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Research Article

Identification of microsatellite markers linked to drought tolerance in rice (*Oryza sativa* L.) through bulked line analysis

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Abstract
Identification of donor parents and markers for traits conferring drought tolerance eventually hastens the breeding for drought tolerance in rice. The extent of variation for water stress indicators, physio-morphological and plant production traits were assessed by evaluating 36 rice genotypes under water stress condition. Significant variation was observed for these traits and thirteen drought tolerant and susceptible genotypes were selected and grouped as tolerant and susceptible bulks based on physio-morphological and biochemical characterization. Genomic DNA was isolated from these rice accessions and pooled, respectively into drought tolerant and susceptible bulks. Bulked line analysis was carried to identify microsatellite markers linked to drought tolerance in rice. Out of 200 SSR primers screened RM 1092; RM 129 and RM157B were polymorphic between the bulks and also co-segregated among the individual genotypes constituting the respective bulks. The genomic regions flanked by these markers have been identified to be associated with various drought tolerance traits in rice and can be useful for marker assisted selection for drought resistance improvement in rice. The genotypes identified as drought tolerant can be used us donars in drought improvement breeding programmes.

Key words
Rice, drought resistance, microsatellite markers, bulk line analysis

Introduction

Rice provides nutrition for more people in the world than any other crop (Phillips *et al*., 2005), especially in Asia, Africa and Latin America and grown world over on 154 million hectares annually under diverse hydrological conditions. Of which, about 28 per cent is grown in rainfed lowlands and about 13 per cent under upland condition (Singh, 2009:www.niaes.affrc.go.jp/marco/marco2009/english/W2-_Singh_P.pdf)**.** These areas experience water deficit due to uncertain and uneven rainfall and yields are seriously affected due to drought. The frequent occurrence of abiotic stresses such as drought and submergence has been identified as the key to the low productivity of rainfed ecosystems. A recent estimate on climate change predicts the water deficit to deteriorate further in years to come and the intensity and frequency of drought are predicted to become worse (Wassmann *et al*., 2009). From the beginning of the Green Revolution era in rice in 1960's till 2013, on 15 occasions, rice production in India failed to achieve the estimated production, drought was the cause of reduced production on 12 such occasions. Severe drought in the wet season during the reproductive stage adversely effect on rice production. Three most recent severe droughts in 2002, 2009 (http://www.dacnet.nic.in) and 2012 witnessed a significant reduction in rice as well as total food production in India.

Considering the frequent occurrence of drought, development of cultivars with inbuilt drought resistance will increase rainfed rice production. However, the progress in breeding for drought resistance is limited because of the low heritability of yield under stress, the complexity of the traits governing drought tolerance and difficulty to screen large germplasm precisely for drought resistance. Molecular markers have the potential to dissect quantitative traits into their single genetic components. Through marker-assisted breeding (MAB) it also assists the selection and pyramiding of the beneficial QTL alleles (Altinkut and Gozukirmizi, 2003). Identification of DNA markers associated with drought tolerance is usually carried out with large mapping populations, where each progeny of the population is genotyped. This is highly demanding in resource, time and often costly. Several strategies have been reported to reduce the number of plants to be genotyped. Bulked segregant analysis (BSA) is one such strategy for identifying DNA markers linked to the trait of interest against a randomized genetic background of unlinked loci (Michelmore *et al*., 1991).

BSA involves bulking of DNAs from selected individuals based on their phenotype. Phenotype based DNA pools might be useful in identifying new DNA markers for the regions of genome known to contain QTLs. BSA is commonly used for finding linkage between simply inherited traits and DNA markers using segregating populations developed from a single cross. However, if cultivars or advanced lines sharing the same

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phenotype can be used instead, identification of DNA markers associated with a target trait will be more flexible (Lawson *et al.*, 1994). Use of bulked line analysis (BLA) by pooling DNAs of genotypes from diverse genetic backgrounds but sharing similar phenotypes (eg. drought tolerance or susceptible) will lead to rapid identification of DNA markers associated with drought tolerance. One requirement of the BLA method is that the bulked lines should have the same target phenotype but vary over non-target phenotypes. The origin of the target gene in bulked lines should be the same for the BLA method to be effective (Tan *et al.*, 1998).

Thus the objectives of this study were to assess the extent of variation in drought response among rice accessions under field conditions and to identify microsatellite markers linked to drought resistance using a method called "bulked line analysis" (BLA).

Materials and methods

Field trial: The rice accessions used in the present study includes landraces and improved cultivars from different locations, known for their varied hydrological habitat (Table 1). Field trial was conducted under upland condition in the experimental fields of Tamil Nadu Agricultural University, Coimbatore, India. Rice accessions were evaluated under water stress and non-stress conditions in a randomized block design with replications. The experimental plot size was 2.0 x 0.2 m^2 with 20 x 10 cm spacing between and within rows, respectively. Seeds were hand dibbled into dry soil and required agronomic practices were followed to maintain healthy crop stand. All the plots were surface irrigated to field capacity once in a week, until water stress was imposed by withholding irrigation to stress plots beginning 87^{th} day after sowing (DAS).

Field measurements: During the initial 14 days of stress period, there were intermittent rains and after that there was 22 continuous rain free days. Changes in soil moisture were monitored periodically in stress plots using a Thetaprobe. Relative water content (RWC) (Barrs and Weatherly, 1962) and chlorophyll meter reading (model SPAD-502) (Monje and Bugbee, 1992) were taken 15 days after last rain fall. Leaf rolling and drying scores were recorded at noon, 16 days after last rainfall during the stress using 1 to 7 scales standardized for rice (IRRI, 1996). Canopy temperature was measured 17 days after last rainfall. Leaf samples for estimation of chlorophyll stability index (Murthy and Majumdar, 1962), cell membrane stability index (Blum and Eberocon, 1981) and proline content (Bates *et al.*, 1973) were taken 17 days after rainfall. Stress was relieved on 37^{th} day by irrigating the plots to field capacity and recovery score was recorded 3 days after irrigation. Following this, both control and stress plots were regularly irrigated until harvest. The plants were harvested 128 DAS and total above ground biomass was recorded.

DNA extraction, pooling of DNA samples and microsatellite marker analysis: Thirteen drought tolerant and thirteen susceptible rice genotypes were selected based on various physio-morphological traits under water stress. Genomic DNA from these rice accessions was extracted using the method described by Gawel and Jarret (1991). The quantity of DNA present in each sample was determined by reading the absorbance at 260 nm in a Hoefer Dyna Quant 2000 fluorometer. To assess the quality, agarose gel electrophoresis was done with 1µL of crude DNA sample on (0.8 %) agarose gel and stained with ethidium bromide. Fifteen nanograms μL^{-1} of diluted DNA each from thirteen drought tolerant and thirteen susceptible rice genotypes were taken and pooled into drought tolerant and drought susceptible bulks, respectively. The bulked DNA samples were screened using 200 SSR primers. Putative polymorphic markers between the bulks were checked for co-segregation among individual genotypes constituting the tolerant and susceptible bulks. PCR reactions were performed in a volume of 15µL reaction mixture in a thermal cycler (PTC-100^{TM}, MJ Research Inc.). The reaction mixture contained each primer at 200µM, dNTPs (2.5 mM), Assay Buffer (10x), *Taq* DNA polymerase (3 units/µL) and template DNA 15 ng/µl and the PCR reaction was programmed for 35 cycles as follows, initial denaturation (95˚C for 5 minutes), denaturation (94˚C for 45 seconds), annealing (55˚C for 45 seconds), extension (72˚C for 1 minute), final extension (72˚C for 5 minutes). An aliquot of 3 µL of DNA (from each PCR reaction) and 2 µL of loading dye (98% Formamide, 10Mm Ethylenediamine Tetraacetate (EDTA), Bromophenol blue, Xylene cyanol), were taken for sample preparation, denatured at 95°C for 5 minutes and separated on 5% polyacrylamide gel (acrylamide:bisacrylamide at19:1), at 1750V for 3h in 1x Tris-Borate-EDTA (TBE) buffer (0.09M Tris-Borate and 0.002 EDTA). Amplification products were detected by silver staining and band sizes were determined by comparison with a 100-bp DNA ladder size standard from Gene craft, Germany. The bands developed were scored as polymorphic or monomorphic between the two bulks.

Results and discussion

Thirty nine rice genotypes were subjected to water stress in a replicated field experiment. Significant variation was noticed among the genotypes for drought response in terms of various water stress indicators, physio-morphological and plant production traits under imposed water stress

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condition. Plant height ranged from 25.50 cm to 81.62 cm with an average of 50.50 cm (Table 2). Water stress adversely affected the growth of the susceptible genoypes which in turn leads to reduction in plant height. The growth of the genotypes was comparable and normal until water stress was imposed, upon stress the susceptible genotypes remain stunted and failed to grow further. Likewise, tillering ability of the tolerant genotypes were profuse in comparison with lanky tillers in susceptible genotypes. Effect of water stress on plant growth and development has been documented in rice in different mapping populations and also in different rice lines (Steel *et al*., 2013 and Manikanda Boopathi *et al*., 2013). Leaf rolling score ranged from 1.6 to 7.0 with a mean of 4.6 amongst the genotypes. The genotypes identified for tolerant bulks remained turgid for longer time, which may be due to deep rooting ability of these genotypes. Similar trend for leaf rolling was reported in rice in various populations (Zhang *et al*., 2001). Correlation among physio-morphological and drought responsive traits under stress reveals that parameters of water stress indicators were correlated morphological traits under stress (data not shown). Gomez *et al.* 2006 reported relationships among physio-morphological and drought responsive traits under water stress condition. In this study most of the genotypes identified as drought tolerant were *indica* land races except TRY-2, PMK-3 and PY-3 growing in different agro-ecological conditions and belong to subspecies *indica.* Lilley and Ludlow (1996) reported that *indica* cultivars tend to be dehydration tolerant, desiccating at a low water potential than their *japonica* counter parts. Drought tolerant and susceptible rice genotypes were selected on the basis of physio-morphological and biochemical traits such as plant height, number of tillers, SPAD chlorophyll value, leaf rolling, leaf drying, drought recovery, relative biomass, relative water content, proline content , chlorophyll stability index and cell membrane stability index under water stress condition. Detailed analysis of these traits enabled to identify thirteen drought tolerant and thirteen drought susceptible genotypes for BLA. The genotypes with extreme values on either side of the grand mean were grouped and bulked as either tolerant or susceptible bulks. The genotypes which had nearer value on either side of the grand mean were omitted and were not considered for bulking in order to have two very distinct bulks amongst the genotypes and to avoid the overlapping of genotypes between the bulks. Therefore, the genotypes which scored high values in all traits and low scores for leaf rolling and drying were considered for the tolerant bulk and those scored lower or negative in all parameters and high scores for leaf rolling and drying were used to form the susceptible bulk.

A total of 200 microsatellite primers (Research Genetics Inc., USA) representing different chromosomes of rice were selected randomly and used to amplify the SSR regions among the bulked DNA samples. Polyacrylamide gel electrophoresis was done with amplified products and was silver stained and appropriate scoring was done. Three primers such as RM1092, RM129 and RM157B were polymorphic between the bulks (Fig. 1 and 2) and were found to be putative markers. The primers showing polymorphism between the two bulks were checked in all 26 (13+13) individual rice genotypes along with the bulks, and perfect co-segregation was observed. These three primers cosegregated between the bulks and among genotypes. All the thirteen genotypes, which were considered as drought tolerant, produced similar banding pattern as produced in tolerant bulk, and this banding pattern was different from the susceptible bulk and susceptible genotypes. Although Near isogenic lines and BSA are effective for the identification of the DNA markers associated with target genes, the BLA method allows the genetic stock to be prepared more quickly**.** In addition, as the BLA method is not based on a segregating population, this method can be useful for asexually propagating organisms where generation of a segregating population is difficult due to various reasons. Although the BLA method cannot be used directly to localize genes, it is useful for the identification of DNA markers that are associated with the target gene and thereby saturating the genomic region of interest. Through such markers, the linked traits can be precisely localized if the markers used have been previously mapped. In this study, the markers deployed were found to be present on the flanking region of previously mapped chromosome region on rice genetic linkage maps for traits associated with drought tolerance. Therefore, the drought linked traits can be localized by the BLA method (Tan *et al*., 1998).

Identification of molecular markers associated with traits requires screening of a relatively large number of individuals in mapping population. BLA was developed to overcome this difficulty, because comparing bulk samples is easier than evaluating many individuals in mapping populations (Sweeney and Danneberger, 1994). Among the identified primers RM129 and RM157B were located on rice chromosome 1 (Fig. 3a). Hemamalini *et al.* (2000) reported that the region between RG173 and Amy 1B/A which flanked RM140, RM129 and RM157B to be associated with root: shoot ratio. Similarly association of this region (A*my* 1B/A to RG 345) with leaf drying, RWC and relative growth rate was observed in IR 64/Azucena DH population under water stress (Courtois et al. 2000). Yadav *et al*. (1997) also observed the association of this region with maximum root length in IR64/Azucena

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DH population. In this study RM1092 is located on rice chromosome 2 (Fig. 3b) between RZ318 and RZ58 markers and this region was reported to be associated with traits *viz.* total root number, maximum root length, root thickness, and deep root per shoot ratio in different mapping population (Hemamalini *et al.,* 2000 and Yadav *et al.,* 1997). Interestingly QTLs for osmotic adjustment were also found in this region in CT9993/ IR62266 double haploid population under drought stress (Zhang *et al.,* 2001).

Conclusion

In summary, the rice genotypes evaluated under water stress showed significant variation for physio-morphological and plant production traits. Some of the lines identified as drought tolerant can be used as donors to improve drought tolerance in rice. From the bulked line analysis microsatellite markers such as RM1092, RM129 & RM157B were found to be associated with the regions mapped for various drought tolerant traits. The markers RM129 & RM157B were ~ 2cM away from the root QTLs and these markers may be useful in marker assisted selection for drought tolerance in rice.

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References

Anderson Altinkut, A., and Gozukirmizi, N. 2003. Search for microsatellites associated with water stress tolerance in wheat through bulked segregant analysis. *Mol Biotech.,* **23**: 97-106.

Barrs, H.D., and Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. Biol. Sci.,* **15:** 413-128.

Bates, L.S., Waldren, I.D., and Teare, R.P. 1973. Rapid determination of free proline for water stress studies. *Plant soil,* **39**: 205-207.

Blum, A., and Eberocon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.,* **21**: 43-47.

Courtois, B., McLaren, G., Sinha, P., Prasad, K., Yadav, R., and Shen, L. 2000. Mapping QTL associated with drought avoidance in upland rice. *Mol. Breed.,* **6**: 55-66.

Gawel, N., and Jarret, R.L. 1991. A modified CTAB DNA extraction procedure for Musa and Ipomoea plant. *Mol. Biol. Rep.,* **9**: 262-266.

Hemamalini, G.S., Sashidhar, H.E., and Hittalmani, S. 2000. Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice *(Oryza sativa L.). Euphytica,* **112**: 1-10.

IRRI. 1996. International network for genetic evaluation of rice: Standard evaluation system for rice. International Rice research Institute, Los Banos, Philippines.

Lawson, W.R., Henry, R.J., Kochman, J.K., and Kong, G.A. 1994. Genetic diversity in Sunflower (*Helianthus annus*. L) as revealed by random amplified polymorphic DNA analysis. *Aust. J. Agric. Res.,* **45**:1319-1327.

Lilley, J.M., and Ludlow, M.M. 1996. Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Res.,* **48**: 185-197.

Manikanda Boopathi, N., Swapnashri, G., Kavith, P., Sathish, S., Nithya, R., Wickneswari, R., and Arvind Kumar. 2013. Evaluation and bulked segregant analysis of major yield QTL *qtl*12.1 introgressed into indigenous elite line for low water availability under water stress. *Rice Sci.,* **20**(1): 25-30

Michael Gomez, S., Satheesh Kumar, S., Jeyaprakash, P., Suresh, R., Biji K.R., Manikanda Boopathi, N., Price, A.H., and Chandra Babu, R. 2006. Mapping QTLs Linked to Physio-Morphological and Plant Production Traits under Drought Stress in Rice (*Oryza sativa* L.) in the Target Environment. *American J. Biochem. Biotech.,* **2**(4): 161-169.

Monje, O.A., and Bugbee. 1992. Inherent limitation of non destructive chlorophyll meter, a comparison of two types of meters. *Hort. Sci.,* **27**: 71-89.

Murthy, K.S., and Majumdar, S.K. 1962. Modifications of the techniques for determination of chlorophyll stability index in relation to studies of drought resistance in rice. *Curr. Sci.,* **31**: 470-471.

Phillips, R.L., Odland, W.E., and Kahler, A.L. 2005. Rice as a reference genome and more pp. 3-15, *In:* D.S. Brar, D. J. Mackill and B. Hardy, (eds.) Rice Genetics V: Proceedings of the Fifth International Rice Genetics Symposium, 19-23 November 2005, The Philippines.

Sweeney, P.M., and Danneberger, T.K. 1994. Random amplified polymorphic DNA in perennial ryegrass: a comparison of bulk samples vs individuals. *Hort. Sci.,* **29**:624-626.

Steele, K.A., Price, A.H., Witcombe, J.R., Roshi Shrestha, Singh, B.N., Gibbons, J.M., and Virk, D.S. 2013. QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. *Theor. Appl. Genet.,* **126**:101-108.

Tan, X.L., Vanavichit, A., Amornsipla, S., and Trangoonrung, S. 1998 Mapping of rice *Rf* gene by Bulked Line Analysis. *DNA Res.,* **5**: 15-18.

DOI: 10.5958/0975-928X.2016.00122.8

Temnykh, S., DeClerk, G., Lukashova, A., Lipovich, L., Cartinhour. S., and McCouch, S. R. 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa*): frequency, length variation, transposon associations and genetic marker potential. *Genome Res.*, **11**: 1441-1452.

Wassmann, R., Jagadish, S.V.K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., Serraj, R, Redona, E., Singh, R.K., and Heuer, S. 2009. Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Advances in Agro.*, **102**: 91-133.

Yadav, R., Courtois, B., Huang, N., and Mclaren, G. 1997 Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theor Appl Genet.*, **94**: 619-632.

Zhang. J., Zheng, H.G., Aarti. A., Pantuwan, G., Nguyen, T.T., Tripathy, J.N., Sarial, A.K., Robin, S., Babu, R.C., Nguyen, B.D., Sarkarung, S., Blum, A., and Nguyen, H.T. 2001. Locating genomic regions associated with components of drought resistance in rice: Comparative mapping within and across species. *Theor Appl. Genet.*, **103**: 19-29.

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Table 1. Details of rice accessions used in the study

S. No.	Accession	Parentage	Hydrological habitat	Source
1	ADT36	Triveni / IR20	Dry and semidry	TNAU, Coimbatore, India.
2	ADT44	Selection from OR128-7-51/CIET 14099 1256/OR142-99	Dry and semidry	TNAU, Coimbatore, India
3	ASD17	ADT31/Rathna//ASP8/IR8	Dry and semidry	TNAU, Coimbatore, India
4	ASD18	ADT31/IR50	Dry and semidry	TNAU, Coimbatore, India
5	ASD19	Lalnakanda/IR36	Dry and semidry	TNAU, Coimbatore, India
6	ASD20	IR18348-38-3/IR25863-61-3-2//IR58	Dry and semidry	TNAU, Coimbatore, India
7	Ashoka200F	Kalinga III x IR64	Irrigated	TNAU, Coimbatore, India
8	Ashoka228	Kalinga III x IR64	Irrigated	TNAU, Coimbatore, India
9	CO43	Dasal/IR20	Irrigated	TNAU, Coimbatore, India
10	CO47	IR50/CO43	Irrigated	TNAU, Coimbatore, India
11	CR1009	Pankaj/ Jaganat	Saline tolerant	DRR, India
12	CSR20	NA	Saline tolerant	CSRI, India
13	IR20	IR262 x TKM6	Irrigated	IRRI, Philippines
14	IR36	IR1561-228/IR244/0/CR9413	Irrigated	IRRI, Philippines
15	IR72	Taichung65x ME80	Irrigated	IRRI, Philippines
16	IR62266	Advanced culture	Irrigated	IRRI, Philippines
17	Jaya	TN1 / T141	Irrigated	DRR, India.
18	Kuliadichan	Land race	Rainfed	TNAU, Coimbatore, India.
19	Kuruvaikalanchiam	Land race	Rainfed	TNAU, Coimbatore, India.
20	Mattaikar	Land race	Rainfed	TNAU, Coimbatore, India.
21	Nootripathu	Land race	Rainfed	TNAU, Coimbatore, India.
22	Norungan	Land race	Rainfed	TNAU, Coimbatore, India.
23	PMK3	UPLRI-1 x CO43	Rainfed	TNAU, Coimbatore, India
24	Puzhudikar	Land race	Rainfed	TNAU, Coimbatore, India
25	PY2	Kannagi / UIL2032	Irrigated lowland	Pondichery, TN, India
26	PY3	IR403-2617(PTB33/IR36)	Rainfed upland	Pondichery, TN, India
27	RM96019	Selection from TGR75	Rainfed	TNAU, Coimbatore, India
28	Sivappuchitraikar	Land race	Rainfed	TNAU, Coimbatore, India
29	TKM9	TKM7/ IR68	Dry and wetland conditions	TNAU, Coimbatore, India
30	TRY1	BR153-23-10-1-3(IR572-172-22/	Saline soils	TNAU, Coimbatore, India
31	TRY2	IET 6238/ IR36	Saline soils	TNAU, Coimbatore, India.
32	Vellaichitraikar	Land race	Rainfed	TNAU, Coimbatore, India
33	Varappukudanchan	Land race	Rainfed	TNAU, Coimbatore, India
34	Vytilla1	PLs from Chootu Pokkali	Irrigated	KAU, Kerala, India
35	Vytilla2	PLs from Cheuvirippu	Irrigated	KAU, Kerala, India
36	Vytilla3	Vytilla1/TN1	Irrigated	KAU, Kerala, India
37	Vytilla4	Chettivirippu/IR4630-22-17	Irrigated	KAU, Kerala, India
38	Vytilla5	Mashuri mutant	Irrigated	KAU, Kerala, India
39	White Ponni	Taichung65 / ME80	Irrigated	KAU, Kerala, India

CSSRI - Central Soil Salinity Research Institute, India; DRR - Directorate of Rice Research, India; IRRI - International Rice Research Institute, Philippines
KAU - Kerala Agricultural University, India; TNAU - Tamil Nadu Agricultural University, India; TN - Tamil Nadu, India; NA - Not Available

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Table 2. Mean variation in physio-morphological traits under water stress among 36 genotypes of rice

S. No.	Accessions	PH (cm)	NT	CHL	LR	LD	DR	RBM	RWC (%)	PROL (µg/g)	CSI (%)	CMS (%)
1	ADT36	29.28	4.50	32.90	5.0	3.6	6	0.35	35.53	1.06	72.02	98.85
2	ADT44	36.83	4.50	32.20	5.6	4.3	4	0.63	39.94	0.43	71.10	83.70
3	ASD17	67.43	5.83	32.5	4.3	2.3	7	0.95	47.16	0.56	91.44	93.78
4	ASD18	33.38	6.83	32.00	4.3	3.6	4	0.24	64.50	0.42	82.37	82.22
5	ASD19	29.05	4.67	31.40	7.0	7.0	2	0.63	30.50	0.16	83.38	83.25
6	ASD20	37.27	6.33	32.60	7.0	7.0	4	0.84	62.50	0.37	73.33	82.16
7	Ashoka200F	45.27	4.00	00.00	7.0	7.0	7	0.77	25.88	ME	ME	ME
8	Ashoka228	56.22	6.00	00.00	7.0	7.0	7	0.83	39.24	ME	ME	ME
9	CO43	36.30	5.83	31.60	5.6	5.6	2	0.54	54.99	0.34	76.62	83.44
10	CO47	30.92	5.17	32.50	6.3	6.3	5	0.43	37.85	0.15	77.49	84.68
11	CR1009	28.77	7.00	32.10	4.3	3.6	3	0.69	70.34	0.13	84.12	83.40
12	CSR20	25.50	5.83	32.50	2.3	1.6	1	0.63	57.39	0.18	98.68	80.58
13	IR20	30.88	5.67	31.40	5.0	4.3	2	0.64	54.75	0.14	84.66	84.14
14	IR36	29.22	5.33	32.70	5.6	3.6	5	0.61	42.69	0.19	83.82	89.90
15	IR72	33.13	3.50	31.90	5.0	5.0	4	0.79	63.18	0.02	81.89	88.48
16	IR62266	29.63	7.50	31.30	6.3	6.3	7	0.24	31.68	1.85	848.08	81.37
17	Jaya	31.93	8.67	32.00	1.6	1.6	2	0.39	62.15	0.29	82.57	84.07
18	Kuliadichan	71.22	10.5	38.10	3.0	3.0	1	0.86	74.49	1.71	96.14	99.83
19	KuruvaiKalanchiam	77.15	8.17	38.00	3.0	1.0	3	0.77	59.48	1.81	93.25	97.30
20	Mattaikar	73.58	8.33	36.70	2.3	2.3	2	0.83	65.90	1.10	93.18	92.22
21	Nootripathu	78.10	7.67	38.00	4.3	2.3	5	0.83	60.67	1.27	86.52	98.93
22	Norungan	81.62	7.50	36.00	3.0	1.6	4	0.84	66.93	1.51	87.36	96.06
23	PMK3	65.92	7.33	37.90	4.3	2.3	6	0.84	71.97	1.48	91.06	91.07
24	Puzhudikar	61.17	10.67	36.70	2.3	1.6	1	0.79	59.37	1.40	91.14	95.76
25	PY2	59.88	8.50	33.00	3.6	3.0	1	0.46	53.45	1.28	82.24	80.72
26	PY3	65.18	10.83	33.80	3.0	3.0	3	0.68	57.25	0.11	80.10	95.41
27	RM96019	42.67	4.67	30.50	5.6	3.6	5	0.20	52.42	0.15	72.94	84.76
28	Sivappuchitraikar	77.27	8.50	36.50	3.0	1.6	2	0.87	65.84	1.70	88.36	88.03
29	TKM9	61.35	6.83	35.80	3.0	1.6	1	0.73	75.91	0.37	76.62	81.61
30	TRY1	34.75	5.83	31.50	6.3	6.3	7	0.47	40.95	1.33	77.85	83.06
31	TRY2	65.40	8.33.	36.10	4.3	4.3	1	0.49	62.75	1.60	87.84	87.53
32	Vellaichitraikar	80.02	8.33	34.20	3.6	3.0	3	0.81	67.02	1.46	94.76	89.08
33	Varappukudanchan	77.13	8.00	34.20	3.6	2.3	3	0.92	68.58	1.07	87.93	89.83
34	Vytilla1	45.95	5.33	32.50	5.6	5.6	3	0.61	57.15	0.42	81.29	84.16
35	Vytilla2	46.28	3.50	30.00	5.6	3.6	6	0.62	45.75	0.15	78.61	84.80
36	Vytilla3	42.42	3.00	32.10	7.0	5.0	4	0.64	46.44	0.10	74.81	80.00

PH = Plant height, CHL = Chlorophyll content, NT = Number of tillers, RBM = Relative biomass, RWC = Relative water content, LD = Leaf drying, LR = leaf rolling, PROL = Proline content, DR = Drought recovery, CSI = Chlorophyll stability index, CMS = Cell membrane stability index, ME = matured early before the stress imposition

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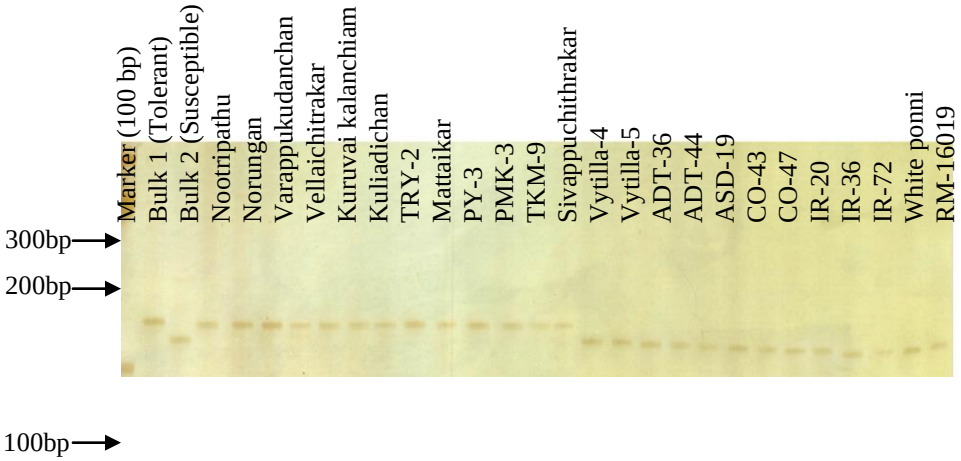

Fig. 1. SSR profile of bulks and individuals using primer RM1092

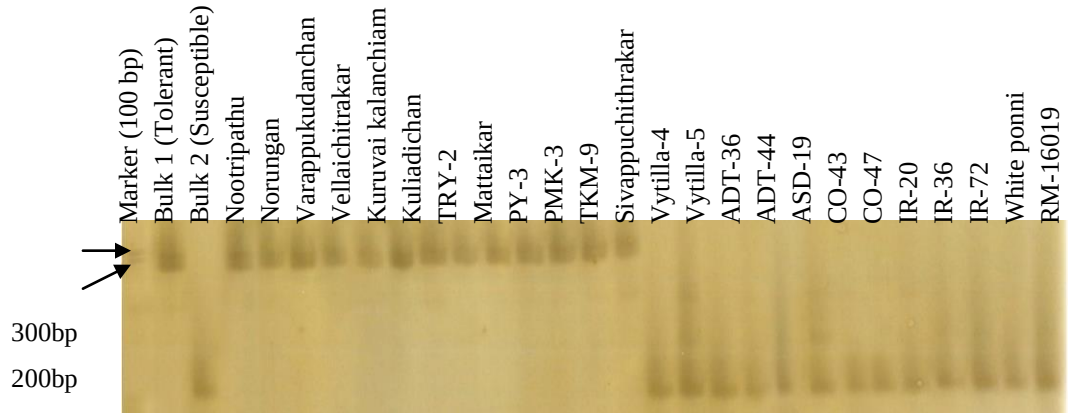

Fig. 2. SSR profile of bulks and individuals using primer RM157 B

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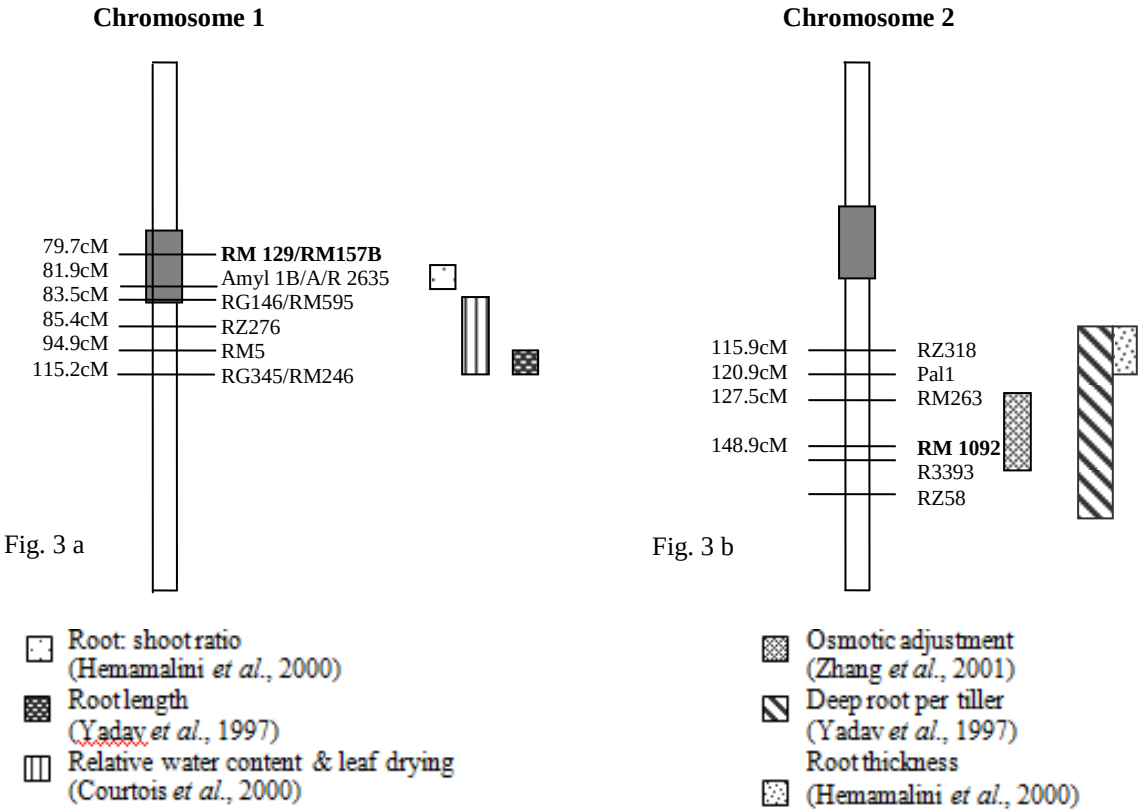

Fig. 3. The genomic regions of rice chromosomes 1 and 2 (Fig. 3a and 3b) showing identified microsatellite markers (in bold) and QTLs for different drought resistance traits across genetic back grounds in rice. The markers were aligned based on the linkage map of Temnykh *et al.*, (2001)