

## QTL Based Screening of Rice Cultivars from Andhra Pradesh Showing Cold Resistance at Germination Stage

Ashutosh Gautam, J. Suresh

Received 4 April 2016; Accepted 9 May 2016; Published online 4 June 2016

**Abstract** Rice is a major source of carbohydrate and staple food of more than 62% people in the world. It is cultivated around the globe, which includes more than 100 countries. The rice cultivated nowadays belongs to species *O. sativa* and *O. glaberrima*, of which former one is widely used for the purpose, *Oryza sativa* contains two major subspecies: *japonica* or *sinica* variety and *indica* variety. Like all other crops, rice too suffers stress conditions caused by various biotic and abiotic factors. Among abiotic factors, the yield loss in rice due to cold or low temperature stress is about 45% at global level. In present study, during

2013-14 we had screened 52 different genotypes of rice, grown extensively across the Andhra Pradesh. Here we used cold shock treatment and germination percent for morphological assessment and used 54 SSR markers for genotypic assessment for level of cold tolerance and to identify QTL associated. Here we found that out of 52 studied genotypes, 13 were highly resistant showing morphological score of 1, among those 13 genotypes BR-11 has shown strong association of five QTLs which are responsible for cold tolerance. Genotype BR-11 may be used as resistant parent in a breeding program for development of cold resistance.

**Keywords** Rice, Cold tolerance, QTL, SSR marker, Germination.

### Introduction

Rice ( $2n=24$ ) is a monocot crop plant belong to family *Poaceae* or *Gramineae* and sub-family *Oryzoidea*, botanically known as *Oryza* sp. ( $2n=24$ ). It is a major source of carbohydrate and staple food of more than 62% people in the world. It is cultivated around the globe, which includes more than 100 countries. Whenever there is a decline in rice production, it affects whole world especially the Asian countries and it may lead to a famine like condition.

Like all other crops rice too suffers stress conditions caused by various biotic and abiotic; among

---

A. Gautam\*  
Department of Genetic & Plant Breeding,  
ANGRAU, Rajendranagar, Hyderabad 500030,  
Telangana, India

J. Suresh  
Assistant Professor,  
Department of Genetics & Plant Breeding,  
ANGRAU, Rajendranagar,  
Hyderabad 500030, Telangana, India  
e-mail : ashutoshgtm9@gmail.com  
\*Correspondence

**Table 1.** Program of INDOSAN single chamber seed germinator.

Sl. No.	Temperature	Treatment hours	Time	Photo-cyclic time
1	8°C	6	00:00 to 06:00	Night
2	10°C	6	06:00 to 12:00	Day
3	10°C	6	12:00 to 18:00	Day
4	8°C	6	18:00 to 00:00	Night

abiotic factors, the yield loss in rice due to cold or low temperature stress is about 45% at global level). Frequently occurring low temperature causes more than 50% yield loss. In China, the recorded yield loss due to low temperature is 3–5 million tons. In 1980, Korea lost an average yield of 3.9 tons per hectare, more than half of its annual yield per hectare [1]. In Australia, the average yield loss due to cold stress was found to be 0.5 to 2.5 t ha<sup>-1</sup>. Low temperature effects on seedlings may be manifested as poor germination, slow growth, and discoloration or yellowing, withering after transplanting, reduced tillering, and stunted growth. All of these responses appear to depend on a common primary mechanism involving loss of membrane function during low temperature. Plant membranes consist of a lipid bilayer interspersed with proteins and sterols.

Here in our study during 2013-14 to overcome the cold stress problem in rice crop we had used QTL approach, which is a part of MAS, using SSR molecular markers. Some important QTLs, which have been reported with high contribution to genotypic variability and included in this study, are qCTSS 8.1, qCTS12a, qCTS12a, qLTB3, qSCT-11, qGR-1, qGI-11 and qSCT-3-1. Their contributions to genetic variability have been reported to be in a range of 22%–74%. Most of the QTLs, which have resistance against low temperature, are clustered on chromosome 1, 2, 3, 4, 7 and 11. QTLs, which contribute 20% or more in phenotypic variability, have been found very suitable to develop tolerance against cold stress.

(Author would like to extend gratitude to Dr M.S. Madhav and Dr L. V. Subbarao, Directorate of Rice

**Table 2.** Score based grouping of genotypes.

Serial No.	Score	No. of genotypes
1	1	13
2	3	16
3	5	11
4	7	6
5	9	3

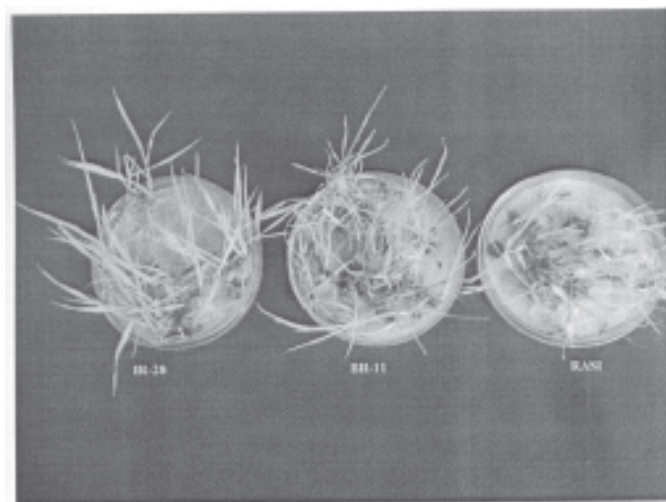
Research, for their kind guidance and Institute of Biotechnology and ANGRAU, Hyderabad for providing necessary facility for this study).

## Materials and Methods

### Plant material and phenotyping

Seeds of total 52 different genotypes of rice, which are grown extensively across the Andhra Pradesh and India, were brought from the DRR (Directorate of Rice Research) Farm, ICRISAT (Hyderabad). In this study, population of total 2000 seedlings was examined in the for the cold stress tolerance. Among 52 genotypes studied, IR-28 and IR-50 were used as reported resistant and susceptible checks respectively, for identification of QTLs on some particular chromosomes.

Seeds were sown in petri dishes (50 seeds from each genotype, 25 seeds in each petri dish) and after 15 days of sowing, seedlings were transferred to seed germinator (INDOSAN, single chamber type) for cold shock treatment in same laboratory. 15 days old seedlings were subjected to cold stress, the exposure period for cold shock was 7 days without any interruption maintaining standard conditions of moisture and light. Here we had used a fully automated germinator INDOSAN, single chamber type); this germinator has a program of 24 h, which is to be specified for every 6 h. We had executed the program following the manufacturer's guidelines and maintained the day temperature at 10° C and night temperature 8°C respectively. The effects of low temperature were re-



**Fig. 1.** Resistant genotypes after cold treatment.

corded in relation to seedling germination, height and color.

#### Scoring of seedlings after cold treatment

After 7 days of cold treatment, the scoring of seedling was done for their color (Table 1). The score were given from 1 to 9. While the score 1 was given to the genotypes which were highly resistance to cold stress, the score of 9 was meant for genotypes with high susceptibility to cold stress. According to scores obtained by genotype, based on their seedling color; five groups were formed. These ground were as high resistance genotype group for dark green seedling, moderate resistance group for light green seedling, medium susceptible group for yellow seedlings, susceptible group for brown seedling and highly susceptible genotype group for dead seedlings. The group wise scoring was 1, 3, 5, 7 and 9 respectively as it is prescribed in IRRI manual of Standard Evaluation System for Rice [2].

Scoring and specification are as follows: 1=Resistant (Dark green seedling), 3=Moderately Resistant (Light green seedling), 5=Moderately susceptible (Yellow seedling) 7=Susceptible (Brown seed-

lings), 9=Highly susceptible (Dead seedling).

#### Molecular analysis

##### *Isolation of Genomic DNA*

Seedlings of each genotype were grown separately as material for the DNA isolation. Samples of 10—15 grams were collected from the 10 days old seedlings for each genotype in early hours of day and were used for extraction of DNA. Genomic DNA was extracted from the samples taken from the seedlings using Cetyl trimethylammonium bromide (CTAB) method.

##### Quality check for DNA/quantification

Extracted DNA samples were run through 0.8% agarose gel ( $0.5 \times$  TBE) and after series of dilutions all samples were brought to uniform concentration of 20—25 ng/ $\mu$ l. Then these samples were tested for amplification using primers and run through 1% agarose gel ( $0.5 \times$  TBE).

##### *Screening of resistant genotypes*

However, IR-28 has been reported to have cold toler-

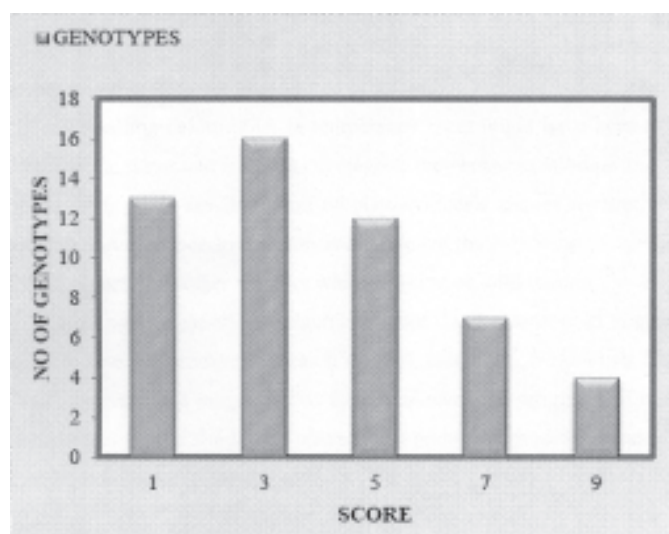


Fig. 2. Score wise grouping of genotypes.

ance QTLs or as donor of QTLs at chromosome 1, 3, 8 and 11 but for other chromosome it is not reliable. For our study we had selected total 18 QTLs from our survey, for most of them checks were not available. For this, we had run the DNA of 5 genotypes namely Sasyasree, Rasi, Krishnahamsa, BR-11 and Tellahamsa (based on results of phenotyping) with flanking markers of respective QTLs, using 3% agarose gel. Those, which were showing amplification with both flanking marker and having polymorphism, were selected as reference check for others. To confirm the presence of QTL on particular chromosome this reference check was also run with interval marker.

#### *Polymorphism in genotypes*

The reference check was now run with all resistant genotype (based on results of phenotyping) using 3% agarose gel to identify the presence of cold tolerance QTL on particular chromosome. Here we had used total 54 SSR markers for checking polymorphism.

#### *Selection of SSR interval marker between flanking region*

1–5 interval SSR markers were selected between the flanking regions of all the QTLs. For this purpose we

had used Rice TOGO Browser software, the software exposes all available SSRs and RFLPs for a given flanking region on a chromosome. These markers were if more than one, then selected at uniform distances between the two flanking markers. Wherever the physical distance between flanking markers was less than 1 Mb, in that case only one interval marker was selected at intermediate distance.

#### *Visualisation of amplified products in agarose gel*

Separation and visualisation of PCR products was done on 3% agarose gel electrophoresis (AGE). Agarose was casted in 3% gel in TBE buffer (0.5 X). Slabs were casted in a horizontal gel frame and products were visualised by incorporating 10 µl ethidium bromide (3, 8-Diamino-5-ethyl-6-phenylphenanthridinium bromide) per 10 ml of gel solution and viewed in a gel documentation system.

## **Results and Discussion**

### **Morphological assessment**

In the present study, an attempt was made for the identification of quantitative trait loci (QTL) conferring cold stress tolerance in germplasm of rice, identi-

fication of genomic SSR among intervals of major reported QTLs and their association with cold stress tolerance. In this study, there were total 30 genotypes, which have shown a germination of 80%, or above along with a minimum 10 cm mean height of seedling and the score obtained by these genotypes were 1 and 3 (Table 2).

Among these 30 genotypes total 13 genotypes got score 1 and showing dark green color of seedling, 17 genotypes scored 3, 11 genotypes scored 5, 7 genotypes scored 7 and 4 genotypes scored 9. IR-28 was among the genotypes which got the score 1 and it also reported cold tolerant line while IR-50 got score 9 and it is also reported highly cold stress susceptible line. Germination of highly resistant genotypes i.e. with score 1 was in a range of 81%–90% and their seedling mean height was in a range of 10.9 cm—15.2 cm and all having dark green color of seedlings. Minimum germination i.e. 81% and minimum mean seedling height, i.e. 10.9 cm in these resistant genotypes was shown by K-332 and Sasyarce respectively. Two genotypes namely IR-28 and Rasi had shown germination of 90%, which is considered excellent performance under cold stress conditions, IR-28 also shown the seedling mean height of 15.1 cm which depicts the highly tolerant nature against cold stress. One more genotype Khiya-Boro, which had germination 86% and shown highest mean height of seedling which is 15.2 cm, is supposed to be promising under low temperature stress, similar results were obtained by in China while working on stress tolerance in local rice germplasm [3].

Among those genotypes which are susceptible to low temperature stress, four genotypes shown less than 40% germination, these are GS-3, Hira, Deval and Jattoo, while there were total nine genotypes which had shown the mean height of seedling less than 7 cm and these are GE-3, Deval, GS-1, Jattoo, GS-2, Matali, Varalu, Tulasi and Begami. Seedling color of these genotypes was varying from yellow to brown, and score were 5 to 9. Among these susceptible genotypes GS-3, Matali and Begami could not stand against cold treatment and died. One more genotype, P-48 was also showing high susceptibility for cold stress and died after cold treatment, however it had shown germination of 65% and seedling mean height

of 8.7 cm.

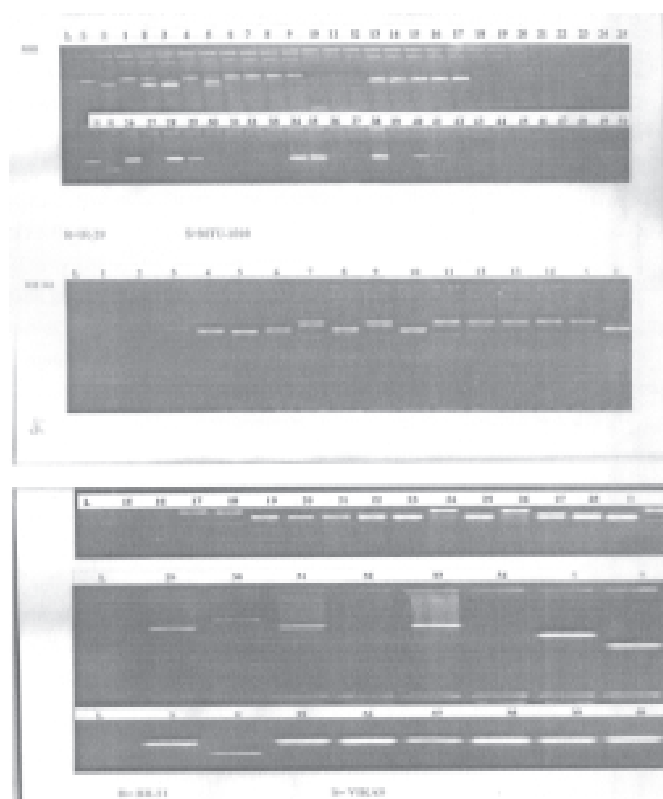
So in this study it had been found that total 13 genotypes having high tolerance and 16 genotypes having medium tolerance against low temperature stress. Among rest 23 genotypes 12 were medium susceptible and 7 were susceptible for cold conditions, 4 genotypes had shown high susceptibility and could not stand against cold condition and died when exposed to low temperature.

#### Identification of Quantitative Trait Loci (QTL) present in studied germplasm of rice

Here in this study one of the important works was identification of QTLs present in studied rice genotypes. We had phenotypically analyzed total 52 genotypes of rice for their cold stress ability and got the results as described in previous section. Now it was necessary to confirm their cold stress tolerance ability at genetic level, therefore we had done the genetic analysis of these genotypes with available resistant and susceptible genotypes. In this genetic analysis, we had studied total 14 QTLs and total 54 SSR markers. The results pertaining to the study showed that among 30 genotypes which showed resistance against low temperature stress, only 11 genotypes confirmed the presence of QTLs for low temperature stress tolerance.

In this study a varying relation of germination per cent, seedling height and seedling color with cold tolerance was found. In some genotype like in Kalinga -2, germination is more than 80% and mean seedling height is more than 10 cm but still they are showing susceptibility for cold stress. Several researchers have used per cent germination as the screening criterion for cold tolerance [4, 5]. However, the present indicates that, although per cent germination is a valuable morphological marker for low-temperature stress tolerance, its reliability depends on the nature and time of the onset of the stress.

Some cold injury symptoms like yellowing of seedling, stunted growth of seedlings, less germination, which were somewhat similar to our findings. It is reported that cold stress significantly reduced seed-



**Fig. 3.** PAGE Result for RM9 and RM341.

ling height development which is one of the most important criterions of cold tolerance [6]. Among 52 genotypes 22 showed susceptibility to low temperature stress with varying degree of effects in terms of germination per cent, seedling height and seedling color. At low temperature there might have been solutes leakage from seedlings to the water and it reflects damage to the plasma membrane and possibly also to the tonoplast. This leads the inhibition of photosynthesis and of respiration reflects injury to chloroplast and mitochondrial membranes. This led the yellowing of seedling as shown in our study and reported by other workers while working on wild tomato [7].

There were 4 genotypes which could not stand against cold treatment and died when exposed to low temperature. Research on fab 1 mutant of *Arabidopsis*

*thaliana* showed that the low temperature in range of 6°C to 8°C allowed the saturation of membrane fatty acids and resulted in loss of fluidity of plasma membrane and gradually caused the death of plant [8]. Similar reasons have been reported for the chilling injury to plants while working on cold acclimation [9]. Workers had showed damage to chloroplast and mitochondria membrane due to low temperature [10].

In this study total 14 QTLs were identified in genetic analysis of the cold tolerance. Out of these 14 QTLs, 9 were confirmed for their presence in particular genotypes showing tolerance to low temperature stress as shown in table 4.4 one of these QTL, qGR-1 showed its presence in two genotypes viz., IR-28 and P-16, qGR-1 provide cold tolerance at germination stage of rice. It is dispersed on chromosome 1 between RM9 and RM 7075 at a map distance of 45.8 cM [10]. qCSH2 another QTL which is present on

chromosome 2 between RM 262 and RM 263 at a map distance of 22.7 cM, provides resistance at seedling stage of rice and minimise the vigor loss due to low temperature. qCSH2 has been shown in linkage with another QTL, qSDW2 which is related to seedling dry weight [11]. In this study qCSH2 has shown its presence on 7 genotypes as given in table 4.4 and one of the most reliable QTL. Among other confirmed QTLs, qPSST-3, qCTS4-land qPSST-7 are other important one which showed their presence on total 4, 3 and 4 genotypes respectively. qPSST-7 provides cold tolerance at seedling as well as reproductive stage [12, 13]. In this study out of 52 genotypes, presence of QTLs was confirmed in 11 genotypes only as shown in Table 2. Among these genotypes IR-28, P-16, k-475 and Rasi showed the presence of 3 QTLs while BR-11 had shown the presence of 5 QTLs. BR-11 satisfy its morphological assessment and is best genotype among all cold tolerant genotypes used in this study.

The full intervals of the QTLs (or the regions covered by the major *G'* peaks) were all very wide, ranging from 8.68 Mb for *qCTSS-5* to 15.36 Mb for *qCTSS-2* and It can be seen that *qCTSS-5*; and the resistant alleles of *qCTSS-1*, *qCTSS-2* and *qCTSS-10* [14].

## Conclusion

Out of 52 genotypes used in present study, 30 are resistant for cold tolerance. Among those 30 genotypes, 13 are highly resistant and can be used in farmer's field. Among 13 highly resistant genotypes, BR-11 is best and can be used as donor parent in a variety improvement program. Out of 54 SSR markers selected among intervals of major reported QTLs, 25 showed to be strongly associated with cold tolerance. Some genotypes e.g. Kalinga-2, shown promising morphological parameters are in an intermediated phase of resistant, susceptible, and prone to yield loss if stress condition occurs. Among the studied 52 genotypes, QTL qCSH2 was confirmed in genotypes.

## References

1. IRRI news (2010).
2. INGER genetic resource center (1996).
3. Gonzales J (1996) Advance in research on cold-tolerance in rice. *Jiangsu J Agric Sci* 7 : 52—56.
4. Nilanjaya TR, Singh IRP, Singh NK (2003) Evaluation of germination, cold tolerance, and seedling vigor of boro rice germplasm. *Int Rice Res Notes* 28 : 19—20.
5. Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y, Takeuchi Y (2004) Mapping of quantitative loci controlling low temperature germinability in rice (*Oryza sativa*). *Theoretical and Applied Genetics*. 108 : 794—799. DOI:10.1007/s00122-003-1509-4
6. Farrell C, Fox KM, Williams RL, Fukai S (2006) Genotypic variation for cold tolerance during reproductive development in rice: Screening with cold air and cold water. *Field Crop Res* 98 : 178—194.
7. Hu Y, Wu Q, Sprague SA, Park J, Oh M, Rajashekar CB, Koiwa H, Nakata PA, Cheng N, Hirschi KD, White FF, Park S (2015) Tomato expressing *Arabidopsis* glutaredoxin gene *AtGRXS17* confers tolerance to chilling stress via modulating cold responsive components. *Horticulture Research* 2, Article number: 15051 (2015), doi:10.1038/hortres.2015.51
8. Xu Z, Wu W (2013) Comparative and joint analysis of gene expression profiles under drought and rewetting in *Arabidopsis*. *Genetics and Mol Res* 12 : 3622—3629.
9. Bai B, Wu J, Sheng WT, Zhou B, Zhou LJ, Zhuang W, Yao DP, Deng QY (2015) Comparative Analysis of Anther Transcriptome Profiles of Two Different Rice Male Sterile Lines Genotypes under Cold Stress. *Int J Mol Sci* 16 : 11398—11416; doi:10.3390/ijms160511398
10. Cruz RP, Sperotto RA, Cargnelutti D, Adamski JM, Terra TF, Fett JP (2013) Avoiding damage and achieving cold tolerance in rice plants. *Food and Energy Security* 2 : 96—119.
11. Wang Z, Wang JI, Bao Y, Wang F, Zhang H (2010) Quantitative trait loci analysis for rice seed vigor during the germination stage. *J Zhejiang Univ Sci B (Biomedicine & Biotechnology)*. ISSN 1673-1581 (Print); ISSN 1862-1783 (Online).
12. Han L, Qiao Y, Zhang S, Zhang Y, Cao G, Kim J, Lee K, Koh H (2007) Identification of Quantitative Trait Loci for Cold Response of Seedling Vigor Traits in Rice. *J Genetics and Genomics (Formerly Acta Genetica Sinica)* 34 : 239—246.
13. Suh JP, Lee CK, Lee HK, Kim JJ, Kim SM, Cho YC, Park SH, Shin JC, Kim YG, Jena KK (2012) Identification of quantitative trait loci for seedling cold tolerance using RILs derived from a cross between japonica and tropical japonica rice cultivars. *Euphytica* 184: 101—108. DOI 10.1007/s10681-011-0575-y
14. Su CF, Wang YC, Hsieh TH, Lu CA, Tseng TH, Yu SM (2010) A Novel MYBS3-Dependent Pathway Confers Cold Tolerance in Rice. *Pl Physiol* 153 : 145—158.