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### GENETIC ANALYSIS AND MARKER ASSISTED SELECTION (MAS) OF COLD TOLERANT RICE

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### ABSTRACT

The present investigations have helped to establish clear-cut identity of all the cultivars under consideration which will be of great utility for the protection of Plant Breeders' Rights. Six markers (RM556, RM562, RM594, RM7075, RM1287 and RM3843) were used to evaluate BPR1 and BPR2 lines with donor parent BRRI dhan36 by amplification of the DNA sequence to cold tolerance. The bands obtained BPR1 and BPR2 lines were compared to the band obtained from cold tolerance BRRI dhan36. The BPR1 and BPR2 lines having similar banding pattern to BRRI dhan36 were considered as cold tolerant. So, cold tolerant QTL has been introduced to these two lines. The average growth duration of BPR1, BPR2, BRRI dhan28 and BRRI dhan36 were 149,148,148 & 145 days with the average yield of 6.50 t/ha, 7.10 t/ha, 7.20 t/ha & 6.10 t/ha respectively. According to all consideration, BPR2 produced highest yield (7.10 t/ha) and cold tolerant performance which was higher than the other genotypes. The objective of this study was to markers related to cold tolerance that can be used to facilitate marker-assisted breeding and evaluation of genotypes in the cold prone environments under the management practices of researchers.

### Keywords: MUS, Extraction, Cold, QTL, Rice

### **INTRODUCTION**

Rice (Oryza sativa L) is the preferred staple food of Bangladesh but the state is a chronically rice-deficit because of floods, cold and/or climatic change. Rice is the most extensively cultivated cereal crop in Bangladesh which covers about 74% of the total cropped area [1]. The total production of rice also has plateaued over recent years with the gradual decrease of rice cropgrowing area because of expansion of other crops, other enterprises and development of infrastructure. The static production of rice is still attributable to the lack of suitable improved cultivars for different agro climatic conditions, particularly unfavorable ecosystems [5]. Low temperature or cold stress is one of the major constraints of rice production and productivity in temperate rice-growing countries and high-altitude areas in the tropics. Cold stress cause seedling mortality and spikelet sterility and eventually, significant yield losses [7]. Fetility as the main index of cold tolerance, addition to morphological and physiological evaluation has made great results in rice cold tolerance germplasm screening and breeding. Among the rice-growing ecosystems, the cold areas are the most challenging due to the prevalence of many abiotic and biotic stresses. Cold is the most important abiotic stress for northern part in Bangladesh. So

far only one high yielding in Bangladesh BRRI dhan36 is reported cold tolerant but cold tolerance is very low. Identification of new genetic sources of cold tolerance is very important to enable rice breeders to develop and grow high yield cold-tolerant rice cultivars in temperate regions.

Since January is the coldest month in Bangladesh have to tackle cold-related diseases affecting their crops. Large number of rice farmers suffers significant losses every year as none of the existing varieties can cope with extreme cold. As the development of molecular markers and linkage maps progresses, marker-assisted selection becomes an effective way to breed cold tolerant cultivars. Many coldtolerance related QTL have been identified in the past 20 years. The *qCTP11* is related to cold tolerance at the germination stage; and *qCtss11* and *qCTS4a* are related to cold tolerance at the seedling stage. Because breeding for abiotic-stress resistance is urgently needed the progress in identification of cold tolerance-related QTL has been a significant development for facilitating molecular marker-assisted selection (MAS). The objective of this study was to markers related to cold tolerance that can be used to facilitate marker-assisted breeding and evaluation of genotypes in the cold prone environments

under the management practices of researchers. Marker assisted backcrossing (MABC) is one of the key components for breeders that facilitates the use of the results of genomics for farmers' benefit [3]. This practical example of marker assisted selection clearly illustrates the superiority of using MAB compared to conventional backcrossing because obtaining such a small donor region within only a few backcross generations would be impossible using conventional methods [2].

### **MATERIALS AND METHODS:**

The following treatments were applied to evaluate cold tolerance at different growth stages:

### **Budding stage**:

Evaluation of cold tolerance at the budding (germination) stage followed protocols adopted at IRRI. To ensure complete dryness and to break any dormancy seeds were placed in a calorstat oven at 45–50°C for 48 h. Ninety well developed seeds of each accession were divided equally, placed in three dishes with wet filter papers, and incubated at 30°C. When shoots were about 5 mm long, the germinating seedlings were subjected to cold treatment at 2°C for 3 days, and then moved to a sunny indoor environment where the temperature was higher than 20°C to ensure normal growth. Seedling survival rates were assessed after 7 days

and cold tolerance evaluation indices were determined as: Seedling survival rate = Surviving seedlings/Budding seeds × 100%.

### Seedling stage:

Seeds were dried and soaked as above and then germinated at 30°C for 7 days. Fifty uniformly germinating seedlings of each line (1 cm radicle, 0.15 cm plumule) were planted in a ceramic pot and grown to the 3–4 leaf stage at 28°C/24°C, after which they were moved to a 5°C growth cabinet for 7 days. Seedling death rates after six days were used to calculate cold tolerance indices [6].

## Genomic DNA extraction and confirmation:

DNA was extracted from young leaves of 21-days-old plants using the Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method at Biotechnology Lab., Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The simplified mini scale procedure for DNA isolation in PCR analysis developed at IRRI [4] was followed. Confirmation of DNA samples were evaluated qualitatively using agarose gel electrophoresis (Fig-1). The quality of the isolated DNA in the protocol was sufficient for PCR analysis.SSR marker genotyping carried out the was development of cold tolerant varieties. Six markers (RM556, RM562, RM594.

RM7075, RM1287 and RM3843) were used for PCR analysis.

### Adaptability of newly developed lines

Two (BPR1 and BPR2) cold tolerant high yielding genotypes along with two standard check varieties (BRRI dhan28 and BRRI dhan36) were evaluated at Rangpur region in the northern part of Bangladesh in the real cold prone environments of the farmers' field. The experiment was laid out in RCB design with three replications. The seed bed management was done in order to raise healthy seedlings. Around forty days old seedlings were transplanted using 2-3 seedlings per hill with the spacing of 20 X 20 cm. The unit plot size was 5.4 m x 12 rows. . Fertilizer doses applied were 270:130:150:120:15 kg/ha Urea, TSP, MP, Gypsum, and ZnSO4. All the fertilizers except N were applied as basal before final land preparation. N fertilizer was applied as top dress with 3 equal splits at 15, 30 and 45 DAT. Furadan 5G was applied twice along with first and second top dresses of urea fertilizer. Standard crop management practices such as weeding, controlling disease and insect pests were followed as and when necessary.

### **RESULTS AND DISCUSSION:**

Among the various DNA based makers currently available, the set of microsatellite markers, used in the present study, provided a positive assessment of the ability of SSR markers in producing unique DNA profiles and establishing discrete identity of rice genotypes, which. otherwise, using were not possible morphological The traits. present investigations have helped to establish clear-cut identity of all the cultivars under consideration, which will be of great utility for the protection of Plant Breeders' Rights. Polymorphism survey of BRRI dhan36 germplasms was carried out using fifty five microsatellite markers. In this study, six SSR markers were evaluated in BRRI dhan36. All six SSRs were polymorphic in BRRI dhan36. Cold tolerant rice was analyzed using a highly repeatable PCR based fingerprinting assay known as Simple Sequence Repeat (SSRs) or microsatellites markers. The microsatellite DNA markers produce a higher level of polymorphism in The DNA rice. microsatellite enriched libraries were constructed using the standard procedure with some modifications. The quality of extracted genomic DNA was also checked by Polyacrylamide gel electrophoresis (PAGE).Six markers (RM556, RM562, RM594, RM7075, RM1287 and RM3843) were used to evaluate BPR1 and BPR2 lines with donor parent BRRI dhan36 by amplification of the DNA sequence to cold tolerance. The bands obtained BPR1 and BPR2 lines were compared to the band

obtained from cold tolerance BRRI dhan36. The BPR1 and BPR2 lines having similar banding pattern to BRRI dhan36 were considered as cold tolerant (Fig.2).So, cold tolerant QTL has been introduced to this two lines. The selected cold tolerant rice lines would be further tested in cold areas yield observed potentiality for to developing high yielding cold tolerant varieties. The present study has the potential applications in future breeding programme for the genetic improvement of cold tolerant rice.

The Rangpur regions at northern zone are coldest of Bangladesh in Boro season .An of validation trials experiment was conducted to evaluate with the performance of yield and yield contributing characters at Rangpur region. The genetic variability among Boro rice genotypes such as BPR1, BPR2 (two lines), BRRI dhan28 and BRRI dhan36 (two varieties) to demonstrate of duration, plant height and yield. The average growth duration of BPR1, BPR2, BRRI dhan28 and BRRI dhan36 were 149,148,148 & 145 days with the average yield of 6.50 t/ha, 7.10 t/ha, 7.20 t/ha & 6.10 t/ha respectively (Table1). The average plant height of BPR1, BPR2, BRRI dhan28 and BRRI dhan36 were 113,114, 95, 94 cm. Among the genotypes BRRI dhan28 were preferred by the farmers for their short growth duration and better yield but it was very cold susceptible. But especially, growth duration of BPR1 was 149 days and BPR2 was 148 days but it was cold tolerant lines. Above all consideration BPR2 produced highest yield (7.10 t/ha) and cold tolerant performance which was higher than the other genotypes. Farmers' opinion and eagerness were also for the evaluated Boro variety to grow for increasing total productivity. The cultivars were fingerprinted and the genomic relationship among cultivars was elucidated and the data will be useful to Bangladeshi rice breeders by improving the selection of parents for crosses, further exploiting the available genetic variation, assigning lines to specific heterotic groups, precisely classifying new accessions and preventing the variety from biopyracy.

Sl. No.	Genotypes	Duration (Days)	Plant Height (cm)	Yield (t/ha)
1	BPR1	149	113	6.50
2	BPR2	148	114	7.10
3	BRRI dhan28	148	95	7.20
4	BRRI dhan36	145	94	6.10

 Table 1: The performance of the entries, Boro2014 at Rangpur region



Fig. 1: Genomic DNA samples extracted from BPR1, BPR2, BRRI dhan36 and confirm by 1.2% agarose gel electrophoresis.

### 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 L



Fig. 2: Microsatellite profiles of three rice germplasms using six primers such as RM556, RM562, RM594, RM7075, RM1287, RM3843 with molecular weight marker (100 bp DNA ladder), Lane 1-6: BRRI dhan36; Lane7-12: BPR1; Lane13-18: BPR2

### **CONCLUSION:**

All consideration, BPR2 produced highest yield (7.10 t/ha) and cold tolerant performance which was higher than the other genotypes. The selected cold tolerant rice lines would be further tested in cold areas to observed yield potentiality for developing high yielding cold tolerant varieties.

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