

Original Research Article

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Impact of LOD Score and Recombination Frequencies on the Microsatellite Marker Based Linkage Map for Drought Tolerance in *Kharif* Rice of Assam

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ABSTRACT

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Intermittent drought stress in rainfed ecosystem significantly limits the production of Ranjit, the most predominant high yielding rice variety of North East India. In order to understand the genetic basis of drought tolerance a mapping population comprising 85 F₄ individuals between 'Ranjit' and a drought tolerant cultivar, ARC10372 was developed and genotyped with 80 microsatellite markers. 7 possible linkage groups were analysed by changing the LOD values and the recombination frequencies in the Join map 4.0 software package. Only 3 linkage groups were considered out of the 7 linkage groups as the map was calculated at LOD threshold 3.0 and above. It could be concluded that, 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 few linkage groups with large number of markers per group.

Introduction

Rice is one of the most widely grown cereal crops in the world and is the staple food of more of the world's population (Chen *et al.*, 2013). In 2008, a total of 661 million tons of rice was produced from 155.7 million ha (IRRI, 2009). Rice is cultivated in a wide range of environments such as irrigated, rainfed upland, rainfed lowland, flooded and saline, and it faces multiple biotic and abiotic challenges. According to the USDA reports, in 2008, more than 430 million metric tons of rice was consumed worldwide and about 3.5 billion people depend on rice for more than 20 per cent of their daily calories. It is estimated that the demand for rice will be 2,000 million

metric tons by 2030 due to population increment (FAO, 2002) and according to another report, production of rice must increase by 60 per cent by the end of 2025 (Chen *et al.*, 2013).

Drought mitigation in rice production to ensure food security to the rising population in Asia can be achieved through development of drought-tolerant rice varieties with higher yields. In Asia, drought stress is a major threat to both rainfed lowland (46 Mha) and upland (10 Mha) rice production, affecting the yield stability (Pandey *et al.*, 2007). In Assam, total cultivated area is approx. 30 lakh hectares. Among them 23.24 lakh hectares of land is under paddy cultivation and usually most of

them are affected by intermittent drought (Directorate of Economics and Statistics, Government of Assam). Ranjit is the leading variety of Assam which is a drought susceptible high yielding variety. ARC 10372 is a drought tolerant moderately yielding variety which matures earlier than the Ranjit. Linkage analysis in a mapping population derived from cross between Ranjit and ARC 10372 will help us to identify the genes contributing to drought tolerance in rice and their relative contribution to the very important trait.

Materials and Methods

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 Gupta *et al.*, 2003. The quality of DNA extracted was checked by electrophoretting the samples using 0.8 percent 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), 2.5mM dNTP, 3 mM MgCl₂, 20pM of each primer, and 50 ng of DNA template with a final reaction volume of 10µL. The PCR reactions were denatured at 94⁰C for 5 minutes followed by 35 cycles of 94⁰C for 1 minute, 55⁰C for 1 minute and 72⁰C for 1 minute.

The final extension was 72⁰C for 5 minutes. The amplified products were resolved in 3.5 percent agarose gel stained with ethidium bromide. The polymorphic SSR markers reported by Verma *et al.*, 2017 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 JoinMap 4.0 (Stam *et al.*, 1993) software. Markers were assigned to linkage groups using the odds ratios and grouping was done by considering the SCL (Strongest cross link) values. 7 possible linkage groups were observed (Table 1).

The linkage parameters like weak linkages with a recombination frequency larger than 0.45 or a LOD smaller than 0.05 or strong linkages with a recombination frequency smaller than 0.01 or a LOD larger than 10 were set in the calculation options along with regression mapping algorithm of the software programme. Kosambi's mapping function was selected and the LOD scores were changed from 1.00 to 8.00 to calculate the map distance.

Results and Discussion

Increase in LOD threshold may decrease the possibility of linkage group establishment. But sufficient linkage was observed in the linkage groups 2, 3, 4, 6 and 7 to get the map distance at recombination frequency 0.40, 0.30 and 0.20. But Group 4, 6 and 7 were only considered as the map was calculated at LOD threshold 3.0 and above (Fig. 1). The markers RM72, RM335 and RM25 were put to the linkage group 2 of 35.6 mb length at LOD threshold 1.0 and 2.0. As per earlier work, RM25 was mapped on chromosome number 8 at a distance 38.1 mb (Cho *et al.*, 1998) and RM72 was mapped on chromosome number 8 at a distance 30.5 mb (*www.gramene.org*) and our results are in agreement with these results. However, the marker RM335 has been mapped on chromosome number 4 at a distance 5.4 mb (*www.gramene.org*), which is in the linkage group with markers from chromosome number 8 in the present study. The map was calculated at LOD threshold 1.0 and 2.0, due to which RM335 came to this group due to low stringency. This can also be explained if there has been any chromosomal

translocation in the population under study. This need to be verified by detailed wet-lab experimentations. Similarly, in linkage group 3, markers RM209, RM202 and RM167 were assigned to the map at 0, 28.7 and 51.9 mb respectively at LOD threshold 1.0. As per earlier work, all the markers RM209, RM167 and RM202 were mapped in chromosome 11 (Septiningsih *et al.*, 2003; Xiao *et al.*, 1998). As such, the results of the present study are more or less in agreement with earlier results. In linkage group 4, the marker RM336 and RM1132 were fall apart in 25.2 mb from each other and the other marker RM182 was assigned at 55.6 mb respectively. As per earlier work, RM336 was mapped in chromosome 7 at a distance 55.7, RM182 was mapped in chromosome 7 at a distance 54.8 mb (IRGSP, 2005) and RM1132 was mapped in chromosome 7 at a distance 23.9 mb (Gramene Annotated Nipponbare Sequence, 2009). In group 6, the marker RM19629 and RM253 were placed in a distance of 19.6 mb and RM253 was mapped in chromosome 6 at a distance 20.4 mb (Xiao *et al.*, 1998). As such, the results of the present study are in agreement with earlier results.

Fig.1 Linkage groups according to LOD scores with ARC10372× Ranjit-F₄ population (Left side of bar represents position of marker in mb and right side of bar represents SSR markers)

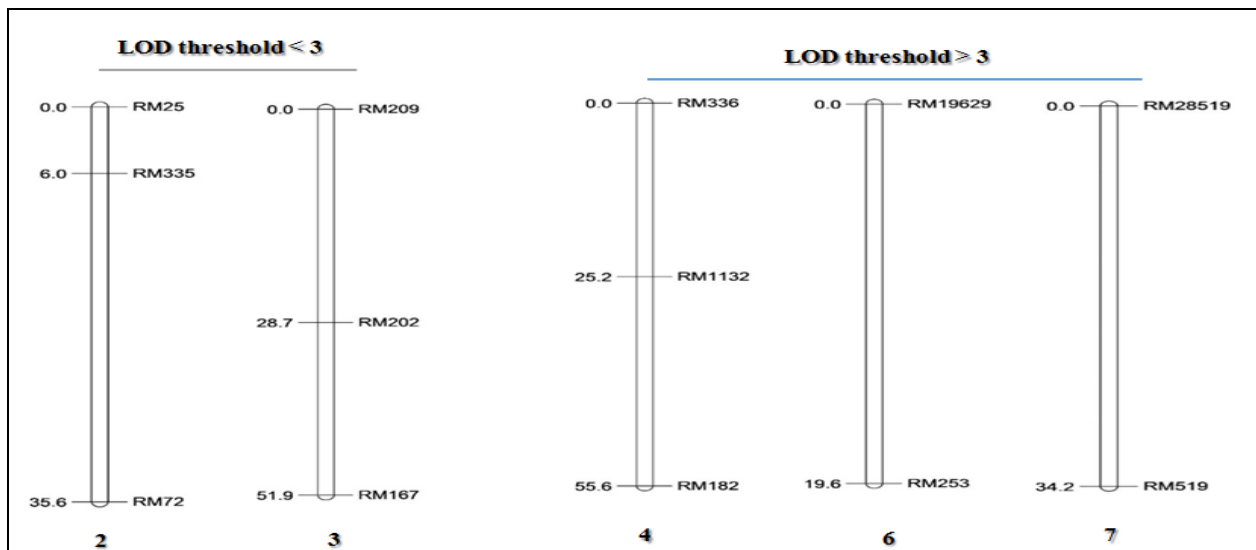


Table.1 Grouping based on LOD score showing SCL values

Nr	Group	Locus	Node	SCL-Nr	SCL-Locus	SCL-Node	SCL Value
3	1	RM24	4.0/1(4)	4	RM243	4.0/51(1)	2.3
25	1	RM273	4.0/1(4)	4	RM243	4.0/51(1)	1.6
7	1	RM5638	4.0/1(4)	4	RM243	4.0/51(1)	3.0
49	2	RM25	4.0/2(3)	47	RM429	4.0/36(1)	1.9
28	2	RM335	4.0/2(3)	39	RM253	4.0/6(2)	1.3
51	2	RM72	4.0/2(3)	15	RM530	4.0/14(1)	1.3
70	3	RM167	4.0/3(3)	72	RM206	4.0/31(1)	2.2
71	3	RM202	4.0/3(3)	43	RM125	4.0/34(1)	2.6
73	3	RM209	4.0/3(3)	4	RM243	4.0/51(1)	1.8
48	4	RM1132	4.0/4(3)	70	RM167	4.0/3(3)	1.8
45	4	RM182	4.0/4(3)	24	RM261	4.0/23(1)	1.2
46	4	RM336	4.0/4(3)	30	RM164	4.0/27(1)	1.3
35	5	RM141	4.0/5(3)	72	RM206	4.0/31(1)	2.1
31	5	RM169	4.0/5(3)	18	RM1256	4.0/17(1)	3.1
32	5	RM249	4.0/5(3)	34	RM574	4.0/28(1)	3.9
42	6	RM19629	4.0/6(2)	18	RM1256	4.0/17(1)	2.5
39	6	RM253	4.0/6(2)	19	RM1352	4.0/18(1)	2.1
80	7	RM28519	4.0/7(2)	76	RM235	4.0/13(1)	3.1
78	7	RM519	4.0/7(2)	53	RM256	4.0/38(1)	2.8

In group 7, the markers (RM28519 and RM519) were placed in 34.2 mb of length from each other in the map. As per earlier reports, both markers (RM28519 and RM519) were mapped in chromosome 12 at a distance 19 mb and 23 mb respectively (Gramene Annotated Nipponbare Sequence, 2009). So, the present genetic map of rice can be used further for introgression of various QTLs identified under drought stress. To construct a saturated linkage map, more number of markers are required.

As less number of markers were found polymorphic in the F₄ mapping population, the length of the linkage map as well as the interval size between the markers were reduced. Genetic maps with good genome coverage and confidence in locus order requires not only large numbers of DNA markers, but also the analysis of large numbers of individuals.

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