Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.)

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Among the abiotic stresses, drought is a serious limiting factor that reduces rice production and yield stability in rainfed ecosystems. Conventional breeding for drought resistance is slow in attaining progress due to poor understanding of genetic control of drought resistance. Molecular markers help in identification of quantitative trait loci (QTLs) associated with drought resistance traits and their indirect selection using marker assisted selection. But QTL mapping requires genotyping of large mapping progenies demanding time and labour. Bulked segregant analysis (BSA) serves as an alternative approach for rapid identification of markers associated with drought resistance traits. BSA was carried out to identify markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20/Nootripathu, two indica ecotypes with extreme drought response. The parents were screened for polymorphism using 1206 rice microsatellite primer pairs. Out of 134 SSR polymorphic primers between parents, three primers showed polymorphism between bulks. These three primers co-segregated among the individual RI lines constituting the respective bulks. The genomic regions flanked by these markers have been reported to be associated with several drought resistance component traits and will be useful in marker assisted breeding for drought resistance in rice.

Keywords: Bulked segregant analysis, drought resistance, microsatellite markers, rice (*Oryza sativa* L.).

RICE (*Oryza sativa* L.) is a dietary staple of more than half of the world[1] and 65% of the Indian population. It is grown globally on 153 million hectares (mha)[2] in a wide range of ecosystems under varying temperatures, altitudes and water regimes. About 45% of global rice area is under rainfed ecosystems[3]. In India, of the 44 mha of total rice area, 33% is in rainfed low lands and 15% in uplands[4]. The major breeding objective in these ecosystems is to improve drought resistance in rice plants but, little progress has been achieved in improving yield under stress due to poor knowledge of the genetic control of drought resistance. Yield improvements under drought stress can be achieved by selecting secondary traits contributing in drought resistance in a breeding programme[5,6] and this has been demonstrated for anthesis to silking interval in maize[7], water-use efficiency in wheat[8] and stay green in sorghum[9]. Several putative traits contributing to drought resistance have been reported in rice[10]. However, phenotypic selection for such traits is labour-intensive. Molecular marker technology serves as a tool for selecting such complex traits and allows breeders to track genetic loci controlling drought resistance traits, without having to measure the phenotype, thus reducing the need for extensive field testing over space and time[11].

Identification of DNA markers linked to drought resistance traits is usually carried out with a large population, each of which has to be genotyped with several markers. This is time and labour intensive and cost ineffective[12]. Bulked segregant analysis (BSA) is one such strategy in which the process of genotyping aids in reducing the sample size to two DNA samples[13] by grouping plants according to their high or low expression of a particular trait[14]. BSA measures the variation in pools of segregants that have sorted according to phenotype and uses the correlation to assign a likely map location. Markers linked to drought resistance traits have been identified using BSA in wheat[15] and maize[16]. The present study has been undertaken to identify molecular markers linked to drought resistance traits in rice using BSA.

The rice lines used in the study are a subset of the recombinant inbred (RI) lines of the cross between IR20, high-yielding drought sensitive variety with shallow root system and Nootripathu, a land race with thicker and deep root system adapted to drought prone rainfed ecosystems of Tamil Nadu, India. The population, consisting of 330 lines was previously tested for drought resistance under rainfed conditions in Agricultural Research Station, Paramakudi of Tamil Nadu Agricultural University[17]. Based on leaf rolling and leaf drying scores under water stress condition, 11 RI lines which performed well (low scores) and 12 RI lines which performed very poorly (high scores) were selected out of 330 RI lines and grouped into drought resistant and drought susceptible lines respectively (Table 1).

Genomic DNA was extracted from parents and the RI lines using cetyl trimethyl ammonium bromide using leaf tissues of 30-days-old seedlings following the protocol described by Gawel and Jarret[18] and treated with RNase to remove RNA contamination. The quantity and quality of DNA was determined by fluorometry and agarose gel (0.8%) electrophoresis with 1 μl of diluted genomic DNA samples and stained with ethidium bromide. After quantification, all the samples were diluted to 25 ng μl^{-1} for bulking and polymerase chain reaction (PCR).

Equal amount and the same concentration (25 ng) of DNA of the 11 drought tolerant and 12 drought susceptible RI lines were pooled into two separate bulks – drought resistant bulk (DRB) and drought susceptible bulk (DSB) respectively. These bulked DNA samples were used to

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Table 1. Recombinant inbred lines of IR20 × Nootripathu selected for BSA and their drought scores under rainfed condition in a field experiment

	Drought tolerant			Drought susceptible		
Sl No.	RIL #	Leaf drying	Leaf rolling	RIL #	Leaf drying	Leaf rolling
1	6	3.0	3.0	97	7.0	7.0
2	14	3.0	2.3	312	7.0	7.0
3	347	2.3	3.0	372	7.0	7.0
4	80	2.3	3.67	371	7.0	7.0
5	266	3.0	3.67	361	7.0	7.0
6	308	3.0	3.67	74	7.0	7.0
7	42	2.3	3.67	38	7.0	7.0
8	37	2.3	2.33	362	7.0	7.0
9	238	3.0	3.0	345	7.0	7.0
10	204	3.0	3.67	334	7.0	7.0
11	295	3.0	3.67	49	7.0	7.0
12	–	–	–	89	7.0	7.0
13	Nootripathu	3.0	3.0	IR20	7.0	7.0

Leaf rolling: 1–7 scale: 1, No rolling; 7, Fully rolled. Leaf drying: 1–7 scale: 1, Full green; 7, Completely dried.

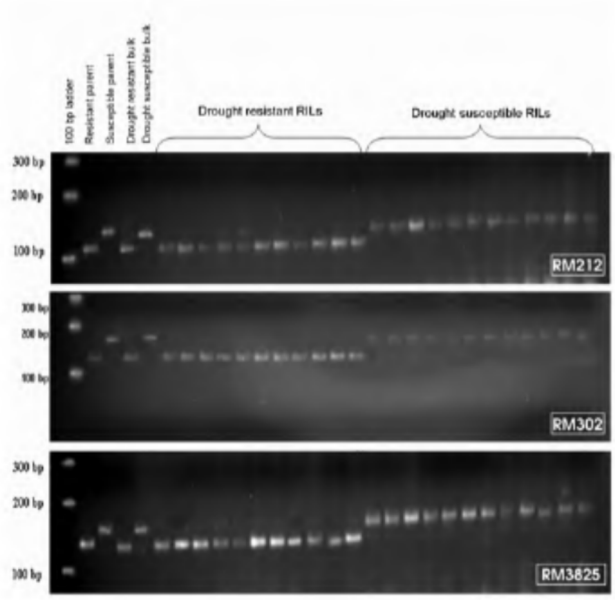

Figure 1. Alleles showing co-segregation of the SSR primers RM212, RM302 and RM3825 among individual rice lines and the bulks.

amplify the simple sequence repeats (SSR) region which showed polymorphism between parents. A total of 1206 rice microsatellite (RM) primers representing different chromosomes were selected randomly and used to amplify the SSR regions among the parents. The PCR products were resolved in vertical mini-gel electrophoresis with 12% polyacrylamide gel with ethidium bromide staining. Primers which showed polymorphism between the parents were tested for polymorphism among bulks. Primers, which showed polymorphism between drought tolerant and susceptible bulks, were checked for their co-segregation in the individual RI lines constituting the bulks.

PCR was performed in a total volume of 15 µl containing 10 × PCR buffer (1 × contains 10 mM Tris Cl, pH 8.8 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$), 15 pmol of each primer (Sigma Aldrich, USA), 25 ng of rice genomic DNA, 100 µM of each of the four dNTPs and 1 unit of *Taq* polymerase (Bangalore Genei, India) with sterile water. Thermal conditions such as initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 72°C for 1 min and a final extension of 72°C for 5 min were common to all. Variations were observed only for the annealing temperature. Amplified products were resolved on 3% agarose gels[19] with ethidium bromide staining.

Out of 1206 SSR pairs used, 134 pairs showed polymorphism between the parents. Out of 134, three primers alone showed polymorphism between the bulks. These three primers, viz. RM212, RM302 and RM3825 showed complete co-segregation among individual RI lines constituting the bulks (Figure 1). These three primers were located on chromosome 1 of rice between 135.8 and 143.7 cM[20]. The markers which co-segregated and linked to drought resistance were compared with the previously mapped quantitative trait loci (QTLs) for drought resistance traits in rice. This region has been found to be linked with several drought resistance traits such as plant height, biomass, deep root mass, leaf drying, relative water content, osmotic adjustment, basal root thickness, tiller number and deep root to shoot ratio[21], grain yield and panicle length, canopy temperature in these IR20/Nootripathu RI lines under drought stress in this laboratory[21–23] (Figure 2). This region was associated with relative water content (RWC) under stress in CT9993/IR62266 doubled haploid (DH) lines[5] and root length, root thickness and root weight in Bala/Azucena RI

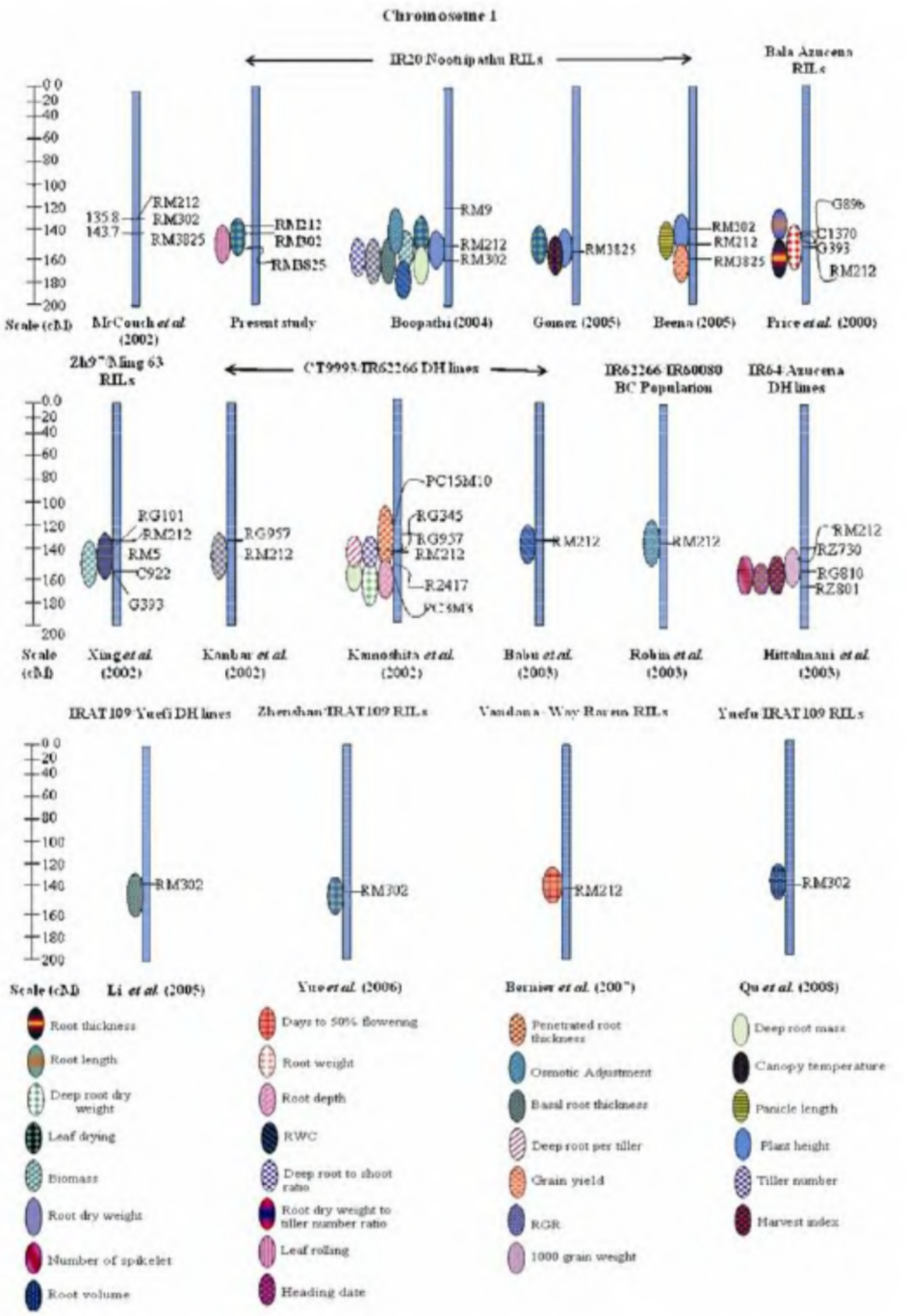

Figure 2. SSR primers linked to drought resistance traits identified using bulked segregant analysis in the present study and co-location of drought resistance QTLs in various rice lines.

lines of rice[24] (Figure 2). Kanbar *et al.*[25] reported this region to be linked to panicle length in CT9993/IR62266 DH lines and days to 50% flowering in Vandana/Way rarem RI lines[26] under stress. RM212 was linked to root depth, penetrated root thickness, deep root to shoot ratio, deep root dry weight, deep root per tiller and deep root mass to be associated with RM212 in CT9993/IR62266 DH lines[10] and a QTL for osmotic adjustment was reported to be close to this region in IR62266/IR60080 backcross progenies[27]. This region was found to be associated with root volume[28] and basal root thickness in IRAT109/Yuefi RI lines[29] and leaf drying in Zhenshen/

IRAT109 RI lines in rice[30]. Hittalmani *et al.*[31] reported that a genomic region of 7.9 cM (from 135.8 to 143.7 cM) on chromosome 1 was associated with drought resistance traits such as leaf rolling, number of spikelets, heading date and harvest index in IR64/Azucena rice DH lines. RM212 was linked to biomass and root dry weight in Zh97/Ming63 RI lines[32]. It is suggested that use of phenotype-based DNA pools might be successful in tagging QTLs of very large effect, but is unlikely to permit comprehensive identification of the majority of QTLs affecting a complex trait. DNA pools constructed from prior information should, however, be useful in identifying new DNA markers for regions of the genome known to contain QTLs of interest[33]. It is evident from our results that the genomic region RM212–RM302–RM3825 on chromosome 1 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice.

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