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Research Article

Screening of Anaerobic Germination Rice Genotypes in Nepal

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Abstract

Poor germination has been the main obstacles to the widespread adoption of rainfed lowland rice production due to the saturated state of the soil, . In order to produce rice types appropriate for direct seeded culture, the current experiment was conducted to find rice with anaerobic germination tolerance. The screening techniques of the tray method were used to assess the anaerobic germination tolerance of 402 different rice genotypes. Under anaerobic conditions, the genotypes 10 genotypes namely, IR 129077:1-1-6-8-B, IR 129077:1-1-6-9-B, IR 129077:2-1-16-8-B, IR 129077:1-1-36-5-B, IR 129077:1-1-41-7-B, IR 129077:1-1-46-6-B, IR 129077:2-1-17-6-B, IR 129077:1-1-31-5-B, IR 129077:1-1-39-8-B, IR 129077:2-1-13-7-B, IR 129077:2-1-17-7-B and IR 129077:2-1-28-7-B recorded the highest germination percentages (> 80%). Under anaerobic germination, the recorded coleoptile length for all genotypes ranged from 0.50 to 10.6 cm. The best performing genotypes were chosen for genotyping using previously published SSR markers based on the screening results. On QTL analysis, anaerobic germination QTL (qAG) present on Chromosome number 11 and chromosome 12 for higher germination and QTL (qCL) on chromosome 12 for longer coleoptile.

Keywords: Anaerobic; Coleoptile; Germination; Genotypes; Rice

Introduction

Rice (*Oryza sativa* L.) is one of the oldest cultivated crops originated as early as 3000 BC and has been the main stay of millions of mankind since down of civilization. Rice is the staple food for 50 percent of the world population (IRRI, 2020). The global rice area and production during the year 2021 was 195.40 million hectares and 1001 million tons, respectively with the productivity of 4.78 t/ha (FAOSTAT, 2022). In Asia, where 90 % of the world rice is grown, it is estimated that rice supplies 50 to 80% of the daily calories (Papademetriou, 2013). The countries having rice as the major food source have an average population growth rate of 1.3%, but the rice yields are increasing by only 1.47% per year (Timsina *et al.*, 2023). These figures are quite important for the countries where agriculture has a prime role in national economy. Agriculture provides employment to 65 % of total population and contributes 20% to the Agricultural Gross Domestic Product (AGDP) and more than 7% to the total GDP of the Nepal economy. Rice has a prime role in Agriculture sector of Nepal as it contributes about 57% of the total food grain production (MoALD, 2022).

Rice cultivation on rainfed condition affected by a number of factors, including poor crop establishment or crop loss entirely as a result of badly leveled fields, severe rainfall, and poor drainage, which causes water to accumulate at various levels right after sowing (Angaji *et al*., 2010). Because rice is so sensitive to anaerobic germination, the risks of flooding right after sowing or during germination can cause anoxia or hypoxia, which hinders seedling growth. Additionally, it results in poor seedling establishment and anchorage issues. In fact, rice coleoptiles can develop longer during anaerobic germination than aerobic coleoptiles whereas the root and main leaf do not (Alpi and Beevers, 1983). Anoxia is a state in which the O_2 concentration is so low that nearly no ATP is created, whereas hypoxia is a state in which the O_2 concentration inhibits ATP generation in the mitochondria. The ability of rice seeds to germinate in anaerobic conditions has been attributed by many scientists to the enzyme -amylase, which can be produced even in anoxia and is thought to be responsible for starch degradation in the anoxic rice endosperm, ensuring a constant supply of energy to the germinating embryo in anaerobic conditions (Guglielminetti *et al*., 1995).

Tolerant AG varieties have evolved a variety of coping mechanisms, including anaerobic respiration to maintain energy production, carbohydrate catabolism in germination seeds, and maintenance of the cellular extensibility of the developing embryo (Ismail *et al*., 2009). The transition from aerobic to anaerobic respiration during germination is the primary adaptation to the constraints of the energy supply under anoxia or hypoxia. Alcohol, lactate, and alanine fermentation routes are used by anaerobic respiration to replenish the NAD+ needed for glycolysis (Kato-Noguchi, 1999). Protein synthesis is redirected to only generate those enzymes required for the digestion of carbohydrates. In the AG-tolerant and moderately-tolerant lines, an ATP generation rate of roughly 10% of the aerated conditions is maintained (Edwards *et al*., 2012). The nitrate that is taken up by AG-tolerant rice seeds is converted to ammonium, which can regulate the pH of the cytoplasm and vacuoles and maintain the pH, allowing for extended periods of anoxic life (Greenway *et al*., 2012). Same as Tolerant rice genotypes store relatively more soluble sugars in their endosperm and have greater ability to break down starch into soluble sugars during the germination than sensitive genotypes (Ismail *et al*., 2012).

Development of high yielding rice varieties that can withstand anaerobic condition during germination and early seedling growth is essential to alleviate the constraints associated with practicing of rainfed rice farming. Therefore, evaluating and revealing the various genetic, biochemical regulatory, and signaling mechanisms associated with tolerance to anaerobic germination would benefit from characterization and screening of different genotypes. Hence, to find the genotypes with AG tolerance by standardized procedures, the diversity among the rice germplasm be evaluated and screened.

Materials and Methods

This phenotypic study was conducted using two BC1F3:4 and BC1F3:5 QTL mapping populations generated by crossing IRRI, a parent with high AG potential IRRI 119, with high yielding but susceptible recurrent parents, Samba Mansuli. It was conducted during rainy season of 2019 and 2020, at Directorate of Agricultural Research, Tarahara, Sunsari, Nepal. In this study, 400 mapping population and 2 parent lines include as check were evaluated under anerobic germination with two replications. Before the conduct of the experiment all these genotypes were tested for viability and the genotypes showing 100 percent germination were further tested under anaerobic condition. Anaerobic germination experiment conducted at lab condition in plastic tray having 15cm height. Soil placed in 2 cm and 50 seeds placed and complete submergence with 10 cm water for 15 days without any disturbance. Observed the germination percentage and coleoptile length in 16th day as per the standard procedure for anerobic germination developed by IRRI. Progenies germinate more than 80% is considered to be tolerant to anaerobic germination because the standard germination percentage required for seed certification is 80% or above.These genotypes also tested in irrigated and drought condition in another experiment.

1k Rice Custom Amplicon (RiCA) SNP genotyping was used in this study along with SSR as additional genotype data (Arbelaez *et al*. 2019). Using KingFisher SBeadix kit (https://www.thermofisher.com), the DNA was extracted from the lyophilized leaves of the BC1F3 source plants and 3 samples each of the parents, IRRI 119 and Sambha Mahsuri. Sequencing was performed using the MiSeq Sequencing-by-Synthesis Technology System as specified by illumina® (https://www.illumina.com) using MiSeq™ System (SY-410-1003). Alignment to the Nipponbare rice genome MSU7 version (Kawahara *et al*., 2013) was done. Final SNP data were merged with SNP map information and encoded with the physical position and chromosome number of the SNP markers in a Hapmap format.

SSR genotyping was done using TPS-Quick and Dirty Method (Collard *et al*. 2005). A 100mg lyophilized leaf samples of for the parents for the polymorphic survey and then later for the 400 progeny lines of BC1F3 of the 400 in 2ml microfuge tube and then cut it finely and a 800ul of the TPS buffer was added to the labeled tubes. The samples were then incubated samples and extracted for DNA.

QTL IciMapping Version 4.1 (Meng *et al*. 2015) was used in building high-density genetic linkage maps for the 285 markers and the generated scores for each progeny lines in each marker for the purpose of mapping.

Results

Phenotypic Variation

One of the characteristics associated to the tolerance of rice genotypes to anerobic germination is capacity of the seed to germinate and grow well. Genotypes germinate more than 80% is considered to be tolerant to anaerobic germination because the standard germination percentage required for seed certification is 80% or above. During our investigation, 80 % germination was recorded by 10 genotypes namely, IR 129077:1-1-6-8-B, IR 129077:1-1-6-9-B, IR 129077:2-1-16-8-B, IR 129077:1-1-36-5-B, IR 129077:1-1-41-7-B, IR 129077:1-1-46-6-B, IR 129077:2-1-17-6-B, IR 129077:1-1-31-5-B, IR 129077:1-1-39-8-B, IR 129077:2-1-13-7-B, IR 129077:2-1-17-7-B and IR 129077:2-1-28-7-B whereas Parents IRRI 119 had 80% and 75% and Samba Mahsuri 10% and 15% in 2019 and 2020 respectively (Table 1). Additionally, when compared to sensitive genotypes, these genotypes showed greater tolerance to flooding during germination. They also appeared to have efficient and quick water uptake during seed imbibition's, better starch breakdown into simple sugar, and a faster starch depletion in their germinating seeds, which may be responsible for their higher germination percentage.

Within 12 hours of imbibition's in both tolerant and sensitive genotypes, anaerobic catabolism also significantly increased the activities of both pyruvate decarboxylase and alcohol dehydrogenase, but it gradually remained higher in the tolerant genotypes over time, indicating that anaerobic respiration is likely one of the key factors contributing to the superior performance of tolerant rice genotypes (Ismail *et al*., 2009).

At 16 days after germination, the coleoptile length of the seeds grew under 10 cm of water submergence, and there was significant variation among genotypes (Table 1). Of the total genotypes that were germinated for the study, 52 were just sprouted and 21 genotypes were germinated but did not survive. Even yet, excessive branch elongation that fails to reach the water's surface will also cause the plant to run out of all its stored carbohydrates, killing it off in the process (Sarkar *et al*., 2006). The lengths of the coleoptile that germinated ranged from 0.5 cm to 10.6 cm (Fig. 1 and 2). The linear regression coefficient found positive with germination percentage and coleoptile length in both years. The coleoptile length coefficient in the regression equation and R^2 is 0.652 and 0.9509 in 2019 and 0.229 and 0.9492 in 2020 (Figures 1 and 2). This coefficient represents the mean increase of coleoptile length in cm for every additional 10% germination and 95.09% and 94.42% of the variance in anerobic germination is explained by coleoptile length, which is commonly seen as a significant amount of the variance being explained.

Rice coleoptiles is one of the few plant organs that could be able to grow under absence or low oxygen conditions and they vary greatly for rates of elongation and alcoholic fermentation. Coleoptiles' rapid extension may make it easier for them to penetrate anaerobic soils and provide oxygen to the developing embryo. Shoot length of seedlings, including the coleoptiles, was employed as a marker to assess anaerobic germination resistance because it is difficult to identify the quantity of alcohol under anoxia (Ling *et al*., 2004).

Table 1: Coleoptile length (cm) and germination percentage of anaerobic germination tolerance rice genotypes during 2019 and 2020

Entry No	Genotypes name	2019		2020	
		Average Coleoptile length (cm)	Survival %	Average Coleoptile length (cm)	Survival %
28	IR 129077:1-1-6-8-B	7.01	100	10.5	100
29	IR 129077:1-1-6-9-B	8.37	100	10.6	98
292	IR 129077:2-1-16-8-B	7.74	100	10.3	90
153	IR 129077:1-1-36-5-B	8.13	90	9.6	80
175	IR 129077:1-1-41-7-B	7.96	90	9.5	80
194	IR 129077:1-1-46-6-B	7.09	90	9.7	80
294	IR 129077:2-1-17-6-B	6.58	90	9.1	80
133	IR 129077:1-1-31-5-B	7.7	80	9.2	80
168	IR 129077:1-1-39-8-B	7.6	80	9.1	80
279	IR 129077:2-1-13-7-B	8.03	80	9.5	80
295	IR 129077:2-1-17-7-B	1.94	80	8.4	80
339	IR 129077:2-1-28-7-B	9.04	80	10.5	80
402	IRRI 119	6.69	80	8.2	75
401	SAMBHA MAHSURI	3.2	10	1.2	15
	Mean	**4.15**	**25.25**	**5.65**	**25.23**
	H^2	**0.50**	**0.76**	**0.68**	**0.78**
	P value	**0.03**	**0.00**	**0.03**	**0.00**
	SED	**2.53**	**16.99**	**2.53**	**19.25**

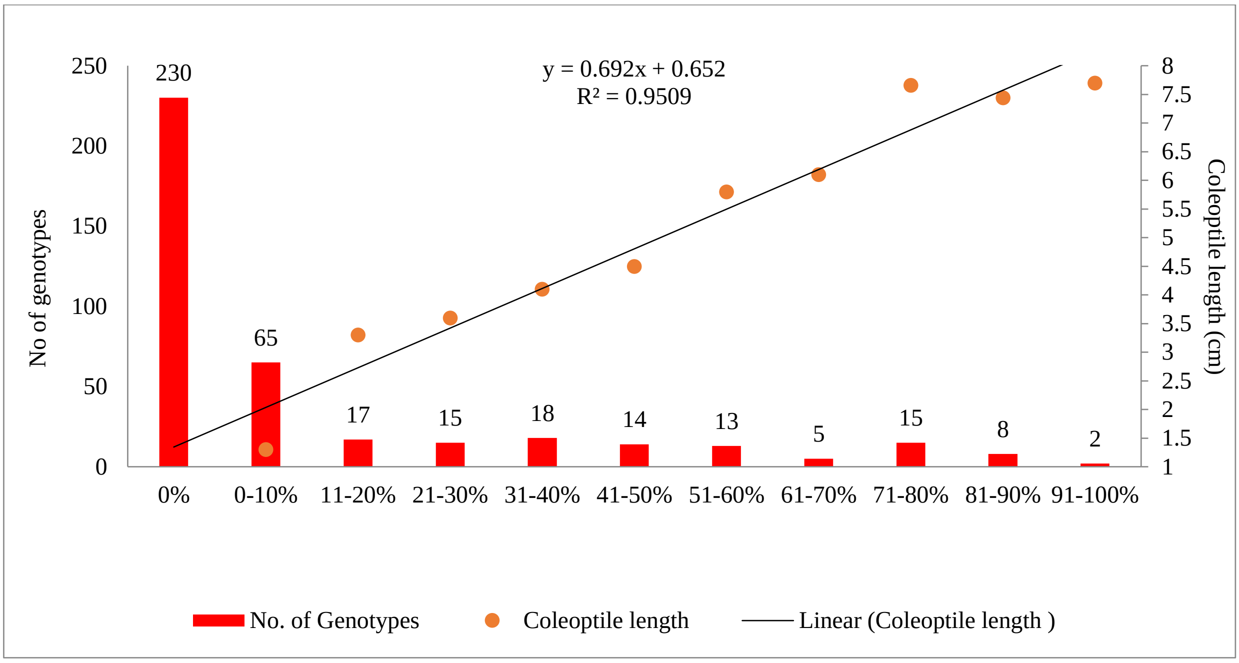

Fig 1: Frequency distribution rice genotypes for germination percentage (%) and Coleoptile length under anaerobic condition during 2019

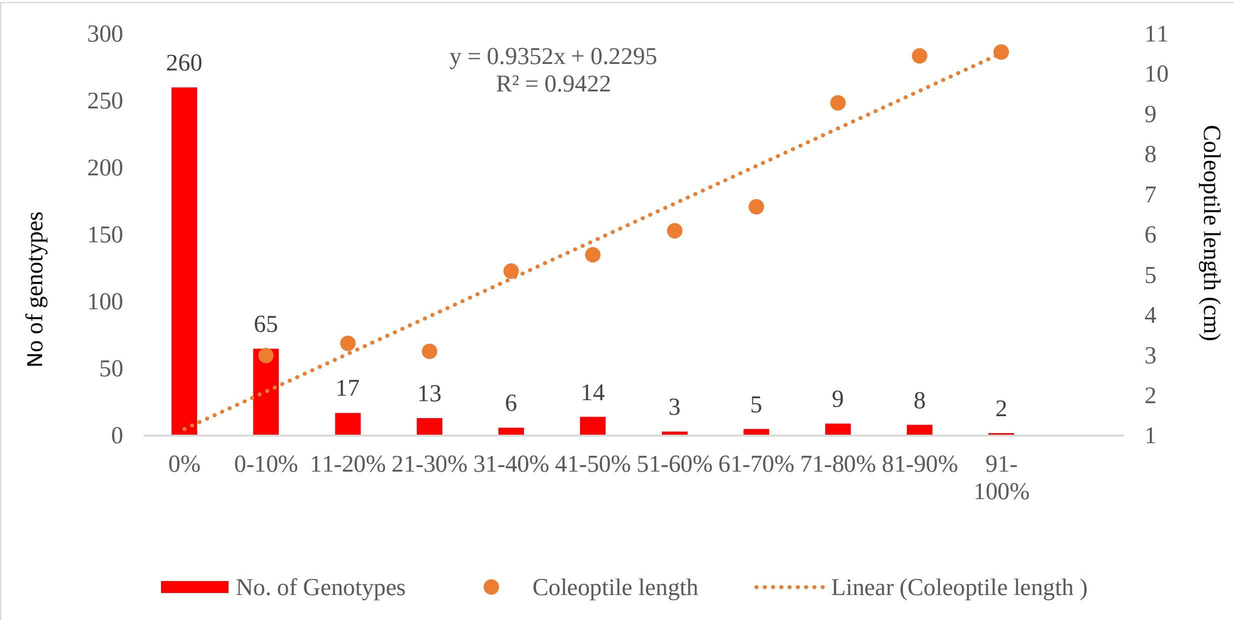

Fig 2: Frequency distribution rice genotypes for germination percentage (%) and Coleoptile length under anaerobic condition during 2020

Yield and Drought Stress Tolerance Capacity of Anaerobic Germinating Rice Genotypes

Among the tested genotypes, traits such as plant height, panicle length, 1000 grain weight, grain yield and harvest index were found significant among genotypes, whereas straw yield was non-significant (Table 2). All anaerobic germinating mapping genotypes had taller plant and log panicle than parent Samba Mahsuri. Likewise, genotypes IR 129077:2-1-17-7-B, IR 129077:1-1-46-6-B, IR 129077:2-1-13-7-B and IR 129077:2-1-17-6-B had higher spikelet fertility percent than both parent IRRI 119 and Samba Mahsuri. Similarly, genotype IR 129077:2-1-16-8-B had bold grain among the anaerobic germinating genotypes. Same as, genotypes IR 129077:1-1-46-6-B and IR 129077:2-1-13-7-B had higher grain yield in stress condition which is also greater than both parent in drought condition. Likewise. genotypes IR 129077:1-1-46-6-B and IR 129077:2-1-13-7-B had higher drought stress tolerance index. Higher STI values indicate genotypes had high grain production capacity in both under drought stress and non-stress conditions (Fernandez 1992). Selection based on STI will be genotypes with greater stress tolerance and yield potential will be selected.

QTL Analysis

The phenotypic data of 2019 was used to map QTLs linked to chr12:24088227 and chr11:2875883 for germination percent and chr12:350933 for coleoptile length. CIM analysis for higher germination showed the presence of a large QTL, qAG12.1 and qAG11 whose LOD peak was detected near chr12:24088227 and chr11:2875883. Likewise, CIM analysis for germination showed the presence of a large QTL, qAG12 whose LOD peak was detected near chr12:350933. The QTL explained 0.06 % and of the phenotypic variance for germination while it explained 0.10 % of phenotypic variance for coleoptile length (Fig 3, Table 3). The QTL had an additive effect of 3.84 and 1.81 for germination and coleoptile length respectively. The BIM analysis conducted to pinpoint the location of the QTL confirmed the presence of the QTL peak at 152 cM near chr12:24088227 and 26 cM near chr11:2875883 for higher germination and QTL peak at 24 cM near chr12:24088227 for longer coleoptile.

Table 2: Mean data of anaerobic germinating genotypes and parent in drought stress condition during 2019 and 2020

Entry No	Genotypes name	Plant height (cm)	Panicle length (cm)	spikelet fertility %	1000 Grain wt. (g)	Grain yield (kg/ha)	straw yield (kg/ha)	Harvest index	Stress Tolerance Index
28	IR 129077:1-1-6-8-B	96.94	23.01	71.66	22.43	1549.79	4159.55	0.27	0.35
29	IR 129077:1-1-6-9-B	89.43	22.19	59.35	21.64	1381.24	4284.08	0.24	0.25
133	IR 129077:1-1-31-5-B	78.09	20.12	75.00	16.29	1443.18	2500.00	0.37	0.46
153	IR 129077:1-1-36-5-B	90.17	22.27	59.86	20.19	1693.21	5332.72	0.24	0.51
168	IR 129077:1-1-39-8-B	83.24	18.96	61.60	21.25	1527.02	4580.05	0.25	0.32
175	IR 129077:1-1-41-7-B	89.56	24.33	48.85	22.80	1582.40	5941.63	0.21	0.37
194	IR 129077:1-1-46-6-B	88.16	22.57	68.74	20.72	1887.32	3632.05	0.34	0.60
279	IR 129077:2-1-13-7-B	89.82	22.14	69.28	18.92	2023.95	4940.11	0.29	0.64
292	IR 129077:2-1-16-8-B	89.26	20.98	46.66	23.65	1528.34	5251.63	0.22	0.39
294	IR 129077:2-1-17-6-B	80.81	23.80	66.02	18.68	1813.09	3456.58	0.32	0.38
295	IR 129077:2-1-17-7-B	79.91	23.40	72.62	15.38	1231.51	4411.58	0.22	0.31
339	IR 129077:2-1-28-7-B	92.82	21.59	58.03	21.26	1085.58	4837.72	0.19	0.19
401	SAMBHA MAHSURI	74.10	18.51	64.87	13.59	715.95	3783.03	0.17	0.16
402	IRRI 119	92.73	22.08	61.50	22.79	1650.88	5426.43	0.23	0.46
	Trait mean	**89.28**	**21.86**	**61.21**	**20.93**	**1423.20**	**4691.47**	**0.24**	**0.36**
	H2	**0.55**	**0.30**	**0.39**	**0.49**	**0.37**	**0.57**	**0.51**	**0.43**
	P value	**0.10**	**0.05**	**0.04**	**0.41**	**0.02**	**0.12**	**0.18**	**0.01**
	SED	**7.63**	**1.95**	**14.46**	**4.82**	**643.49**	**1553.87**	**0.07**	**0.09**

Table 3: QTLs identified for anaerobic germination and coleoptile length

Trait	QTL	Chr	Marker	Position (BIM)	LOD	A	R^2
Germination	qAG12.1	12	chr12:24088227	152	5.70	-11.93	0.06
	qAG11	11	chr11:2875883	26	3.35	3.84	0.04
Coleoptile length	qCL12	12	chr12:350933	24	6.85	1.81	0.10

Note: Chr: chromosome, A: additive effect, R^2: Phenotypic variance explained.

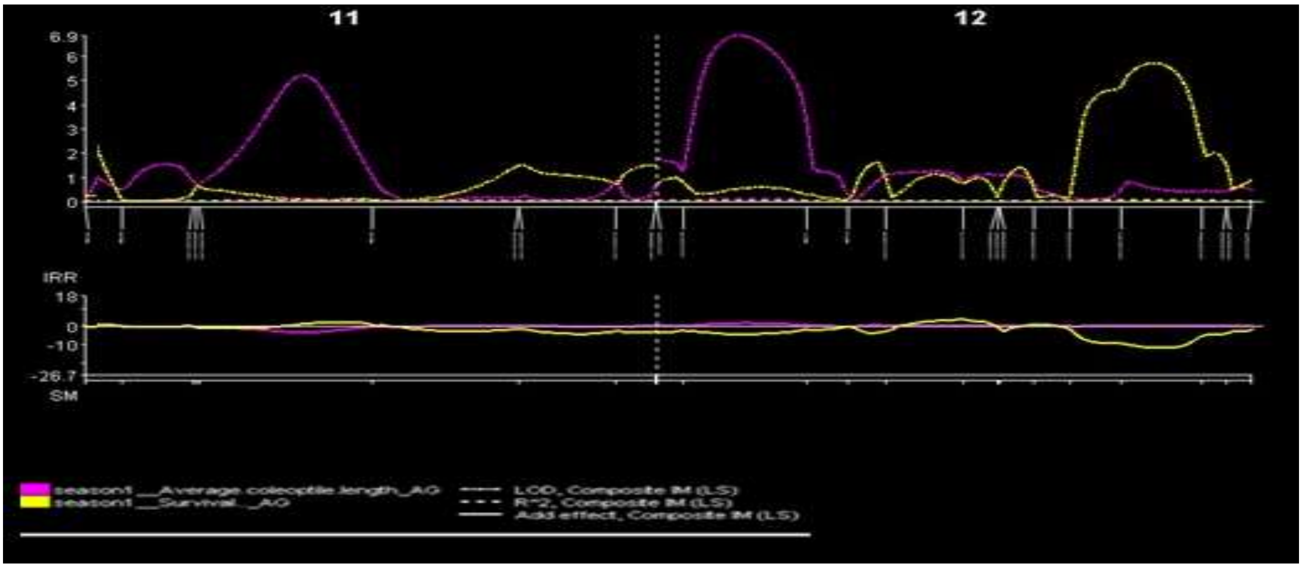

Fig. 3: Distribution of QTLs for anaerobic germination and coleoptile length identified in the interspecific rice mapping population

Discussion

High germination rates, quick shoot and root elongation, and other significant features closely connected to seedling vigor were shown to be crucial for the best seedling establishment under submerged conditions. These findings concur with prior research by Barik *et al*. (2019). The term "AG" refers to a plant's capacity to effectively germinate under water and grow roots and branches. For various underlying features that are important for optimal germination under water, this innate phenomenon serves as the primary attribute to identify genetic diversity among genotypes. Because it reflects rapid coleoptile elongation, which is frequently used to study the genetics of anaerobic germination, coleoptile length is, along with AG, the second most crucial trait. In all indices of the mapping populations, it is evident that the progenies' ranges of variation were greater than their parents', showing transgressive segregation and a contribution from both parents. Similar results for AG by Septiningsih *et al*. (2013), Hsu, and Tung (2015) for coleoptile elongation features were also reported. Two highly significant QTLs for anaerobic germination were found on chromosomes 11 and 12 (qAG11 and qAG12), according to the findings of the QTL analysis (Fig. 3). The biggest impact QTL for AG was qAG12.1, which was followed by qAG11. On chromosome 12 (qCL12), one highly important QTL for coleoptile length was found.

Conclusion

Enhancing rice's ability to tolerate submersion during germination would make it easier to employ in lowland rice ecosystems, which provides farmers with several advantages through efficient water management. The current study represents a preliminary step in identifying the morphological, genetic, and physiological factors underpinning the rapid development of rice seedlings in anaerobic environments. When choosing rice genotypes suited for anaerobic conditions, high germination percentage and long seedling length could be targeted. These are essential for a high seedling establishment rate. Therefore, high germination percentage and high seed vigour index of 10 tolerant genotypes viz., IR 129077:1-1-6-8-B, IR 129077:1-1-6-9-B, IR 129077:2-1-16-8-B, IR 129077:1-1-36-5-B, IR 129077:1-1-41-7-B, IR 129077:1-1-46-6-B, IR 129077:2-1-17-6-B, IR 129077:1-1-31-5-B, IR 129077:1-1-39-8-B, IR 129077:2-1-13-7-B, IR 129077:2-1-17-7-B and IR 129077:2-1-28-7-B are important criteria for breeding cultivars with strong early growth under anoxia or hypoxia. Likewise, in these genotypes, anaerobic germination QTL (qAG) present on Chromosome number 11 and chromosome 12 for higher germination and QTL (qCL) on chromosome 12 for longer coleoptile. Thus, to increase their establishment rates, these traits could be bred into elite cultivars through various breeding programs.

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Authors’ Contribution

All authors contributed equally at all stages of research work and manuscript preparation. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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