for Anaerobic germination (AG) was carried out using 188 $F_{2:3}$ population of Swarna Sub1/AC39416A at RARS (APRRI), Maruteru. The mean anaerobic germination per cent recorded after the two weeks of submergence ranged from 0% to 95% with overall mean of 47.51% whereas, for three weeks of submergence, the mean anaerobic germination per cent recorded between 0 and 95%, with overall mean of 37.66%. 134 (19.42%) out of 687 Simple Sequence Repeats (SSR) markers surveyed were polymorphic between the parents. Linkage analysis was done with 83 SSR markers showing polymorphism clearly using the integrated software called QTL IciMapping software version 4.1.0. The length of linkage map constructed across whole genome was 3600.8 cM and identified seven QTLs *viz*., *qAG2, qAG3, qAG7-1, qAG7-2, qAG9, qAG10* and *qAG12*. All these seven QTLs explained phenotypic variance of about 37.47% collectively for AG trait, with their individual contributions ranging from 3.5% to 8.67% of phenotypic variation and LOD scores of 2.6 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.

Conclusion: QTL "*qAG12-1*" identified in this study may be considered for introgression into popular elite rice varieties otherwise susceptible for anaerobic germination after fine mapping studies.

Introduction

Rice production is influenced by many of the biotic and abiotic stresses throughout the world, where abiotic stresses alone contribute to nearly 50% of the total losses in the yield. Under coastal irrigated ecosystem major abiotic stresses *viz*., floods, cyclones (causes lodging of the crop) and salinity resulting in decline in the productivity of rice. Severe and unexpected heavy rains leaves no time to leach excess water in to ground which leads to flooding, a major climate change challenge severely affecting productivity and often place 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 levelled fields or 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 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 coleoptiles emergence and partial growth, but not adequate to triumph over the stress (Miro et al., 2017). Semi-aquatic nature of rice

made it to survive few days of submergence and broad genetic diversity among the rice landraces and traditional varieties has enabled its cultivation in different agro ecological zones and water regimes. Although rice could tolerate flooding, its germination is limited to coleoptiles elongation as in susceptible genotypes; however root and primary leaf fall short to develop normally (Kumar et al., 2018). Tolerance of rice crop to flooding stress through enhanced germination and early growth of the seedlings is a prerequisite for successful cultivation in regions where floods is a recurrent problem.

Anaerobic germination is characterized by rapid elongation of coleoptile under submergence, with concomitant delay in development of radical (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. 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 (Magneschi and Perata. (2009) and 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 adaptation of rice flora to submergence have newly been discovered.

Identification of the molecular markers linked to QTLs or genes controlling tolerance to submergence during germination would assist selection for this character, which have low heritability (Angaji et al., 2010). Screening of markers for polymorphism between the parents forms the basis for tagging of the desired gene, fine mapping of the gene in the rice chromosome and in the subsequent Marker Assisted Selection (MAS) programmes (Reddy *et al*., 2018). 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 are cost efficient (Gonzaga et al., 2015). The quantitative trait locus (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 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 of Jiang et al., 2004; Jiang et al., 2006; Angaji et al., 2010; Septiningsih et al., 2013b and Baltazar et al., 2014. QTLs reported previously for anaerobic germination in rice were listed in (Table. 4).

Breeding for increased anaerobic germination or flooding tolerance during germination 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 vision the importance of anaerobic germination the present investigation was planned and executed using the parents Swarna Sub1 and AC39416A for generating $F_{2:3}$ mapping population in lieu of identification of QTLs responsible for AG.

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 (formerly APRRI), Maruteru, West Godavari district of Andhra Pradesh. Swarna sub1 is a variety developed by IRRI, Philippines by introgression of *sub1*gene into a mega rice variety Swarna. It has submergence tolerance during vegetative stage for 7–10 days, but lacks tolerance to submergence during germination. Several donors were identified for anaerobic germination tolerance across the world. In RARS, Maruteru the cultures, AC39416A and AC39397 were identified as good donors for the anaerobic germination trait. Hence, in the present study the parents Swarna sub1 and AC39416A were used for generating mapping population. The 188 $F_{2:3}$ lines along with their corresponding contrast parents 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 at RARS, Maruteru, West Godavari district of Andhra Pradesh, located at an altitude of 5m above MSL, 81.44^0E longitude and 26.38^0N latitude, using complete randomized design with two replications in concrete tank as per Septiningsih et al. (2013b). Initially anaerobic stress is created and then level of their tolerance was recorded. From each line about 25 healthy seeds were soaked for a period of about 24 hours and incubated in dark or closed chamber yet again for 24 hours so that seeds will start germinating. Then pre-germinated seeds were sown in pro-trays which are filled with well puddled soil in such a way that the sprouted portion facing upwards or top end. And finally the pro-trays were arranged randomly inside the concrete tank and submergence is imposed by filling water up to 15 cm above the trays. Constant depth of water is maintained throughout the submergence treatment *i.e* two weeks and three weeks separate experiments. After submergence treatment for two weeks and three weeks pro-trays were kept outside of the concrete tank for about one week of de-submergence treatment during which watered daily, finally record the data from survived lines.

Genotyping of mapping population:

Fresh, young leaf samples were collected from all 188 $F_{2:3}$lines and parents Swarna Sub1 and AC39416A at tillering stage (45 DAS) during early hours of a day and stored at -20°C. Genomic DNA isolation was done using the modified *Cetyl Tri Methyl Ammonium Bromide* (CTAB) method Zheng et al. (1995). Parental polymorphism survey was conducted using a total of 687 SSR (Microsatellite) markers. The SSR primer sequences and other information like physical position, annealing temperature, expected PCR product size and sequences were obtained from Gramene markers database (http://www.gramene.org.in). Genotyping of entire population was done using the polymorphic SSR

markers. 7.5 µl of master mix was added to each well of PCR plate having 2.5 µl of template DNA to make the final volume to 10 µl per cell. Then PCR plate was kept in a thermal cycler for the reaction to take place.PCR amplified products *i.e* DNA samples (10 µl) were loaded into wells of 3 %agarose gels and run for 2 hours at constant mode with 110 volts where, DNA molecules with in an agarose gel matrix were subjected to steady electric field; it will migrate through the gel towards the positive electrode, anode since DNA has a strong negative charge at neutral P^H. The pores in the gel separate the linear fragments of DNA according to their size. The DNA fragments were then visualized under UV-trans-illuminator as bands and documented using gel documentation system (SYNGENE Gene flash U.K.).

Linkage map construction:

Linkage between the markers and QTL was detected by a statistical test called the Logarithm of Odds (LOD) score method. Integrated software called QTL IciMapping software version 4.1.1 (Wang *et al*., 2016) was used for linkage analysis using 83 polymorphic SSR markers for which map positions were taken from http://www.gramene.org. Grouping of all the 83 markers across 12 linkage groups (chromosomes) was done based on anchor information, for removal of unanchored markers and select anchor order, for ordering the markers for fitting on the best positions. And then outputting is done after which map show tool is selected to draw linkage map of SSRs. Linkage maps were constructed using linkage map construction tool in biparental populations (MAP) of ICIM software following Kosambi mapping function.

QTL analysis:

QTL IciMapping software version 4.1.1 is integrated software for linkage analysis and genetic mapping in biparental populations. 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 V.4.1 software (Wang *et al*., 2016). QTLs were detected by Single Marker Analysis (SMA) and Inclusive Composite Interval Mapping for the additive QTL (ICIM-ADD) methods in the present study.

Results

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. The number of seedlings survived after submergence treatment was counted and expressed as anaerobic germination percent. The mean anaerobic germination per cent recorded after the two weeks of submergence among the population of 188 lines ranged from 0–95% with overall mean of 47.51% whereas mean AG per cent of the contrasting parents SwarnaSub1 and AC39416A was 40% and 85% respectively indicating significant differences for the trait. For three weeks of submergence treatment, the mean anaerobic germination per cent recorded between 0 and 95%, with overall mean of 37.66% and the mean AG per cent 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)

Plant survival rate was calculated by counting the seedlings survived after one week of de-submergence. The average survival rate of Swarna Sub1 and AC39416A was 35% and 80% respectively after two weeks of submergence, whereas for $F_{2:3}$ mapping population it ranged from 0 to 95% with overall mean of 36.74%. The plant survival rate after three weeks of submergence for Swarna Sub1 was 17.6% whereas 72% for the donor AC39416A. The average survival rate of population was 15.5% which indicated clear cut variation and following normal distribution. (Fig. 2)

SSR marker based linkage map construction:

A total of 687 SSR markers covering all the 12 chromosomes of rice were used for parental polymorphism survey, of which 134 (19.42%) SSR markers were found to be polymorphic. But, only 83 (12.08%) SSR markers which have shown clear distinct polymorphic bands are further used for generating genotypic data for construction of the linkage map and QTL analysis. The level of polymorphism was ranged from 7.50–27.59% with an average of 13.25% for all the chromosomes. (Table 1). Linkage map provides information about the number of markers on each chromosome, marker order, name and position on the chromosome. We used integrated software called QTL IciMapping software version 4.1.1 (Wang *et al*., 2016) for linkage map construction using 83 SSR markers for which map positions were taken from http://www.gramene.org. The whole genome length of linkage map constructed using 83 SSR markers was 3600.8 cM. (Fig. 3) The map length of each chromosome varied with 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.

Table 1
Chromosome wise list of markers screened, number of polymorphic markers along with per cent of polymorphism.

Chromosome Number	Number of Markers			Polymorphism (%)
	Surveyed	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**

QTL mapping analysis:

QTL analysis was performed with 83 SSRs. In Single Marker Analysis, six markers *viz.*, RM 15554, RM 401, RM 5711, RM 21700, RM 28073 and RM 1584 were found to be linked with anaerobic germination trait which are located on chromosome 3, 4, 7, 7, 12 and 12 respectively. All these QTLs individually accounted for a total of 4.24–10.02% phenotypic variance (R^2). Highest phenotypic variance (10.02%) was recorded on chromosome 12 by *qAG12-1* with peak marker RM 1584. (Table 2)

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-3*	3	RM15554	2.97	4.24	-3.40	12.52
2	qAG-4 **(novel QTL)**	**4**	**RM401**	**3.31**	**4.72**	0.24	-16.04
3	*qAG-7-1*	7	RM5711	3.86	5.46	-1.36	14.48
4	*qAG-7-2*	7	RM21700	3.88	5.49	2.17	-15.29
5	*qAG-12-1*	12	RM1584	7.39	10.02	-7.03	-18.73
6	*qAG-12-2*	12	RM28073	4.29	6.03	6.23	13.47

A total of seven putative QTLs *viz*., *qAG2, qAG3, qAG7-1, qAG7-2, qAG9, qAG10* and *qAG12* were identified and mapped using Icimapping (ICIM) method with manual input threshold LOD score of 2.5. (Table 3) Out of 7 QTLs found, 2 QTLs were located on chromosome 7, 1 QTL on each of the chromosomes 2, 3, 9, 10 and 12. All these 7 QTLs explained phenotypic variance of about 37.47% collectively for AG trait, with their individual contributions ranging from 2.99–8.67% of phenotypic variation and LOD scores of 2.65 to 5.86. The phenotypic variance explained by *qAG2*, was highest (8.66%) followed by *qAG10* (8.60%) which were mapped on chromosome 2 and 10 respectively. Whereas, the highest LOD score (5.86) was shown by *qAG10*and *qAG-7-2*.

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

S.No	QTL	Chromosome	Flanking Markers	LOD	PVE (%)	Add	Dom
1	*qAG-2*	2	RM263 - RM6933	2.73	8.60	-15.01	13.94
2	*qAG-3*	3	RM15554 - RM15561	2.65	5.15	7.44	23.93
3	*qAG-7-1*	7	RM6697 - RM5711	5.05	3.53	-0.64	15.91
4	*qAG-7-2*	7	RM418 - RM21700	5.86	3.62	1.08	-16.27
5	*qAG-9*	9	RM23958 - RM1553	2.90	4.91	-12.95	2.20
6	qAG-10 **(novelQTL)**	**10**	**RM25735 - RM591**	**5.86**	**8.67**	**-2.39**	**23.76**
7	*qAG-12*	12	RM28759 - RM1584	5.47	2.99	-4.52	-14.20

Table 4
List of QTLs reported by earlier workers for anaerobic germination in rice (*Oryza sativa* L.).

S. No.	Parents	QTLS	Chromosome	Flanking Markers	Reference
1	IR64/ Kharsu 80A F2:3	qAG3	3	id3002377-id3004190	Baltazar et al. (2019)
		qAG7.1	7	id7000519-id7002260	
		qAG7.2	7	id7002427-id7003359	
		qAG7.3	7	id7003853-id7004429	
2	Tai Nguyen/ Anda F2:3	qAG1a	1	43902 -48,214	kim and Reinke *et al*. (2018)
		qAG1b	1	id1006871-327,392	
		qAG8	8	id8001299-8,107,849	
		qAG11	11	id11003544-1,194,923	
3	IR64-AG1, Ciherang-Sub1AG1 and Bg 358, BC_1F_1	qAG9-2	9	RM 24161	Sartaj *et al*. (2016)
4	48 rice genotypes	qAG2	2	RM 341	Reddy et al. (2015)
		qAG11	11	RM 206	
5	IR64/Nanhi F2:3	qAG2.1	2	id2001831-id2003094	Baltazar et al. (2014)
		qAG2.2	2	id2006621-id2007526	
		qAG3	3	id3007932-id3010875	
		qAG7	7	wd7000465-id7002784	
		qAG11	11	id11009201-id11010245	
6	IR42/Ma-Zhan	qAG2	2	RM263–RM5378	Septiningsih et al.

S. No.	Parents	QTLS	Chromosome	Flanking Markers	Reference
	Red F2:3	qAG5	5	RM5361	(2013b)
		qAG6	6	RM204–RM402	
		qAG7.1	7	RM3583–RM21427	
		qAG7.2	7	RM7338–RM346	
		qAG7.3	7	RM21803–RM234	
		qAG9	9	RM553–RM3808	
		qAG12	12	RM313–RM28766	
7	IR64/Khao Hlan BC2F2 population	qAG1-1	1	RM582-RM10713	Angaji et al. (2010)
		qAG1-2	1	RM11125-RM104	
		qAG2-1	2	RM327-RM6318	
		qAG3-1	3	RM7097-RM520	
		qAG7-1	7	RID12i-RM5606	
		qAG7-2	7	RM21868-RM172	
		qAG8-1	8	RM210-RM149	
		qAG9-1	9	RM8303-RM5526	
		qAG9-2	9	RM3769-RM105	

Discussion

In this study $F_{2:3}$ mapping population of Swarna Sub1/AC39416A was used for mapping QTLs for anaerobic germination. Phenotypic screening experiment revealed significant differences among the population for the traits studied. Among 188 $F_{2:3}$ population of Swarna Sub1/AC39416A studied 12 lines for two weeks treatment and five lines for three weeks treatment have shown AG percent on par with the donor parent AC39416A. Barik et al., (2019) reported similar trend of variation in anaerobic germination per cent. Doley et al., (2018) noticed that survival per cent was correlated positively with coleoptiles elongation which helps in obtaining oxygen from surroundings. Greater variability in germplasm lines screened for anaerobic germination was also described by Umarani et al., (2018) which is in accordance with the present results. Similar pattern of variation in survival per cent of population was described by Septiningsih et al., (2013b) in the $F_{2:3}$ population of IR 64 / Ma-Zhan Red and Baltazar et al. (2014) in $F_{2:3}$ population of IR 64 / Nanhi during screening for tolerance to anaerobic conditions during germination.

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 al., 2013b).

Polymorphism is a measure of genetic diversity and varies with the parental combinations. The contrasting parents Swarna Sub1 and AC39416A selected for development of mapping population were initially surveyed for polymorphism using SSR markers to identify polymorphic markers between them. Only 134 (19.42%) SSR markers were found to be polymorphic among 687 SSRs screened. But, only 83 SSR markers shown clear distinct polymorphic bands are further used for generating genotypic data for construction of the linkage map and QTL analysis. The polymorphism percentage of markers on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 was 18.92, 27.59, 17.19, 11.11, 7.50, 8.20, 12.90, 10.29, 7.81, 10.53, 12.24 and 14.52 per cent respectively. Among the 12 chromosomes surveyed, chromosome 3 recorded the maximum number of polymorphic markers (eleven) followed by chromosome 12 (nine) and chromosome 2 and 7 (eight). The polymorphism percentage was reported to be highest in chromosome 2 (27.59%) and lowest in chromosome 9 (7.58%). 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. Integrated software QTL IciMapping V.4.1 software (Wang *et al*., 2016) was used for linkage map construction.The whole genome length of linkage map constructed using 83 SSR markers was 3600.8 cM. Lal *et al*. (2018) Performed linkage mapping with 60 SSR primers using QTL ICIM software version 4.0 Software. Whereas Pramudyawardani et al. (2018) used QTL ICIMapping V3.2 software for construction of linkage map using 97 SNP and 7 SSR markers.

QTL analysis in the present research using the software was performed using two mapping methods namely Single Marker Analysis (SMA) and Inclusive Composite Interval Mapping for the additive QTL (ICIM-ADD). Single marker analysis (SMA) revealed that six markers were found to be linked with anaerobic germination in the $F_{2:3}$ population of Swarna Sub1 / AC39416A. LOD score of 2.96 and

phenotypic variance of 4.24% has been recorded with RM 15554 on chromosome 3, whereas RM 401, RM 5711, RM 21700, RM 28073 and RM 1584 have varying LOD scores 3.31, 3.85, 3.88, 4.28 and 7.39 and phenotypic variance of 4.71%, 5.45%, 5.48%, 6.03% and 10.01% respectively. A total of seven QTLs were identified and mapped using inclusive composite interval mapping (ICIM-ADD) method for anaerobic germination. *qAG2* was found to be flanked between RM 263 and RM 6933 on chromosome 2 with LOD score of 2.73 and phenotypic variance of 8.60%. *qAG3* identified on chromosome 3 was flanked between RM 15554 and RM 15561 and explaining 5.15% of phenotypic variation with LOD score of 2.65. The QTLs *qAG7-1, qAG7-2* on chromosome 7 were flanked between RM 6697 and RM 5711, RM 418 and RM 21700, have LOD scores of 5.05, 5.85 and phenotypic variation of 3.52% and 3.62% respectively. On chromosome 9, QTL *qAG9* was identified flanking between RM 23958 and RM 1553 with 2.89 and 4.90% of LOD score and phenotypic variation respectively. Whereas *qAG10* on chromosome 10 and *qAG12* on chromosome 12 were flanked between RM 25735 and RM 591, RM 28759 and RM 1584 with 5.86, 5.47 and 8.67%, 2.99% of LOD scores and phenotypic variation respectively.

Among the QTLs identified for AG in the present investigation *viz qAG2, qAG3, qAG4, qAG7-1, qAG7-2, qAG9, qAG10, qAG12-1* and *qAG12-2* using SMA and ICIM methods, *qAG2, qAG3, qAG7-1, qAG7-2, qAG9, qAG12-1* and *qAG12-2* were also reported in earlier studies. The novel QTLs identified in the present study are *qAG4* with LOD score of 3.31 and phenotypic variance of 4.72% and *qAG10* with LOD score of 5.86 and phenotypic variance of 8.67%. In both the SMA and ICIM methods the QTLs *viz qAG3, qAG7-1, qAG7-2* and *qAG12* are commonly identified. Among the QTLs identified the QTL *qAG12-1*, has shown 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.

Similar results of QTL analysis using QTL cartographer was reported by Angaji (2008) where *qAG2* located on chromosome 2, with LOD score of 4.44 and phenotypic variation of 14.5%. QTL *qAG12* on chromosome 12, with LOD of 5.71 and phenotypic variation of 29.24% by IM method was also found to linked with peak marker RM 28759 in the present investigation. QTLs reported for tolerance of flooding conditions during germination on chromosome 2, 3, 7 and 9, with highest LOD and phenotypic variation of 15.32 and 20.59 respectively has noted on chromosome 9 for QTL *qAG9-2* by Angaji et al. (2010) are in line with identified QTLs of present investigation. Similar QTLs were identified by Septiningsih et al. (2013b) on chromosome 2, 7 and 12, for submergence tolerance during germination. They reported that the QTL *qAG2* has peak marker RM 263 with 3.7 and 9.3% of LOD value and phenotypicvariance respectively, whereas in the present investigation it has recorded 2.73 and 8.60% of LOD score value and phenotypic variance respectively. Baltazar et al., (2014) also identified similar QTLs, *qAG2-2* on chromosome 2 having LOD value of 2.43 and phenotypic variation of 9.79% and *qAG7* on chromosome 7 with LOD score and phenotypic variation of 13.93 and 14.06% respectively. The QTL, *qAG3* flanked between RM 15554 and RM 15561 in the donor parent AC39416A was also identified in RILs developed with same donor in the earlier studies conducted at RARS, Maruteru during 2017-18 (Annual report, RARS, Maruteru, 2017) Similar QTLs on chromosome 3 and 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 for anaerobic germination after characterization of the mechanism underlying anaerobic germination and fine mapping.

Abbreviations

QTLs: Quantitative Trait Loci

SSR: Simple Sequence Repeats

AG: Anaerobic Germination

QTL Icim: QTL Inclusive Composite Interval Mapping

Declarations

Author Contribution Statement: Dr. N. Chamundeswari, planned the experiment and contributed to development of mapping population. Dr. N. Chamundeswari and Dr. N. Veronica helped in conducting the experiment. Dr. T. Haritha and Dr. Reddy Yamini helped in writing and reviewing manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest: The authors declared that there is no potential conflict of interest.

Research is not involving Human Participants and/or Animals

Informed consent: Nil

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Figures

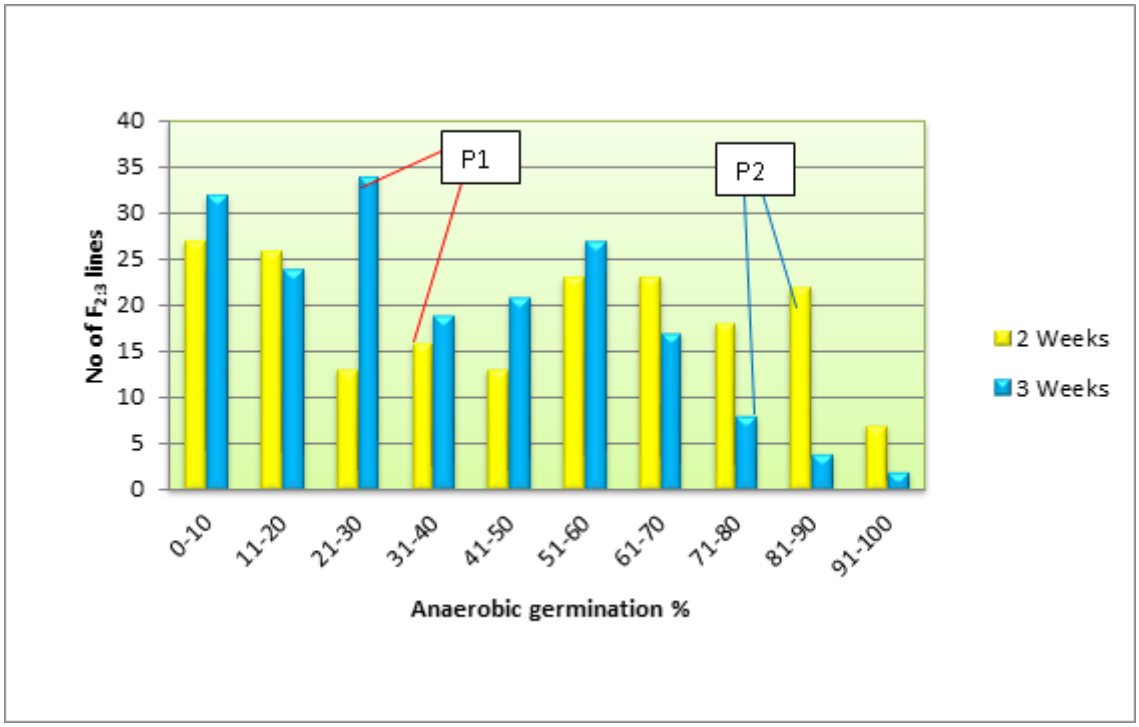

Figure 1

Frequency distribution for Anaerobic Germination (%) in F2:3 mapping population of Swarna Sub1 (P1) / AC39416A (P2).

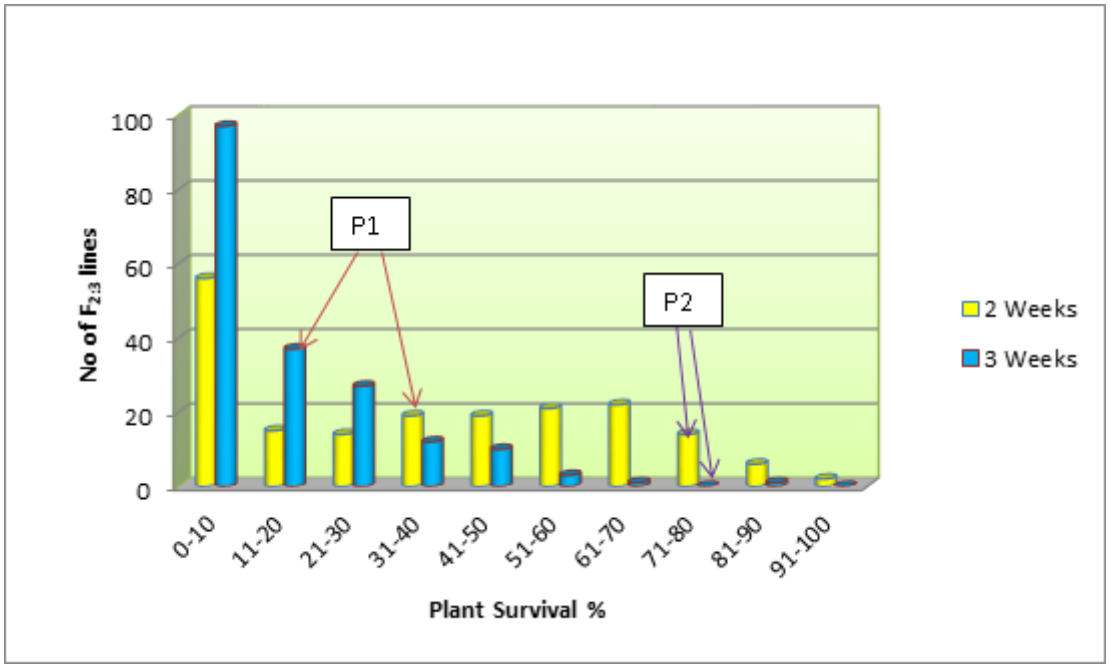

Figure 2

Frequency distribution for Plant Survival (%) in F2:3 mapping population of Swarna Sub1 (P1) / AC39416A (P2).

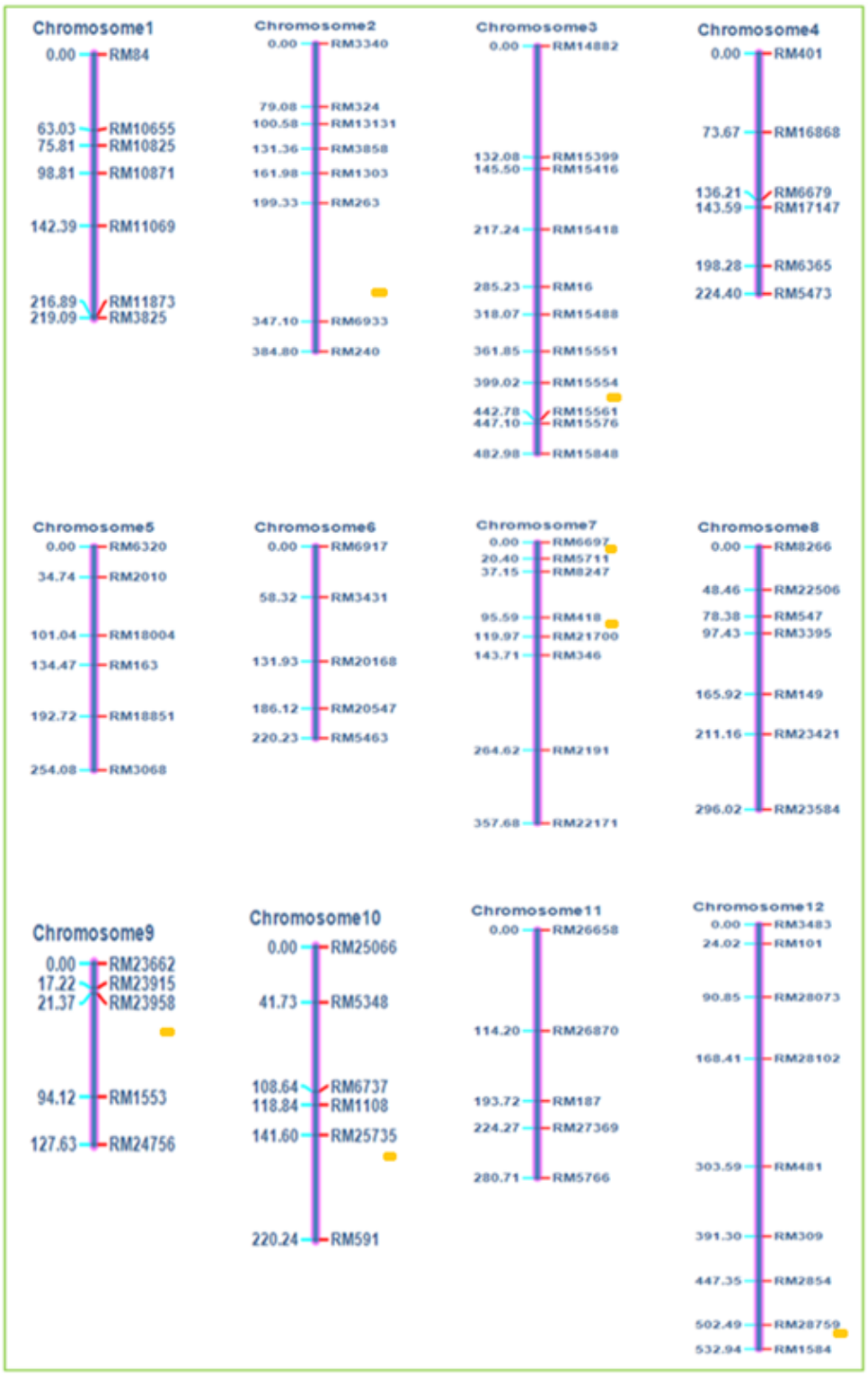

Figure 3

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

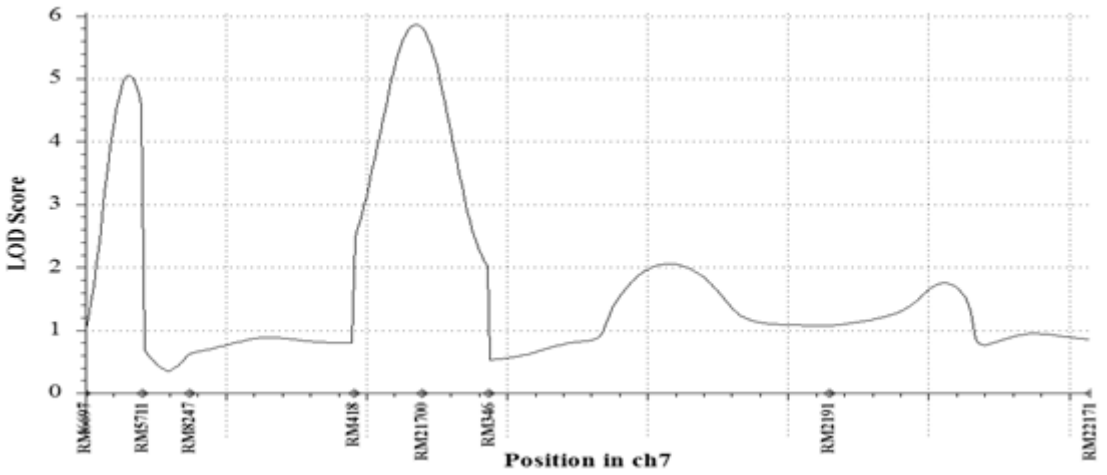

Figure 4

Graphs showing LOD scores for AG QTLS on Chromosome 7 (ICIM).

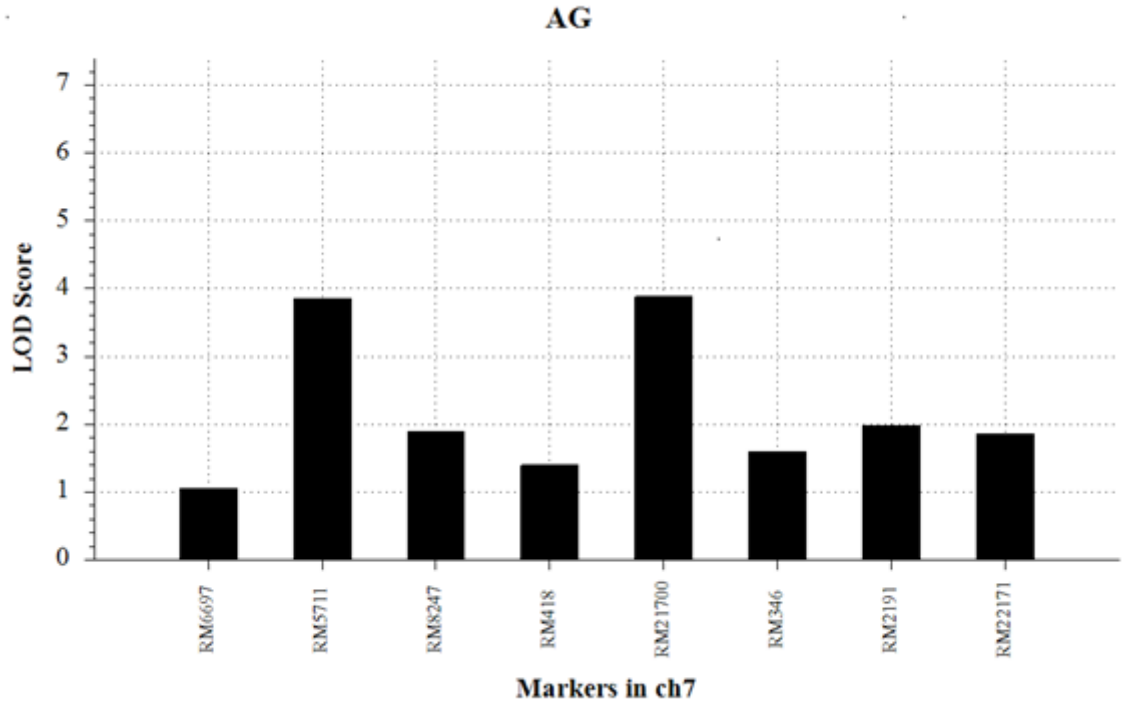

Figure 5

Depiction of LOD score for AG on Chromosome 7 (SMA).