

# Identification of QTLs for tolerance to hypoxia during germination in rice

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**Abstract** Direct seeding of rice as a method of crop establishment is increasingly being adopted by farmers as a means of saving labor and reducing costs. However, the method often results in a poor environment for germination as excessive water levels after seeding can cause poor seedling establishment and a concomitant reduction in yield potential, especially in submergence-prone areas. In this study, we discovered QTLs associated with tolerance of anaerobic germination (AG) in new genetic accessions using genotypic data derived from the Illumina 6K SNP chip. The mapping population developed for QTL analysis comprised 285  $F_{2:3}$  plants derived from a cross between Tai Nguyen and Anda. In order to evaluate

AG tolerance within the mapping population, phenotyping was carried out under anaerobic conditions for 21 days. Three QTLs associated with AG tolerance were identified in the population, *qAG1a* and *qAG1b* on chromosome 1 and *qAG8* on chromosome 8 using composite interval mapping (CIM). The percentage of variance explained by these QTLs ranged from 5.49 to 14.14%. The lines with three QTLs (*qAG1b* + *qAG1a* + *qAG8*) demonstrated an approximate 50% survival rate under anaerobic conditions, while lines with two QTLs including *qAG1b* demonstrated survival rates of 36 and 32% after the treatment, respectively. The QTLs detected in this study may be used to improve AG tolerance during germination and may be combined with other QTLs for anaerobic germination to enhance adaptation to direct seeding and to broaden the understanding of the genetic control of tolerance of germination under anaerobic conditions.

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## Introduction

Rice is able to grow under many environmental conditions across a range of ecologies, including under water-limited conditions under the upland component

of the toposequence, in drought-prone rainfed lowland conditions, under optimal conditions in the irrigated lowlands, and under exposure to deep flooding and submergence in the rainfed lowlands. Traditional plant establishment in most rice cultivation areas include transplanting and direct-seeding methods. Rising labor costs and the need to intensify rice production through double and triple cropping have provided clear economic incentives for a switch from transplanting to direct seeding, accompanied by significant improvement of direct-seeding methods (Pandey and Velasco 2002). In tropical areas, direct seeded rice is most often planted by broadcasting germinated seeds onto the puddled soil surface after drainage of standing water or by broadcasting into standing water followed by drainage (Yamauchi et al. 1993). In the case of submergence-prone areas, however, this method often results in waterlogging and flooding problems.

Flooding is one of the most common environmental stresses that affects plant growth and development and often places major limits on plant productivity. In particular, when flooding comes soon after seeding, the damage may result in poor crop establishment or even a total loss of the crop stand and this is intensified by improperly leveled fields and the combination of heavy rainfall and poor drainage after seeding (Septiningsih et al. 2013). Enhanced tolerance of flooding during germination and early seedling growth could therefore help improve crop establishment and promote more widespread adoption of direct seeding (Ismail et al. 2009).

In general, starchy seeds (rice, wheat, sorghum, maize, etc.) with carbohydrate reserves have been shown to have greater tolerance of anaerobiosis during germination than nonstarchy seeds (lettuce, sunflower, soybean, flax, etc.) with fatty acid reserves. Although there is considerable variation in the ability to germinate under anaerobic conditions, seeds utilizing starch as an energy source are able to maintain a high energy metabolism under oxygen deficiency in comparison with those reliant on fatty acid reserves (Al-Ani et al. 1985; Raymond et al. 1985; Magneschi and Perata 2009). Among the cereals, after establishment, rice is well adapted to waterlogged and submerged soil because of its well-developed aerenchyma system that facilitates aeration of the roots and the rhizosphere (Ella and Setter 1999; Jackson and Ram 2003; Setter and Waters 2003; Jiang et al. 2006; Ismail et al. 2009).

While rice seeds can germinate and the coleoptile can extend under low oxygen, there remains a need to breed for better seedling vigor and higher tolerance for anaerobic conditions during germination to improve the sustainability of direct seeding for rice crop establishment. However, donors showing tolerance for anaerobic conditions during germination are limited or unavailable and little is known of the physiological and genetic basis of tolerance for anaerobic germination (Biswas and Yamauchi 1997; Yamauchi et al. 2000). Consequently, Angaji et al. (2010) screened over 8000 gene bank accessions from IRRI's Genetic Resources Center, including breeding lines, to find genotypes with tolerance for flooding during germination, and reported a only few tolerant lines. Jiang et al. (2004) also tested germinability under anoxia using 359 rice varieties submerged under 0.2 m of water. Their results indicated that the most varieties had relatively low tolerance in general, however *japonica* rice was shown to be more tolerant than indica rice. In addition, constraints such as the poorly adapted genetic backgrounds of the limited number of donors, low heritability of the trait and lack of well-defined and repeatable screening methods still hinder progress in gaining knowledge and improvement of the trait. This is in spite of two decades of attempts to improve the trait.

The identification of QTLs linked to tolerance of anaerobic germination (AG) would provide breeders with the possibility of improving this trait, in addition to providing insights into the underpinning genetic mechanism. Jiang et al. (2004) reported five QTLs for seed anoxia germinability in 81 RILs derived from a Kinmaze/DV85 cross and the variation explained by each QTL ranged from 10.5 to 19.6% on chromosomes 1, 2, 5 and 7, respectively. The study also reported three pairs of epistatic loci located on chromosomes 2, 3, 5 and 11 with significant effects ranging from 16.7 to 48.8%. A later study reported two QTLs for anoxia germinability located on chromosomes 5 and 11 explaining 15.51 and 10.99% of the phenotypic variation respectively (Jiang et al. 2006). Angaji et al. (2010) reported five putative QTLs, viz, *qAG* 1-2, *qAG* 3-1, *qAG* 7-2, *qAG* 9-1 and *qAG* 9-2, on chromosomes 1, 3, 7 and 9, respectively, explaining 17.9–33.5% of the phenotypic variation. This study was based on the survival rate of BC<sub>2</sub>F<sub>2</sub> lines derived from a Khao Hlan On/IR64 cross germinating under 10 cm of water. Septiningsih et al. (2013) identified

six significant QTLs on chromosomes 2, 5, 6 and 7, which were contributed by Ma-Zhan Red, a highly AG-tolerant landrace from China. In particular, the largest QTL was on chromosome 7, having a LOD score of 14.5 and an  $R^2$  of 31.7%; this was further confirmed using a  $BC_2F_3$  population. Baltazar et al. (2014) detected four QTLs related to AG tolerance using a 384-plex SNP *Indica/Indica* set on the Illumina BeadXpress reader. A major QTL, *qAG7*, for AG derived from the cultivar Nanhi, an upland landrace from India, was detected on chromosome 7 with an LOD of 13.93 and explaining 22.3% of the phenotypic variance. Given that the relevance of the trait for improved adaptation to direct seeding, it is imperative to find new sources of tolerance and to conduct genetic studies and QTL identification to facilitate practical deployment in rice breeding programs.

In this study, the identification of AG QTLs from a new genetic source using polymorphic markers derived from the Illumina 6K SNP chip will provide rice breeders with options for marker-assisted selection and gene pyramiding to improve adaptation and crop establishment under directed-seeded conditions.

## Materials and methods

### Plant materials and mapping population

We conducted QTL analysis for AG tolerance using an  $F_{2:3}$  population. It was derived from a cross between Tai Nguyen (TN), an AG-tolerant indica landrace from Vietnam and Anda, a Korean Tongil-type (*indica/japonica*), AG-susceptible rice under well-controlled submerged, hypoxic conditions. The  $F_2$  seeds were obtained from  $F_1$  plants, which had been confirmed as  $F_1$ 's by purity inspection using PCR. The seeds were grown in the field to produce the  $F_2$  plants for genotyping and  $F_{2:3}$  populations for phenotyping. A total of 285  $F_{2:3}$  families were used for QTL analysis for AG tolerance.

### Evaluation of AG tolerance

To evaluate AG tolerance of the population, the screening was conducted following Septiningsih et al. (2013). We used seedling boxes (dimensions 72 × 28 × 4 cm) with 72 wells (3 × 3 cm) in each

box. Fifteen dry seeds from each line were sown, a single seed per well, in the seedling box pre-filled with 3.5 cm of fine soil. After sowing, the seeds were covered with an additional 1 cm of soil with high bulk density to prevent seed floating as the boxes were submerged, or after submergence. The parent lines, TN and Anda, were each sown in six wells per each seedling box, respectively. The  $F_{2:3}$  lines and parents were completely randomized within each box with two replications. To ensure consistent hypoxic screening conditions, nitrogen gas was bubbled through a water reservoir until the dissolved oxygen content of the water was 4.0 ppm or lower before flooding the seedling boxes. The dissolved oxygen value was monitored for the screening period using a portable dissolved oxygen measuring instrument. After sowing, the seedling boxes were submerged carefully in water tanks filled such that 10 cm of water were maintained above the soil surface in the boxes. The water tanks were sealed with adhesive transparent vinyl to maintain hypoxic conditions and to maintain the water depth. The vinyl shield was removed about 14 days after sowing (DAS) when the coleoptiles start to emerge from the covered soil. Evaluation of AG tolerance of each line was measured using the seedling survival rate at 21 DAS.

### Genotyping

Leaf samples from 285  $F_{2:3}$  individuals were collected to extract genomic DNA using PlantTrak Hx<sup>TM</sup>. DNA extraction was performed using the automated magnetic bead system, oKtopure. The final concentration of the genomic DNA sample was normalized to 40–60 ng/μL which was suitable for SNP genotyping. For high density genotyping of the tested lines, the Infinium platform comprised several subsets of markers from the BeadXpress 384-SNP sets and these were used to detect SNP alleles. The 384-plex genotyping on the BeadXpress platform, with the oligo pool assay (OPA) customized for the *Indica-Japonica* SNP chip (Illumina OPA ID: GS0011862-OPA), is a robust and efficient method for marker genotyping in rice (Thomson et al. 2012). The Infinium 6K BeadChip used for the genotyping contains about 6000 bead types and these results were used for the QTL mapping. The fluorescence intensity of the beadchip was scanned using the Illumina BeadXpress Reader (Genotyping Services Lab, IRRI).

## Linkage mapping

Of the complete set of SNP data points generated by the Infinium 6K chip, only those SNPs showing polymorphism between the parents were selected for analysis of the mapping population. The selected SNPs were used to construct a genetic linkage map using linkage mapping software QTL IciMapping version 4.0 (Meng et al. 2015). To remove SNPs presenting segregation distortion, the observed segregation of each SNP was compared against the expected segregation ratio (1:2:1) using the MAP functionality on the software. A Chi square test was used to determine the degree of marker distortion based on the value of 9.04 ( $P < 0.01$ ). The Kosambi mapping function was used to transfer recombination frequency to mapping distance in linkage map construction and “By LOD” and “By Input” options were applied, respectively, for grouping and ordering of all markers.

## QTL analysis

The mean survival rate across both replications of each line was used to detect QTLs related to the phenotypic measure of AG tolerance. Missing phenotypic data values were excluded from QTL mapping. QTL analysis was conducted using the phenotypic data and SNP genotypic data with the conventional Interval Mapping for additive QTL (IM-ADD) and the inclusive composite interval mapping for additive QTL (ICIM-ADD) (Zhang et al. 2008). Significant threshold values of LOD scores for QTL detection were determined using permutation tests (Churchill and Doerge 1994) with 1000 replicates. The Type I error to determine the LOD threshold from permutation tests was defined at  $P \leq 0.05$ .

## Results

### Construction of the genetic linkage map

Of 5274 possible SNP markers, 1405 were polymorphic between the parents and were used for constructing the linkage map. The polymorphism rate ranged between 19.9 and 33.9% with an average of 26.6% for whole chromosomes. To select proper SNP markers for the linkage mapping, a test of segregation

distortion was performed using an  $\chi^2$ -test goodness of fit for the expected allelic frequency of 1:2:1 confirmed at  $P < 0.01$ . The results indicated that 62% of the markers (872 SNPs) could be used for linkage mapping out of complete set of 1405 polymorphic markers. Using these markers, the high-density genetic linkage map constructed for this study had a mean of 67 markers harbored on each chromosome (Fig. S1), a total length of 1448.45 cM and an average interval length of 1.7 cM. However, 236 markers detected to be overlapping, were stacked in the same position with 0 cM interval. Ultimately, about 10% of the 5274 possible SNPs were able to be anchored on the linkage map using the mapping population from a cross between *japonica* and indica lines. On the resulting linkage map, there were two long gaps detected on chromosomes 9 and 10. One region flanked by two markers, id9003562 and 9712393, had a genetic distance of 26.6 cM on chromosome 9 and the other had a genetic distance of 31.7 cM between id10534927 and 10703329 on chromosome 10 (Fig. S1). The gaps were produced by the absence of markers in the regions, owing to SNPs which were not selected as a result of a lack of polymorphism and/or segregation distortion of markers (Table 1).

### AG phenotyping

Two hundred and eighty-five  $F_{2,3}$  families were able to be harvested from 300  $F_2$  plants and these were used for AG phenotyping. The seedling vigor and germination rate of the mapping population and parents were previously checked under normal germination conditions before conducting the phenotyping test for tolerance for AG. Both parents, TN and Anda, had a germination rate of more than 95% and all test lines also normally germinated with a range of germination rates from 85 to 100%. Therefore, families could be used as reliable plant materials for QTL analysis without any exception. The average survival rates of the contrasting parents (TN and Anda) under submerged hypoxic conditions were 54 and 5% at 21 days after treatment (DAT), respectively (Fig. 1). The range of survival rates of  $F_{2,3}$  families ranged from 0 to 75% and the distribution approximated a normal distribution suggesting that the trait is quantitatively controlled by minor genes (Fig. 1). The distribution pattern of seedling survival was slightly biased

**Table 1** Summary of the linkage map for QTL analysis

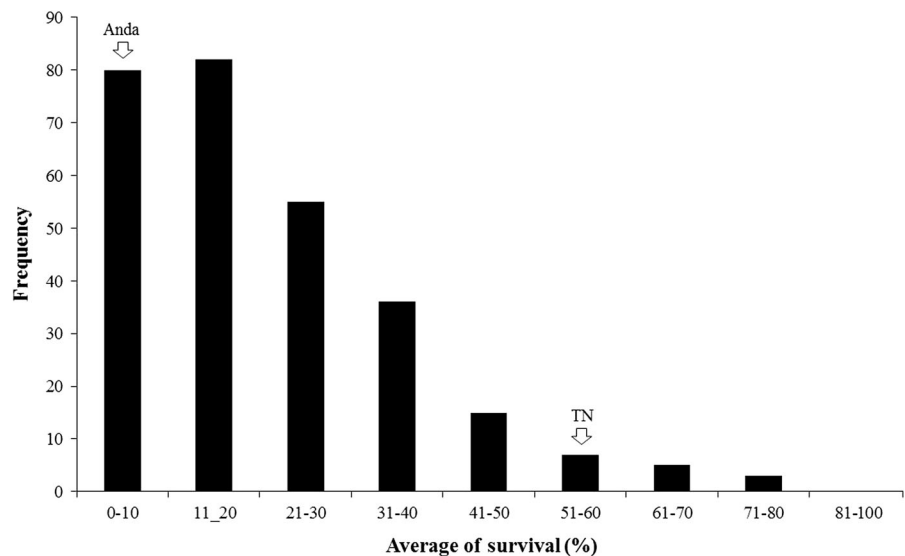
Chromosome	Used markers	Polymorphic markers	Polymorphism (%)	Anchored markers <sup>a</sup>	Length (cM) <sup>b</sup>	AMI (cM)
1	603	139	23.0	85	175.96	2.1
2	508	101	19.9	81	163.32	2.0
3	541	139	25.7	91	152.27	1.7
4	493	125	25.4	73	131.60	1.8
5	428	121	28.2	90	125.36	1.4
6	430	135	31.4	97	132.03	1.4
7	420	101	24.0	66	89.68	1.4
8	389	132	33.9	97	119.17	1.2
9	340	68	20.0	34	88.17	2.6
10	316	81	25.6	48	83.15	1.7
11	410	129	31.4	64	71.39	1.1
12	396	134	33.8	72	116.35	1.6
Total (average)	5274	1405	26.6	872 (67.3)	1448.45	1.7

AMI average marker interval

<sup>a</sup>Number of markers selected by Chi square test

<sup>b</sup>Length: chromosomal length

**Fig. 1** Distribution of survival rate of the 285 F<sub>2:3</sub> lines derived from a cross between TN and Anda under submerged hypoxic conditions. The graph shows the phenotypic distribution of AG tolerance of the tested lines along with the average survival rate of both parents, TN and Anda

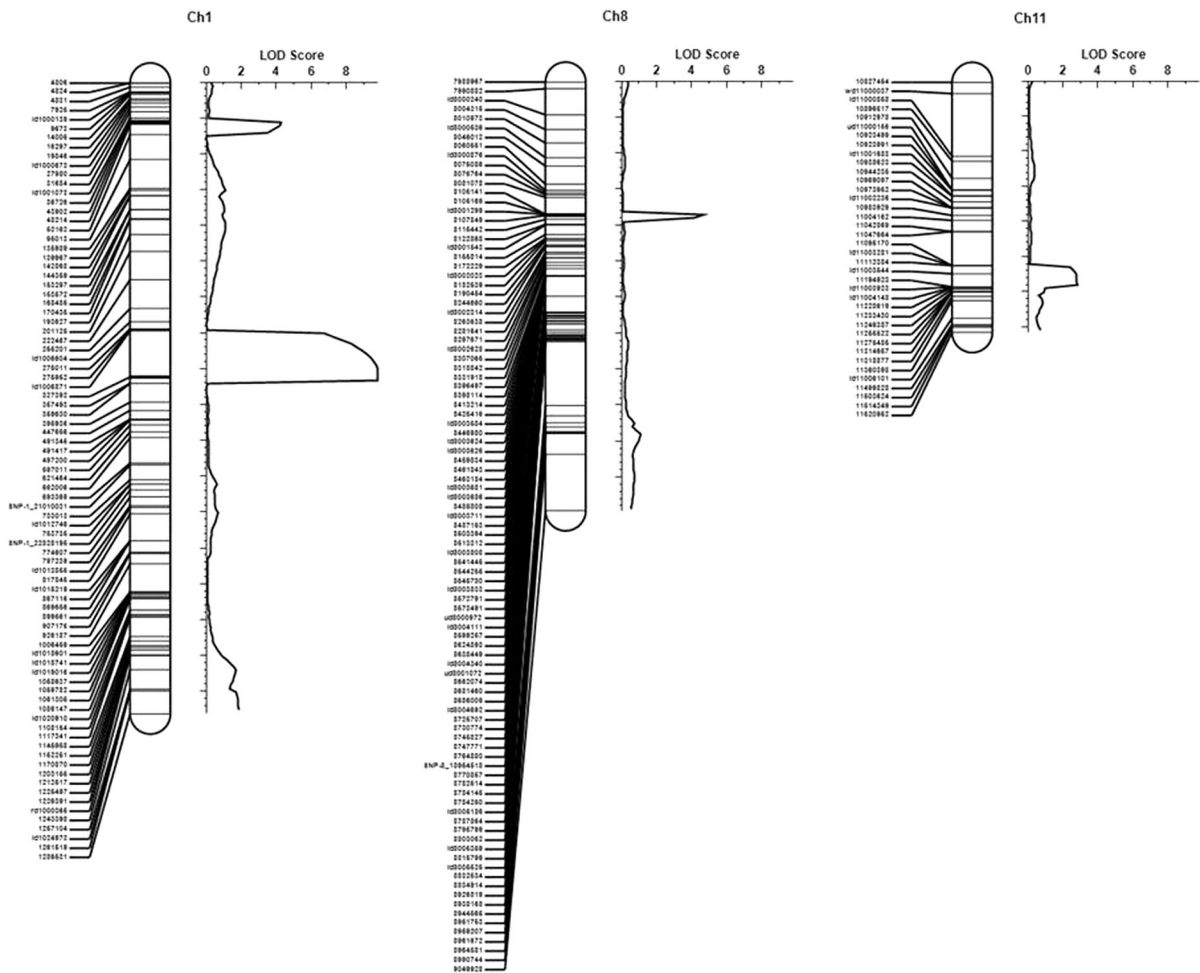


towards susceptibility and showed positive skewness (1.20) and leptokurtosis (1.3).

### QTL analysis

Phenotypic data for the QTL analysis were derived from assaying the 285 F<sub>2:3</sub> families for tolerance for AG conditions using submerged hypoxic conditions

from sowing until 21 DAS. Following the treatment period, the plants exhibited a continuous distribution for AG tolerance consistent with quantitative inheritance (Fig. 2). From the phenotypic data, three QTLs were identified and mapped at an empirical threshold of LOD 3.82 declared by 1000 permutations at  $P \leq 0.05$ . All QTLs showed a positive effect of the alleles derived from the AG-tolerant landrace, TN



**Fig. 2** Indication of detected QTLs in the linkage map. Three identified QTLs were positioned in the whole genome. Dotted line is the threshold ratio 3.8, which is calculated by the permutation test with 1000 repetitions

**Table 2** Chromosomal location of the QTLs for AG tolerance detected in an F<sub>2</sub> population derived from a cross TN/Anda

QTL	Chromo-some	Position <sup>a</sup>	Left marker	Right marker	LOD <sup>b</sup>	PVE <sup>b</sup> (%)	Add <sup>d</sup>	Dom <sup>e</sup>
<i>qAG1a</i>	1	10.0	43902	48,214	4.29	5.49	0.54	- 0.06
<i>qAG1b</i>	1	78.0	id1006871	327,392	10.05	14.14	0.86	- 0.14
<i>qAG8</i>	8	37.0	id8001299	8,107,849	4.63	5.90	0.55	0.06
<i>qAG11</i>	11	58.0	id11003544	11,194,923	2.82	3.54	0.38	0.33

<sup>a</sup>Marker position in cM on the chromosome

<sup>b</sup>LOD experiment-wise  $P = 0.05$  was equivalent to critical LOD score threshold of 2.5

<sup>c</sup>Percent phenotypic variation explained by the QTL

<sup>d</sup>Positive values indicate additive effects from TN

<sup>e</sup>Estimated dominance effect of the QTL

(Table 2). The percentage of phenotypic variation ( $R^2$ ) explained for each QTL ranged from 5.45 to 14.14%. The QTL *qAG1b* explained 14.14% of the total

phenotypic variation in the population and mapped within a 12.5-cM interval proximally flanked by the markers, id10006871 and 327392. On the same

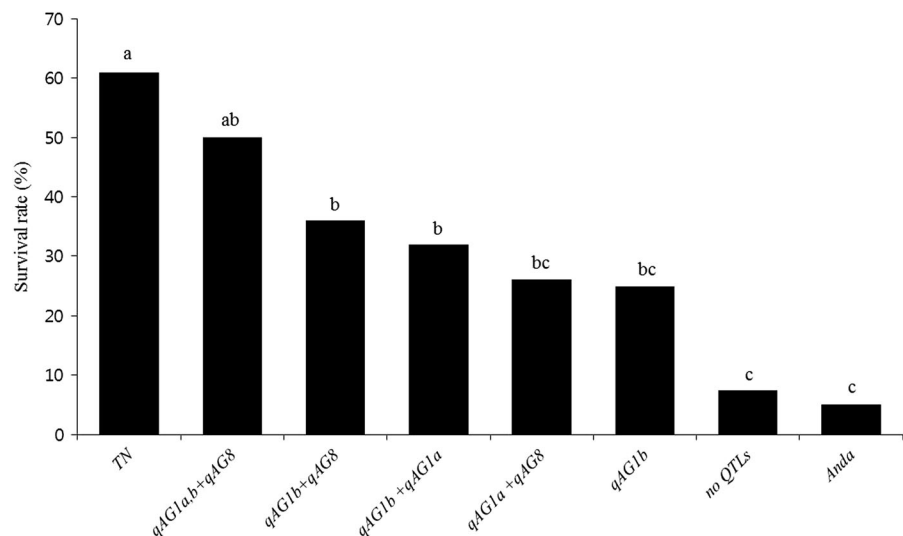


chromosome QTL, *qAG1a* was identified by ICIM analysis, which had a LOD value of 4.25 and an  $R^2$  of 5.49% within two flanking markers, 43,902 and 48,214. A additional QTL, *qAG8*, was detected on chromosome 8, which explained the phenotypic variation of 5.90% between the id8001299 and 8107849 markers. Another two QTLs (*qAG1c* and *qAG11*) detected by CIM in ICIM had LOD scores below the permutation threshold.

### Effect of detected QTLs

To confirm QTL *qAG1b* with the largest effects on AG in this study, the distribution of QTLs in individual plants was analyzed based on the relationship between survival rate and number of QTLs present (Fig. 3). The lines identified with three QTLs (*qAG1b* + *qAG1a* + *qAG8*) showed about a 50% survival rate under the AG-stress conditions, while lines with two QTLs including *qAG1b* indicated 36% (*qAG1b* + *qAG8*) and 32% (*qAG1b* + *qAG1a*) survival rates after the treatment, respectively. The survival rates of the remaining combination excluding *qAG1b* and lines with only *qAG1b* were 26 and 25%, respectively. Lines without the identified QTLs showed a similar survival rate (7.5%) to the susceptible check line Anda (5.1%). All QTL combinations, including *qAG1b*, showed a significantly different survival rate to others (Fig. 3). In particular, the value of three QTL combinations was not significantly different to that of the AG-tolerant donor TN at  $P \leq 0.05$ .

**Fig. 3** QTL effect by percent survival rate of genotypes derived from TN homozygous allele labeled as *qAG1b*. The other QTL's allele included both of homozygous and heterozygous type in every two QTL combinations. Letters from **a** to **c** indicate significantly different values according to statistical analysis using Duncan's Multiple Range Test ( $\alpha = 0.05$ ) in R



### Discussion

Rice seeds can germinate and coleoptile growth can occur under anaerobic conditions because rice has the complete set of enzymes required to enable starch degradation as an energy source for the rice seedling. Amylases were reported as key components for starch breakdown in seed (Murata et al. 1968) and the induction of  $\alpha$ -amylase occurs in rice seeds during germination under anoxic conditions (Perata et al. 1992, 1993). However, coleoptiles and shoots from different rice varieties differ greatly in rates of elongation and alcoholic fermentation under anoxia (Gibbs et al. 2000). Further, the physiology and genetics of AG tolerance in rice are still not sufficiently elucidated. To improve AG tolerance in practical rice breeding programs, key donors with AG tolerance are required, however, such donors with tolerance for low oxygen conditions during germination are relatively rare in rice (Ismail et al. 2009; Angaji et al. 2010).

In this study, the Vietnamese landrace, TN, was used as a donor for development of the mapping population for to examine the QTLs associated with AG tolerance. The donor showed a survival rate under hypoxic flooded conditions of 51%, which was similar to that of the check line, KHO (Khao Hlan On) at 56.0% (Table 3), while the susceptible parent, Anda, had a survival rate of about 5%. The phenotypic variation of the population developed from a cross between these parents showed transgressive

**Table 3** Comparison of survival rates in tested lines between hypoxic flooded conditions (submergence in the water with 4 ppm or lower of dissolved oxygen) and flooding condition (submerged condition without oxygen value control of water in reservoir)

Tested line	Hypoxic flooded condition (%)	Flooding condition (%)
TN	62	78
KHO	64	81
Anda	5	12

segregation at both the tolerant and susceptible extremes, with 15 lines showing a survival rate greater than TN, while 32 lines had a survival rate less than that of Anda. The population distribution was skewed towards susceptibility, possibly as a consequence of the consistently hypoxic screening conditions in which the DO value was maintained at < 4.0 ppm until the coleoptiles emerged from water surface. Screening methods, which involve flooding with 10 cm of water depth, are commonly used to evaluate the AG-tolerance level of rice seedlings (Angaji et al. 2010; Septiningsih et al. 2013; Baltazar et al. 2014), however, none report monitoring oxygen levels in the floodwater. Flooding in combination with the maintenance of consistent hypoxic conditions is a new evaluation method for AG tolerance used in this study. We prevented the occurrence of algal blooms (which change DO levels markedly) and found that the imposition of consistent hypoxic conditions exerted sufficient stress during screening to differentiate AG tolerance among lines.

For comparison, we monitored untreated water held in a reservoir in the glass house ( $25\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ ) during the 21-day treatment period. In general, the solubility of oxygen is inversely proportional to temperature. However, the DO value of water in the reservoir was greater than that expected (data not shown). To examine the impact of DO levels in floodwater, we measured the survival rates of TN and Anda (parents) and KHO (AG-tolerant check line) using untreated water and treated water (DO < 4.0 ppm). The survival rate was more than 25% higher using untreated floodwater than for the treated floodwater with low levels of dissolved oxygen (Table 3).

Germination is controlled by many genetic factors in a number of plant tissues, including the embryo, endosperm and pericarp. Under stress conditions in

particular, seed germinability is affected by a range of complex pathways during germination. In previous studies, seed germinability or seedling development in rice was examined under anoxic or anaerobic conditions (Ishizawa and Esashi 1984; Pegoraro et al. 1988; Jackson and Ram 2003; Jiang et al. 2004; Han et al. 2004; Jiang et al. 2006; Magneschi and Perata 2009). Increased levels of indole-3-acetic acid (IAA) level were found in coleoptiles of seedlings under hypoxic conditions (Pegoraro et al. 1988). Increased levels of GA, ethylene and IAA were found to enhance coleoptile growth under anaerobic conditions, but ABA and kinetin were inhibitory and, in particular, ABA is known to be involved in the phenomenon of dormancy (Horton 1991; Hoffmann-Benning and Kende 1992; Mapelli et al. 1995; Rock 2000). To identify the QTLs associated AG tolerance, the mapping population from a cross between TN and Anda was used to construct a linkage map and to detect novel QTLs associated with the trait. Three significant QTLs associated with AG tolerance were identified in the populations, *qAG1a* and *qAG1b* on chromosome 1 and *qAG8* on chromosome 8 (Fig. 1) using the CIM method. The percentage of variance explained for these QTLs ranged from 5.49 to 14.14%. Relatively few QTL studies have been conducted for germinability under anoxic or anaerobic conditions. Jiang et al. (2004) reported five QTLs for germinability under anoxic conditions in a recombinant inbred population, with QTLs detected on chromosomes 1, 2, 5 and 7, respectively. Of these, three QTLs, *qAG1*, *qAG2* and *qAG7*, derived from Kinmaze (*japonica*) acted to increase germinability under anoxia. Two QTLs for the same trait, designated *qAG-5* and *qAG-11*, mapped to chromosomes 5 and 11, with a LOD value of – 3.78 and 2.97, respectively by Jiang et al. (2006). The percentage of variance explained by these loci was 11.0 and 15.5%, respectively. Alleles from USSR5 (*japonica*) acted to increase the germinability (Jiang et al. 2006).

Angaji et al. (2010) conducted QTL mapping for seedling emergence rate under flooded conditions using the rice landrace KHO as an AG-tolerant donor. Five putative QTLs (*qAG 1-2*, *qAG 3-1*, *qAG 7-2*, *qAG 9-1* and *qAG 9-2*) contributed from the indica landrace, KHO, explained from 17.9 to 33.5% of the phenotypic variation. In a later study, six QTLs identified on chromosomes 2, 5, 6 and 7 came from another donor for AG tolerance, Ma-Zhan Red. The



largest QTL, *qAG7.1*, located on chromosome 7 explained 31.7% of the variation, having an LOD score of 14.5 (Septiningsih et al. 2013). Similarly, a major QTL for AG derived from Nanhi, an aus landrace from India, named *qAG7*, was detected on chromosome 7 with an LOD of 13.93 and 22.3% of the phenotypic variance explained (Baltazar et al. 2014).

In subsequent research, the KHO-derived *qAG9-2* was fine mapped to a ~ 50 kb region on chromosome 9 that contains four genes and, of these, the trehalose-6-phosphate phosphatase gene, *OsTPP7*, was identified as the genetic mechanism underlying *qAG9-2* (Kretzschmar et al. 2015). QTLs for AG tolerance have been detected in eight chromosomes in rice, except chromosome 4, 10, 11 and 12, and the major-effect QTLs have generally been identified on chromosomes 7 and 9. Interestingly, the QTL regions overlap with a number of loci, including seed germinability at low temperature, seed longevity and seed dormancy. In our case, the location of *qAG8* matches with that of *qCTS-8* associated with seedling cold tolerance on chromosome 8 (Lou et al. 2007). However, the other two QTLs identified in this study, *qAG1a* and *qAG1b*, were located on chromosome 1 which were not co-located with any previously defined loci. A possible reason for this less common result is that QTL studies for AG tolerance have not been extensively reported and the genetic pool of genotypes with known AG tolerance is relatively limited in comparison with other traits.

In this study, novel QTLs were identified using 6K SNP chip analysis under a combination of flooded and hypoxic conditions and explained a sum of 25.5% of phenotypic variation in survival under AG conditions. However, the QTLs explain a relatively modest percentage of the phenotypic variation, hence pyramiding with other QTLs may be necessary to improve AG tolerance using marker-assisted selection. Further studies are in progress to develop *japonica*-type NILs having two KHO-derived QTLs, *qAG 7-2* and *qAG 9-2*, in addition to fine mapping to define the position of *qAG1b* by backcrossing with a *japonica* recurrent parent. These results may be useful to improve tolerance to AG conditions when rice is established from direct seeding and to broaden the understanding of AG tolerance mechanisms.

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