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QTL mapping: a conceptual approach to improving cold tolerance at seedling stage in rice (*Oryza sativa. L*)

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Abstract

Much of what is known about the process of technological innovation in agriculture has yet to be captured in the discussions of abiotic stress plant tolerance as well as rice cold tolerance. The development of research and technological solutions to minimize risks of current abiotic stresses to the plant can lead to two possible outcomes: increase in agricultural productivity and assist the future of plant breeding work. Research efforts about the role of technological development, driven by abiotic stress constraints, are pivotal in making any assertion about the likely tolerance of plant to abiotic stress. Drawing upon the hypothesis of QTL mapping, this research investigates on detection of QTLs for cold tolerance at the seedling stage in rice (*Oryza sativa. L*), QTLs identified from a BC_1F_2 breeding population derived from the cross between Chomrongdhan, a donor parent tolerant with Vary botry a susceptible parent, that lead to increase rice productivity in Madagascar. Using a controlled environment and molecular work, out of total 500 BC_1F_2 segregating plants, 144 plants were used for genotyping based on of visual seedling stage cold tolerance symptom. A total of 4606 SNP markers evenly spread throughout the whole 12 rice genome was used for parental polymorphism survey. The 34% polymorphic markers were used for QTL mapping for cold tolerance at seedling stage. QTL analysis using inclusive composite interval mapping detected four QTLs on chromosome 2 and 10 with phenotypic variances (R^2) of 11.11, 7.55, 12.8 and 8.8%, respectively. The position of QTL on chromosome 2 was flanked by 2262412 and 2237404, three other QTLs were detected on chromosome 10 conferred cold tolerances for seedling growth and leaf growth at 14day after recovery and appear to be a novel QTLs. Selected tolerant plant in this research should be useful for the farmers and the markers flanking those identified QTLs should be useful for molecular marker assisted breeding for cold tolerance for the breeder.

Keywords: QTL mapping, cold tolerance, seedling, rice.

1. Introduction

Despite the evidence that technological innovation has been fundamental to growth and development of agriculture around the world, the development of research and technological solutions to minimize risks of current abiotic stresses to the plant can lead to two possible outcomes: increase in agricultural productivity and assist the future of plant breeding work. Research efforts about the role of technological development, driven by abiotic stress constraints, are pivotal in making any assertion about the likely tolerance of agriculture plant to abiotic stress. Drawing upon the hypothesis of QTL mapping, this research investigates on detection of QTLs for cold tolerance at the seedling stage in rice (*oryza sativa. L*).

Rice is a staple food for half of the human population. Rice belongs to the genus *Oryza*. It contains more than 20 species, and two of which are referred to as cultivated rice: *Oryza sativa* Asian cultivated rice, and *Oryza glaberima* African cultivated rice. *Oryza sativa* is cultivated worldwide and has two subspecies *Indica* and *Japonica*. Subspecies *indica* widely cultivated in the hot and humid regions of Asia, Africa and Latin America, and accounts for 80% of world rice production (Jena *et al*., 2010). Subspecies *japonica* is cultivated in the temperate, sub-temperate and high-altitude regions of Asia, Europe, Latin America, North America and Oceania (Mackill and Lei, 1997).
Oryza glaberrima is an upland crop, but it is being replaced by *Oryza sativa,* it is planted on a limited scale in West Africa (Li *et al.,* 2011).
Oryza sativa was introduced in Madagascar from India, the Malay Peninsula and Indonesia approximately 800–1400 years ago (Mather *et al.,* 2010). Rice is both the main crop and the staple food of the majority of the population of Madagascar with an average annual consumption of rice by an individual estimated at 154 kg/capita/year (paddy equivalent) (FAO-STAT, 2014; Raboin *et al.,* 2014; Min Agri, 2015).
Due to its origin in tropical and subtropical regions, Low temperature is one of the most important constraints not only in many rice producing of the high altitude areas but also in high altitude area in the tropical and

subtropical countries (Ye *et al.,* 2009; da Cruz *et al.,* 2013). In Africa, by seasonal temperature variation in Sahel regions of West Africa and because of the elevation in high-altitude regions of East Africa (Madagascar, Ethiopia, Rwanda, Burundi, Tanzania), low-temperature damage could occur, resulting in considerable yield losses (Wainaina *et al.,* 2015).
In Madagascar, Rice is grown also in the Central part of the Malagasy highlands at elevations up to 1500- 1900 meter above the sea level. The Highland region is a densely populated area, with 102 inhabitants per km^2 (Gastineau *et al.,* 2010). Temperatures normally drop with increasing altitude (0.6^oC per 100 meters); therefore, an estimated about 12,889ha (10.48%) of cultivated rice areas was affected by the cold at seedling stage (Kumashiro, 2011; DRR, 2014, 2015). At the seedling stage of rice, the occurrence of low temperature stress inhibits seedling development and eventually leads to stunted growth and delayed heading or incomplete grain filling (Andaya and Tai 2006), about 70 to 85% of yield was lost (GRISP, 2013; DRR, 2014, 2015). Cold tolerant varieties would help to solve this problem.

This paper reports on a series of control condition experiment and associated laboratory works. The aims of these studies were twofold. Firstly, to evaluate the effect of cold stress on cold tolerance related traits at seedling, and secondly, to identify quantitative trait loci (QTLs) related to cold tolerance at seedling stage.

2. Materials and Methods

2.1. Plant materials and mapping populations

"Vary botry" (*Oryza sativa ssp*. *Indica*) was used as a recurrent parent. "Chomrongdhan", temperate *japonica* rice originated from Nepal and well adapted to the cold conditions of high altitude in Nepal was used as a donor parent. A total of 500 BC_1F_2 plants from a cross between "Vary botry" and "Chomrongdhan" were used for cold tolerance evaluation and QTL identification.

2.2. Evaluation of cold tolerance

Evaluation of cold tolerance at seedling stage under control condition

The experiment was conducted in a greenhouse and the cold room at KARLO Njoro, Kenya. BC_1F_2 seeds and the parents were soaked in distilled water in a Petri dish and were germinated in a growth chamber at 32^oC for 48h. Both parents Vary botry and Chomronghdan were used as susceptible and tolerant checks, respectively. Germinated seeds with 10cm coleoptiles were planted in a pot filled with soil around 5kg and animal manure (5t/ha) and they put in the greenhouse under the natural conditions until seedlings reached at four leaf stage. When seedlings reached at four leaf stage, the BC_1F_2 seedlings and checks took about 10 days to cold room with a constant temperature of 12°C and an artificially lighted within photoperiod of 12h light and /12h dark and light intensity was about 15000 LX (Yang *et al.,* 2013). After the cold treatment, the seedlings were moved back to the greenhouse to allow seedlings to recover and resume normal growth. Seedling survival rate ((Surviving seedling x Total seedling treated)/100) were then, seedling growth, seedling vigor, and seedling leaf growth at 0, 7 and 14 days after cold treatment. Visual observation of leaf injury symptom, leaves number and tiller number and the degree of leaf wilting were used to evaluate clearly and score those traits followed by Standard evaluation system (SES) for rice developed by the International rice research institute (IRRI, 2002) and Suh *et al*., 2012 visual scaling approach.

The experiment was conducted as augmented randomized complete block design; constructed the layout by using the Plant Breeding Tools software (IRRI, 2014). It was comprised ten blocks, within the donor and recurrent parent used as checks that appear exactly once in each block, and the new BC_1F_2 plants that are replicated once in the trial. Analysis of variance (ANOVA) of the evaluated trait between BC_1F_2 plants and parents also were analyzed by using the SAS version 9.1.3 with proc Mixed procedure (SAS 2000).

2.3. QTLs Identification

DNA was extracted from the young leaves of each individual BC_1F_2 plant and their parental using CTAB (Cetyltrimethylammonium bromide) method with modifications based on the procedure described by Murray and Thompson (1980). The Infinium SNP chip rice 6K contained 4606 SNP markers that evenly distributed on 12 rice chromosomes were used to genotype the BC_1F_2 and the parents. Polymorphic SNP markers were used for construction of linkage map and QTL analysis. Mapping and QTL for Cold tolerance were detected using

phenotypic data at the seedling stage of the BC_1F_2 plants. QTL analysis was conducted by the inclusive composite interval mapping using QTL IciMapping version 4 software (Wang *et al*, 2014).

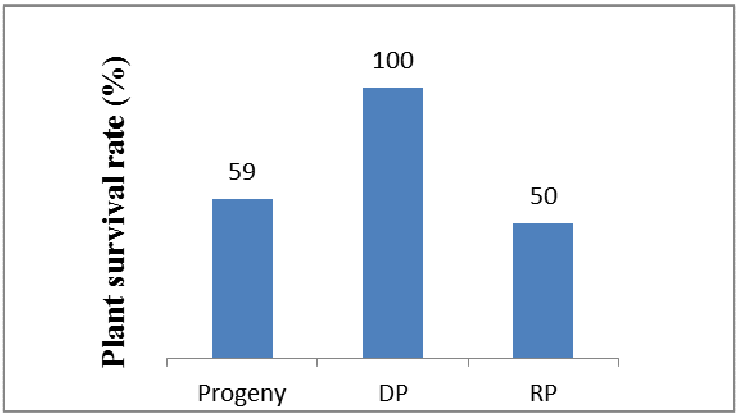
A logarithm of Odds (LOD) threshold of 2.5 was initially used to declare major QTL in this study. After that, 1,000 permutations at a probability of 0.05 were used to declare definitive QTL. A chromosomal walk speed of 1.0 cM and the default window size of 8cM were used for all QTL estimation.QTL effects were estimated as the proportion of phenotypic variance explained (PVE) by the QTL within positive or negative additive effects that were used to identify the origin of the favorable alleles. Positive additive effect indicates that the allele from the donor parents contributed the phenotypic values, while a negative additive effect indicates provenience from the susceptible parent.

3. Results

3.1. Evaluation of cold tolerance at seedling stage under control condition

3.1.1. Plant survival rate

"Chomrongdhan" the donor parent showed the highest percentage of survival of 100% (Figure1). The seedlings of this variety were classified as tolerant to cold at seedling stage. "Vary botry", the recurrent parent, exhibited an intermediate reaction with 50% of plant survival. Regarding the BC_1F_2 plants, 59% of the plants were survivals that classified as an intermediate reaction to cold at seedling stage (Figure 1), indicating a dominant gene action for cold tolerance at seedling stage was not expressed on plant survival.



DP: Donor parent; **RP**: Recurrent parent

Figure 1: Frequency distribution of seedling survival percentage after cold treatment at seedling stage

3.2. Evaluation of BC_1F_2 segregating plants

Results showed that two hundred two (202) were killed during cold treatment at seedling stage and thus they were left out from the analysis, 154 BC_1F_2 individual plants yielded poor and thus couldn't be genotyped, the DNA sample was degraded during the transfer of DNA sample from KARLO/ Njoro, Kenya to GSL/IRRI the Philippines.144 of BC_1F_2 plants were used to map QTL.

3.2.1. Evaluation of seedling growth

The seedling growth evaluation result is given in table 1, at the beginning of seedling growth, at 0 day after recovery (0 DAR) majority of BC_1F_2 seedlings showed a moderately susceptible (53.47%) reaction. Only 35.42% of BC_1F_2 seedlings exhibited as moderately tolerant and 3.47% as tolerant. At 0 DAR, normal distribution frequency for seedling growth was observed (Figure 2a).

At 7 day after recovery (7DAR) the frequency distribution trend changed, a relative similarity between the percentages numbers of moderately tolerant (38.19%) and the moderately susceptible (33.33%) of BC_1F_2 seedlings were observed (Figure 2b). 14 days after recovery (14 DAR), the result showed a normal distribution of seedling growth, but slightly skewed towards the donor parent (Figure 2c). Analysis of variance of BC_1F_2 seedlings was exhibited highly significant difference (7 DAR) and 14 days after recovery (14 DAR) (Table 1).

3.2.2. Evaluation of Seedling vigor

The majority of the BC_1F_2 plant was revealed as moderately susceptible plant (seedling at 4-leaf stage and no tiller formation), a total of 87.5, 75.69, and 60.42% at 0 DAR, 7 DAR and 14 DAR, respectively (Table 2). Distribution frequency of seedling vigor did not fit the normal distribution; it was skewed toward the sensitive parent (Figure 3). Analysis of variance of seedling vigor showed significant differences between BC_1F_2 plants at 7 DAR, and as well as 14 DAR (Table 4).

3.2.3. Evaluation of Seedling Leaf growth

Seedling leaf growth result is given in table 3At 7 DAR, a majority of BC_1F_2 plant showed susceptible to highly susceptible visual scores (score 7 to score 9). They have wilted leaves while some showed seedling apparent death (42.36%), and the plants that showed as moderately susceptible was 37.50% of the total number of plants which showed moderately tolerant and tolerant BC_1F_2 were few At 14 DAR, the state changed, a majority of BC_1F_2 plants showed as moderately susceptible (score5) (53.47%) and the seedling plants as tolerant and moderately tolerant increased (29.86%) (score3). The distribution frequency, at 7 DAR did not fit the normal distribution and was skewed in the direction of the susceptible parent Figure 4 (a-b).

Table 1: Evaluation of seedling growth

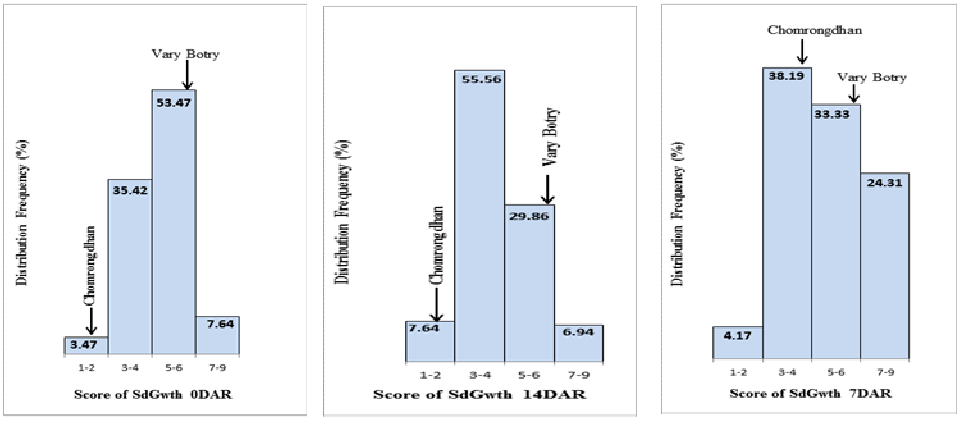
Scores	Frequency distribution (%)		
	Seedling Growth0DAR	Seedling Growth7DAR	Seedling Growth14DAR
1-2	3.47	4.17	7.64
3-4	23.42	38.19	55.56
5-6	53.47	33.33	29.86
7-9	7.64	24.31	6.94

Table 2: Evaluation of seedling vigor

Scores	Frequency distribution (%)		
	Seedling Vigor 0DAR	Seedling Vigor 7DAR	Seedling vigor14DAR
1-2	4.17		6.94
1-3	-	24.31	-
3-4	8.33	-	32.64
4-7	-	75.69	-
5-7	87.50	-	60.42

Table 3: Evaluation of seedling leaf growth

Scores	Frequency distribution (%)	
	Seedling Leaf growth 7DAR	Seedling Leaf growth 14DAR
1-3	20.14	29.86
4-6	37.50	53.47
7-9	42.36	16.67



2a **2b** **2c**

Figure 2(a, b, c): Distribution frequency of seedling growth of BC_1F_2 plants and the parent after cold treatment at seedling stage

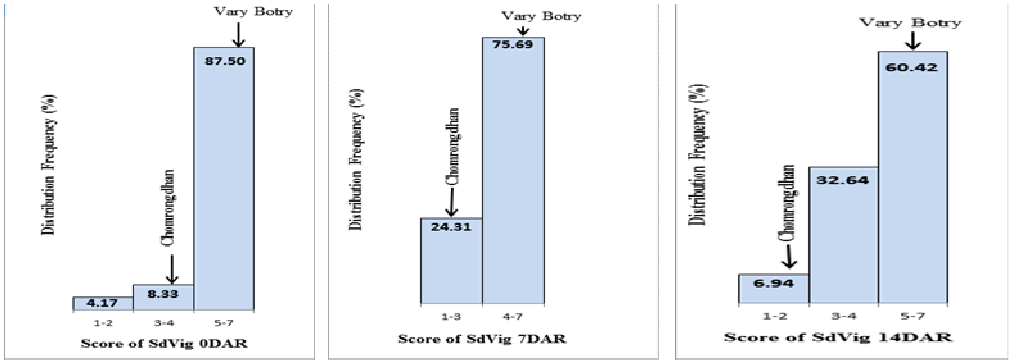
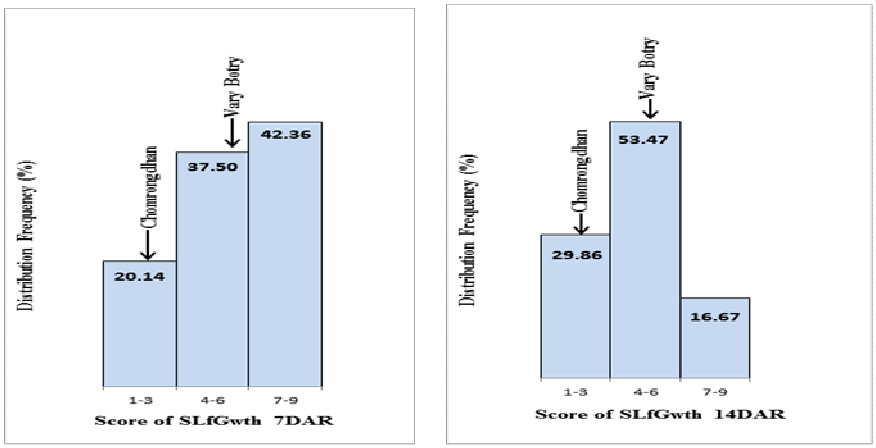


Figure 3: Distribution frequency of seedling vigor of BC_1F_2 plants and the parent after cold treatment at seedling stage



4a **4b**

Figure 4 (a, b): Distribution frequency of seedling leaf growth of BC_1F_2 plants the and parent after cold treatment at

Table 1: Analysis of variance of seedling growth, seedling vigor and leaf growth scale at seedling stage of BC_1F_2 plants

Source of variation	DF	Mean square		
		SdVig14	LfGwth7	LfGwth14
Block	9	5.5674	1.7925	1.5391
Checks	1	21.198*	51.2*	36.2264*
Tested Plant	143	5.1206	5.9215**	6.02**
Error	12	2.544	1.7888	1.9055
CV (%)		29.8382	23.1586	26.8607

Table 1 (continued)

Source of variation	DF	Mean square				
		SdGwth0	SdGwth7	SdGwth14	SdVig0	SdVig7
Block	9	5.5674	1.7925	1.5391	1.9407	1.4962
Checks	1	21.198**	51.2*	36.2264*	57.80*	57.800*
Tested Plant	143	5.1206	5.9215**	6.0200**	2.8501	3.2147***
Error	12	2.544	1.7888	1.9055	1.5444	1.2111
CV (%)		29.8382	23.1586	26.8607	18.32	17.196

***Significant at p≤ 0.05;** significant at p≤0.01, * significant at p≤0.001; SdGrwt0DAR: seedling growth at 0 day after recovery; SdGwth7 DAR: seedling growth at 0 day after recovery; SdGwth14DAR: seedling growth at 14 day after recovery; SdVig7DAR: seedling vigor 7 day after recovery; SdVig14DAR: seedling vigor 14 day after recovery; LfGwth7: seedling leaf growth7 day after recovery; LfGwth14: seedling leaf growth 14day after recovery.

3.3. Identified Quantitative trait loci

Four putative QTLs were identified and mapped by Inclusive Composite Interval Mapping onto rice chromosomes 2 and 10 (Table 2, Figure 5). Three QTLs (*qSdGwth14-10-1*, *qSdGwth14-10-2* and *qLfGwth14-10-1*) were detected on chromosome 10 conferred cold tolerances for seedling growth and Leaf growth at 14day after recovery. One QTL, *qSdVig0-2-1*, located on Chromosome 2 was identified for seedling vigor at 0 day after recovery.

qSdGwth14-10-1 and *qSdGwth14-10-2* for chromosome 10 was positioned at 10 and 20cM respectively. *qSdGwth14-10-1* were flanked to id10000391 and 10099158 with LOD scores 3.66 and the phenotypic variation expected (PVE) by this QTL was 11.11% with negative parental additive effect (-0.4573). While, *qSdGwth14-10-2* were flanked with 10465477 and 10469362 with LOD scores 2.65, expected 7.55% of phenotypic variation and positive parental additive effect (0.2422). *qLfGwth14-10-1* for 10 was positioned at 10cM, it was flanked by 2262412 and 2237404 with LOD scores 3.65 and phenotypic expected variation was 12.80% with negative parental additive effect (-0.4357). *qSdVig0-2-1*, located on Chromosome 2 was at 130cM, this QTL was flanked to 2262412 and 2237404with LOD scores 3.65 and phenotypic variation explained by the QTL as 12.80%, and negative parental additive effect (-0.1055).

Table 2: Quantitative Traits Loci Identified at seedling stage

[a]QTL	[b]Ch	[c]POS	[d]LM	[e]RM	[f]LOD	[g]PVE(%)	[h]Add
qSdGwth14-10-1	10	10	id10000391	10099158	3.6631	11.1104	-0.4573
qSdGwth14-10-2	10	20	10465477	10469362	2.6568	7.5502	0.2422
qSdVig0- 2-1	2	130	2262412	2237404	3.6576	12.8046	-0.1055
LfGwth14-10-1	10	10	id10000391	10099158	2.8964	8.8726	-0.4357

[a]QTL identified at in the present study; [b]Chromosome on which QTL for cold tolerant is located; [c]POS Position of QTL on chromosome (cM),; [d]LM Left marker; [e]RM Right marker; [f]LOD scores (Log10-likelihood ratio) offer the strength of the data supporting the existence of a QTL in a defined interval at LOD≥2.5 at P≤0.0001;

[g]PVE(%), Phenotypic variance explained by identified QTL;[h]Add Additive genetic effects of QTL, Positive and negative value indicates that alleles resulting in an increasing tolerance are from Vary botry and ChomrhongDhan.

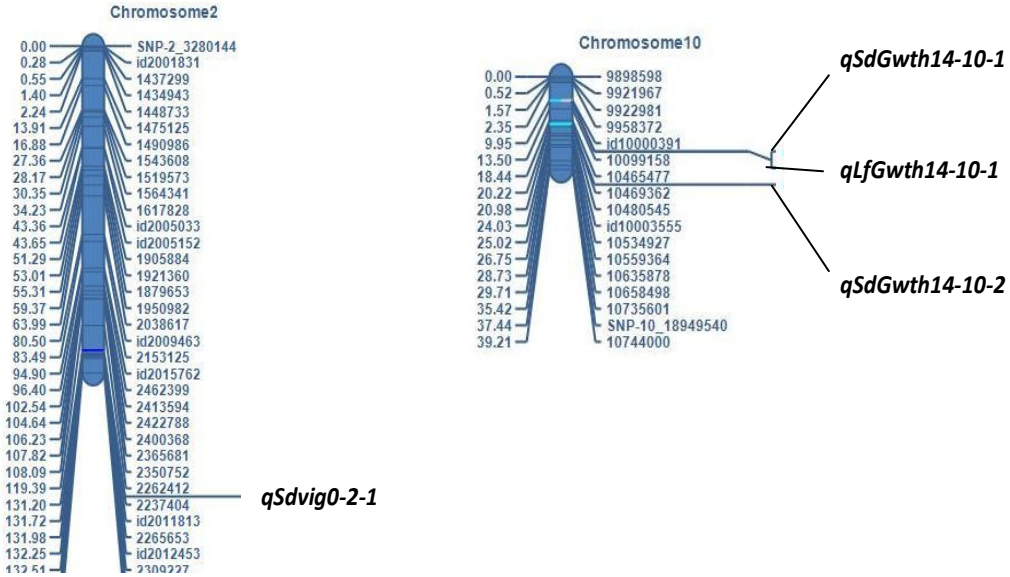


Figure5: Genomic location of Quantitative trait loci affecting cold tolerance of BC_1F_2 plants from Vary botry//Chomronghdan at seedling stage. Map units are expressed in centimorgan (cM)

4. Discussion

4.1. Plant survival rate

BC_1F_2 seedlings, donor parent and recurrent parent showed a clear difference in their rate of survival plant under the described cold stress condition at seedling stage.

The *indica* parent exhibited survival rate at 50% compared with the donor *japonica* parent (100%). On the other hand, the japonica parent presented a relatively higher percentage of survival of the plants as stated by Ma *et al.,* (2015). It confirms that Chomronghdan, the donor parent used in this study is a typical *japonica* rice and it exhibits more cold tolerance than *indica* varieties (da Cruz *et al.,* 2013; Ma *et al.,* 2015).

4.2. Phenotypic variation of BC_1F_2 progeny

In this study, the results indicated that a good selection method to evaluate cold tolerance in segregating populations is by the use of controlled air. Screening rice genotypes by imposing controls air provides a favorable environment for selection, as it allows to correct measurement of traits associated with cold tolerance, and it is considered a reliable method of phenotyping for cold tolerance (Suh *et al*., 2010). Screening for cold tolerance using screen house and greenhouse with temperature controls successfully allows for evaluation of substantial differences in cold tolerance related traits, and thus were suitable for the QTL study.

In the present study exposing BC_1F_2 rice progeny to the temperature of 12°C for 10 days at seedling stage allowed for the distinction between cold tolerant and cold sensitive of BC_1F_2 plants, this result confirmed that optimum conditions are needed to evaluate stress tolerance (da Cruz *et al*, 2013).

4.3. Quantitative trait loci analysis

4.3.1. Parental Diversity

The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotypic level for QTL analysis to be effectively carried out (Semagn *et al*., 2006c). The significant differences were found between donor "Chomrongdhan" and "Vary botry" recurrent parent for the morphological studied traits. This allows for diversity studies using these parents. The recurrent parent with relative number reduction was due to the cold stress and this confirmed the sensitivity of "Vary botry" and tolerance of "Chomrongdhan" under cold stress.

The polymorphism level (34%) exhibited by the parents in this study was acceptable when compared with some studies. Therefore, it was good enough to be used for QTL analysis and linkage map as well. For example, Xiao *et al* (2014), also mapping QTL for cold tolerance of rice roots at seedling and mature stages, only 113 markers showed the polymorphisms between the two used parents with a total of 653 SSR.

In this study, phenotyping data were relatively significant for any related study of the traits at seedling stage after cold tolerance stress. Having confirmed acceptable Polymorphism for the genotyping data, the BC_1F_2 mapping population derived from this cross between being suitable for mapping QTL for cold tolerance traits.

4.3.2. Identified Quantitative Traits Loci

This study allowed identification of QTLs linked to seedling growth, seedling vigor, and seedling leaf growth *at* seedling stage on chromosome number 2, and 10.

Both donor and recurrent parents were found to possess QTL alleles which increased phenotypic values.

The contribution of the parents to increase one trait was confirmed by the additive effect observed during QTL identification. The result suggested that recurrent parent (Vary botry) was contributed 0.45, 0.10 and 0.43 by the alleles from the recurrent parent to increase *SdGwth14-10-1, SdVig0-2*-1, and *LfGwth14 -10-1* respectively. While, the donor parent (Chomrongdhan) contributed 0.24 by the alleles from the donor parent to increase *SdGwth14-10-2*. This parental contribution values was relatively similar with the result of Liu *et al* (2015), they identified seven QTL and the susceptible parent contributed (-) 0.03 only to increase seedling cold tolerance when they were mapping QTL cold tolerance at the early seedling stage in Landrace rice Xiang 743 using $F_{2:3}$ populations derived from a cross between cold tolerant Landrace rice Xiang 743 and cold-sensitive variety Katy.

In addition, for this study at seedling stage, two cold tolerance loci *qSdGwth14-10-1* and *qLfGwth14 -10-1* with LOD 3.66 and 2.89 respectively coincided with the SNP markers flanking between id10000391 and 10099158 on chromosome 10 in position 10 (Figure 5), moreover on chromosome 10 contained more than one QTL; it has up to three QTL for seedling growth and seedling leaf growth, hence *qSdGwth14-10-1* and *qSdGwth14-10-2*, those traits were considered a major QTL (PVE >10%).

4.3.3. Comparative analysis of identified QTLs

In this present study, seedling growth 14DAR (*qSdgwth14-10-1*) and leaf growth 14DAR (*qLfGwth14-10-1*) QTLs were identified on chromosome 10 at the same position at 10 cM on. The fact that those QTL were located at the same position, suggested that one or group of genes controls the assessed trait. This means that the genes controlling both traits are pleitropic on the other hands, and this might contribute to significant positive correlation observed between seedling growth 14DAR and seedling leaf growth 14DAR in the BC_1F_2 Plants.

Two QTLs (*qSdGwth14-10-1* and *qSdGwth14-10-2*) which were located on chromosome10, one (*qSdGwth14-10-1*) was closed to id10000391 at the 20cM position while the other one (*qSdGwth14-10-2*) was in 20cM position from 10465477. These results suggest that these traits were controlled by multiple genes (polygenes) under cold stress.

In this study QTL associated with seedling growth were identified on chromosome 2 and 10, in a previous similar study, Andaya and Tai (2007), Suh *et al.,* (2012), identified QTL associated with seedling growth on chromosome 4 (*qCTS4, qCTS4a*, and *qCTS4b*) at seedling stage and Lou *et al.,* (2007), identified QTL

associated with seedling growth on chromosome 2 (*qCTS-2*) at seedling stage. In contrast, Andaya and Mackill (2003a) identified QTL associated with seedling growth on chromosome 12 (*qCTS12a*), Zhang *et al.,* (2005) on chromosome 3, 5, 8 (*qSV-3-1/2, −5, −8-1/20*), Koseki *et al.,* (2010) on chromosome 11 (*qCtss11*). Several studies related to identification of QTLs for cold tolerant rice at seedling using different mapping population and markers in different locations have been undertaken; it is difficult to compare the chromosomal locations of QTLs directly because different materials and molecular markers were used. However, this present study has come up with molecular markers which have identified suggested positions of QTLs related to cold tolerance at seedling stage.

5. Conclusions

The application of molecular marker assisted selection accelerates the selection of rice plants that have QTL for cold tolerance. In addition, the development of molecular markers and linkage maps allowed the detection of many QTL related to cold tolerance at seedling and reproductive stage. An experiment was conducted in a greenhouse followed by cold treatment using a control condition at seedling and reproductive stage to phenotype BC_1F_2 plants derived from crosses between *indica* susceptible parents (Vary botry) with tolerant *japonica* (Chomrongdhan).

There was variation within the new progeny under cold stress;

BC_1F_2 plants showed significant differences for most of the assessed traits, and some new progeny had good performance under cold stress compared to the susceptible parent;

The polymorphism level existing in the parents in this study was suitable for mapping QTL for cold tolerance traits.

The mapping population developed from the cross between Vary botry//Chomrongdhan were suitable to genotype QTLs for cold tolerance, therefore, Chomrongdhan, a tolerant check was found to be a novel source of cold tolerance;

6. Recommendation

Cold is still one of the factors that decrease rice production in the high altitude region of Madagascar, this situation indicates that there is still need to continue and accelerate as well as breed for cold tolerance rice in Madagascar. Introgression of tolerant QTLs/genes identified in this study could be useful to enhance the level of cold tolerance through marker assisted selection.

The gap between greenhouse research and field application is a major concern in cold tolerance research. Evaluating cold tolerance using open field criteria directly in the field should be continued to validate the result of this study;

Screening of segregating populations from this study should be continued until, hopefully, a variety or more will be released that are cold tolerant;

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