



# Genetic Diversity Analysis of Rice Germplasm in Tripura State of Northeast India Using Drought and Blast Linked Markers



Alpana ANUPAM<sup>#</sup>, Jahangir IMAM<sup>#</sup>, Syed Mohammad QUATADAH, Anantha SIDDAIAH, Shankar Prasad DAS, Mukund VARIAR, Nimai Prasad MANDAL

(Central Rainfed Upland Rice Research Station, Hazaribagh 825301, Jharkhand, India; ICAR Research Complex for North Eastern Hill Region, Tripura Centre, Lembucherra, Tripura 799210, India; <sup>#</sup>These authors contribute equally to this study)

**Abstract:** We genotyped 74 rice germplasms including Tripura's local landraces, improved varieties, cultivars and breeding lines and other rice varieties using molecular markers for genetic diversity, drought QTLs, and blast resistance genes. The number of alleles per locus ranged from 2 to 5 with an average of 2.9. The polymorphic information content value per locus ranged from 0.059 (RM537) to 0.755 (RM252) with an average of 0.475. Cluster analysis based on 30 simple sequence repeat markers revealed 5 clusters and also indicated the presence of variability within the rice accessions. The drought QTL *qDTY2.1* was found in 56.0% of germplasms and *qDTY1.1* was detected in only 6.8% of the germplasms. Out of seven rice blast resistance genes screened, only two rice varieties, RCPL-1-82 and Buh Vubuk (Lubuk), were positive for four blast resistance genes while only Releng possessed two blast resistance genes. Among 74 rice germplasms, only three accessions, Releng, RCPL1-82 and Buh Vubuk (Lubuk), possessed both drought-related QTLs and blast resistance genes. Overall, the 74 indigenous rice genotypes showed low level of genetic diversity, which is in contrast to high level of genetic diversity among rice varieties in northeast India, where highlights the good farming practice, conservation of germplasms and the limitation of molecular markers employed in this study. The presence of both drought related QTLs and blast resistance genes in some of the germplasms can be useful in future breeding programmes.

**Key words:** drought; blast disease; quantitative trait locus; genetic diversity; gene; molecular marker; rice; germplasm

Northeast India is a centre of diversity for rice (*Oryza sativa* L.), which may serve as a valuable genetic resource for future crop improvement to meet the ever-increasing demand for food production. High rainfall, humidity, varied topography, and altitude have made the region rich in both floristic and crop diversities. Tripura is one of the important rice growing states of the northeast region and 75% of its cropped area is devoted to the production of rice. The region has been found to be the richest reservoir for genetic variability in agri-horticultural crops. Biotic as well as abiotic stress results in low productivity of rice in this region (Das et al, 2011). Farmers in drought-prone

areas cultivate either high-yielding varieties with good grain quality that are drought susceptible or low-yielding traditional ones that are drought tolerant but have poor grain quality and less input-use efficiency. High incidences of blast and brown spot have been observed in drought-prone areas. Several studies have shown that indigenous crop varieties traditionally cultivated and maintained by farmers contain high level of genetic diversity and can serve as potential genetic resources for improving yield, resistance to pests and pathogens, and agronomic performance (Choudhury et al, 2013). These traditionally cultivated diverse landraces are generally considered as important

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**Corresponding author:** Nimai Prasad MANDAL ([npmandal@hotmail.com](mailto:npmandal@hotmail.com))

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natural resources to meet the food demand in the present scenario of climate change (Pusadee et al, 2009).

Rice varieties grown in Tripura are susceptible to multiple blast races and of serious concern. In Northeast India, mainly Tripura state, rice blast is endemic causing yield loss ranging from 40% to 46% (Ngachan et al, 2011). Interestingly, only a few of the genes identified/cloned are effective against the different lineages prevalent in the region. Mahender et al (2012) demonstrated the presence of six to seven genes related to high level of resistance in rice accessions from the northeast state of Manipur. The increased frequency of drought in upland areas threatens rice production and demands the development of rice varieties which are capable of high yield under stress. The dryland cultivated rice varieties show adaptations to a wide range of ecological conditions including low levels of soil moisture in areas at high altitudes (Choudhry et al, 2013).

Molecular markers are the new approaches for discovering and tagging novel genes and alleles. These technologies can be effective in breeding programs through their use in marker-assisted selection (MAS). MAS for yield-related QTLs under drought stress has been effective in rice (Bernier et al, 2009b; Vikram et al, 2011). Whole plant selection for yield under both drought and non-stress conditions has successfully identified rice genotypes with high yield in upland condition (Verulker et al, 2010). Recent efforts to identify major QTLs with a large and consistent effect on grain yield under drought condition have marked a new strategy (Bernier et al, 2007; Kumar et al, 2007; Venuprasad et al, 2009; Vikram et al, 2011). Most of the QTLs have shown a similar effect in diverse environments (Bernier et al, 2009a). Zhang et al (2010) explained that the use of random markers for assessing genetic diversity might not reflect the functionally useful variations prevalent at the coding regions of the genome, a crucial requisite for the breeding programs, where the diversity occurring at identified 'heterotic loci' should be taken into account for selection of suitably diverse parental lines. In rice, diversity analysis through molecular markers is abundant, however, allelic diversity linked with drought tolerance in Tripura's rice germplasm is rare.

Molecular genetic markers are now widely used to characterize gene bank collections that contain untapped resources of distinct alleles which will remain hidden unless efforts are initiated to screen them for their potential use and function. The

abundance of single nucleotide polymorphisms (SNPs) have made it an attractive tool for allele mining and MAS. SNPs can be detected using allele-specific PCR primers and typed by the presence or absence of PCR amplified products on standard agarose gels. In this study, local landraces from Tripura, improved varieties, cultivars and breeding lines were analyzed for genetic diversity using drought-related simple sequence repeat markers. The aim of this study was to assess genetic diversity among indigenous rice varieties in the Tripura's germplasm, screen the drought tolerant QTLs with drought tolerant linked markers, and identify the different blast resistance genes in a collection of 74 rice germplasms from Tripura using PCR-based SNP markers.

## MATERIALS AND METHODS

### Rice materials and DNA isolation

A total of 74 rice germplasms consisting landraces, improved varieties, cultivars and breeding lines from Tripura, India were used for the study at Central Rainfed Upland Rice Research Station (CRURRS), Hazaribag, Jharkhand, India. Seeds were germinated on moistened filter paper at 30 °C in a dark plant growth chamber. After 2 d, seedlings were planted in pots and shifted to green house. After 4 weeks, leaves were collected and stored at -80 °C. Genomic DNA was isolated from leaves using a modified cetyl trimethyl ammonium bromide method (Doyle and Doyle, 1987). Purity and concentration of genomic DNA were checked in Nanodrop system 2000c (Thermo, USA). Quality of DNA was checked in 1% agarose gel. DNA was diluted upto 100 ng/μL with Tris-EDTA buffer and stored at -20 °C.

### Markers specific for rice blast R genes and linked to yield under stress

The germplasms were screened for the presence of seven major blast resistance (R) genes, *Piz*, *Piz-t*, *Pita*, *Pita-2*, *Pib*, *Pi2* and *Pi9*, using a set of six SNP and gene-based markers, which are selected according to Variar et al (2009) and Imam et al (2014a). PCR-based allele-specific SNP and gene-based sequence-tagged site markers were obtained for identification of blast resistance genes in the selected germplasms. The 30 selected SSR markers dispersed across the genome linked with different drought QTLs were used to analyze genetic diversity among the 74 rice germplasms from Tripura. The germplasms were also

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