Linkage analysis for drought tolerance in kharif rice of Assam using microsatellite markers

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Drought stress in rainfed ecosystem significantly limits the production of Ranjit, the most predominant high yielding rice variety of Assam. A mapping population comprising 85 F4 individuals between Ranjit and a drought tolerant cultivar, ARC10372 was developed and genotyped with 80 microsatellite markers in order to understand the genetic basis of drought tolerance. The linkage map constructed based on a framework linkage map using these markers showed that the marker loci were distributed across 12 chromosomes spanning a distance of 273.4 cM with an average interval of 3.41 cM between marker loci. Most of the marker loci were found to be in good fit with the expected Mendelian segregation ratio; however, thirteen marker loci in total showed segregation distortion on six chromosomes. The linkage map generated in the study will facilitate mapping of quantitative trait loci imparting drought tolerance in rice of Assam and their map-based cloning.

Keywords: Drought, Linkage map, Rice, Simple sequence repeats (SSR)

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Agricultural production in 21st century is greatly hampered by drought or moisture stress that prevents the crop plants to demonstrate their full genetic potential. According to the IPCC (Intergovernmental Panel on Climate Change) report, more than 60% yield losses could alone be accounted to drought over the last 50 years, highlighting the tremendous impact of drought on agricultural production. Drought stress in field crops is one of the major constrains in Agricultural production that perturbs the crop plants to demonstrate their full genetic potential. As a cereal crop, rice is the second most important crop after wheat and constitutes an integral part of human diet mainly in Asian countries. There is a need to increase its production to 2,000 million metric tonnes by the year 2030, however, a substantial decline in yield has been reported in majority of the rice growing areas in the world as a result of frequent occurrence of drought in the face of rising climatic uncertainty. Nearly one third of the global rice growing area encounters periodic drought resulting in annual yield loss of 18 million tonnes amounting to US $3600. In Asian countries, drought affects 23 million hectares of rainfed rice in South and Southeast Asia. In fact, yield reduction up to 40% has been reported in rice under severe moisture stress conditions in India.

Northeast India (NE) particularly Assam is a rich source of genetic diversity in rice. The farmers of this region still use their elite cultivars which not only suit their taste but also provide crop security. Rice is a part of all meals in Assam. Traditional breakfast consists of chira with yoghurt and jaggery and special class of rice preparations, called ‘pithas’ are generally made only on traditional occasions. Due to an increase in demand of feeding, Assam Agricultural University is trying to develop high yielding rice varieties using the advance breeding strategies and the biotechnological approaches. Breeding for drought stress remains a challenging task due to its complex genetic mechanism and unpredictable occurrence. In recent years, the analysis of genomic regions using genome wide molecular markers has resulted in genetic dissection of drought tolerance with enhanced resolution across a number of crop species. Linkage mapping with simple sequence repeat (SSR) or microsatellite markers allows genetic analysis of complex quantitative traits which enables the localization of genes and used extensively in rice because of high level of polymorphism. Further, it provides a framework with physical maps based on chromosome translocation and also allows the map-based cloning of genes responsible for economically
important traits. Among the high yielding rice varieties released by the Assam Agricultural University and recommended for this region, the variety, Ranjit is the most accepted variety by the farming and tribal community. However, the variety is susceptible to drought extremes and its yield is reduced to a significant level when it encounters drought due to which the cultivation of Ranjit is problematic in the rain shadow areas of Assam, particularly. Therefore, elucidation of the genetic basis for drought tolerance/susceptibility in Ranjit background will enhance our understanding to manipulate in a breeding programme and cater to the need of the farming community. Here, we report genetic linkage map in F2 derived F4 population between Ranjit and ARC10372 based on a framework linkage map and estimate the segregation statistics.

Methodology

Plant materials

The mapping population comprised 85 F4 lines derived from a cross between Ranjit × ARC10372. ARC10372 was used as a drought tolerant parent and a widely cultivated HY rice variety of North East India, Ranjit was used as the susceptible parent. The parents were crossed to raise F1s. True F1s were identified using polymorphic SSR marker and selfed to raise the F2 plants. The F2 plants were harvested and bulked to raise F3 population. Seeds of 85 F3 lines were developed in this way and the population was advanced to F4 generation which has been ultimately used as mapping population in this study.

Genotyping and construction of genetic linkage map

Plant genomic DNA was extracted from young leaf tissue for each of the 85 F4 lines along with parents, as described in Singh et al., 2003. The quality of DNA extracted was checked by electrophoretizing the samples using 0.8% agarose gel and quantified using Nanodrop® ND-1000 Spectrophotometer. Polymerase chain reactions for SSR analysis were carried out under standard conditions for all the primer pairs using 1 U of Taq polymerase with 1X polymerase chain reaction buffer (100 mM Tris-HCl at pH 9, 500 mM KCl, and 15 mM MgCl2), 2.5 mMdNTP, 3 mM MgCl2, 20 pM of each primer, and 50 ng of DNA template with a final reaction volume of 10μL. The PCR reactions were denatured at 94°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. The final extension was 72°C for 5 minutes. The amplified products were resolved in 3.5% agarose gel stained with ethidium bromide. The polymorphic SSR markers reported after the parental polymorphism survey, by Verma et al. were used for genotyping of 85 F4 plants in order to study the segregation pattern of markers.

Statistical analysis

The PCR fragments were scored for presence and absence. Spurious and missing data were repeated for verification. Chi-square test was conducted to compute the segregation pattern of each SSR marker against the expected ratio in F4 generation at 0.01 probability level. Linkage analysis was performed by using Join Map software. It develops the linkage map in sequential steps, and a numerical search is made at each step for best fitting order of loci. It uses weighted least squares method for estimation of map distance from recombination frequencies. Kosambi’s mapping function was used by considering the number of double crossovers and interference to establish the maps. Marker loci were grouped by two-point linkage analysis with a logarithm of odds ratio (LOD) threshold of 3.0.

Result and discussion

Segregation analysis

For each segregating marker, a chi-square analysis was performed to test the deviation from the expected segregation ratio for the mapping population. Here the degree of freedom for this test was found to be 2 as we had three parameters, a (parental type – Ranjit), b (parental type – ARC10372) and h (heterozygote). For each marker, the observed segregations were tested against the expected Mendelian ratio of F4 generation. Segregation analysis data showed that 67 markers followed the Mendelian ratio of the segregation in the progeny (Table 1), whereas 13 markers significantly deviated from it (p<0.01). Maximum number of 4 distorted SSR markers was found on chromosome 9 and minimum of one marker on chromosome 3,5,6,7 and 10. However, segregation distortion was not observed on chromosome number 1, 4, 9, 8 and 12. A total of 7 markers on chromosome 1, 8 markers on chromosome 4, 7 markers on chromosome 8 and 6 markers on chromosome 12 were found to be in good fit with the expected Mendelian ratio. Occurrence of distorted segregation ratio is quite common for a pulled population with minimum skews. Elimination
of gametes due to the effect of a lethal factor located adjacent to the marker loci is one of the main reasons for segregation distortion. It may also occur due to statistical bias, chromosome loss, incompatibility genes, chromosome arrangements or non homologous pairing. However, the observed segregation distortions may be due to the small population size, i.e., only 85 F₄ plants. It is a problem often encountered in mapping studies conducted on small mapping populations. Segregation distortion had very little effect both on marker order and map length. When the skewed markers were removed from the linkage analysis, there was a decrease in the number of linked markers and in the total length of some chromosomes, resulting in low coverage of the genome. Moreover, estimation of recombination frequencies among co-dominant markers is less affected by segregation distortion than that of dominant markers. Therefore, all the skewed markers were retained for linkage analysis.

### Linkage analysis of SSR markers

The genetic map was comprised of 12 linkage groups, equalling the number of rice chromosomes, spanning a total of 273.4 cM of the rice genome at an average marker density of 3.41 cM (Fig.1). The linkage groups ranged in size from 10.1 to 29.5 cM, with a mean value of 22.78 cM. The number of markers on each linkage group ranged from 5 (LG3 and LG 5) to 8 (LG2, LG4 and LG 6). The length of each linkage group was determined by the total number of markers and the recombination frequency among them.
group ranged from 10.1 cM (LG12) to 34.5 cM. The average distance between adjacent markers ranged from 1.68 cM (LG12) to 4.52 cM (LG3) respectively. Marker pairs with a LOD score above a critical ‘3’ were considered to be linked whereas those with a LOD score less than ‘3’ were considered unlinked. Higher critical LOD values will result in more number of fragmented linkage groups, each with smaller number of markers while small LOD values will tend to create fewer linkage groups with large number of markers per group. It was claimed that, two to four markers on a chromosome of about 100 cM distances could provide adequate coverage of the genome in backcross programs through a marker-assisted breeding simulation study.\(^{16}\)

**Conclusion**

The present genetic map of rice can be successfully utilized further for introgression of various QTLs identified under drought stress. The map described in the present study is an additional source of information for the genetic study of kharif rice of Assam, including QTL mapping and map based cloning of important genes imparting drought tolerance. The addition of novel polymorphic microsatellite markers to the constructed map will make it useful for molecular mapping of drought tolerance in other breeding populations of rice also.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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