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Response of Selected Recombinant Inbred Lines for Yield and Yield-Attributing Traits under Aerobic Conditions and Marker-Assisted Graphical Representation of Superior Rice Genotypes

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ABSTRACT

Rice is one of the most important food crops drastically affected by drought in the rice ecosystem. Among grain crops, rice is the single biggest user of water, requiring 2 to 3 times more water per unit of grain produced. Recognizing the water constraints to rice yield, an alternative method of rice production using available water is necessary. One such method is aerobic cultivation which requires about 50% of water as puddle cultivation. To generate new varieties suitable to aerobic cultivation, 41 early duration recombinant inbred lines developed using high-yielding (IR50) and deep-rooted (Moroberekan) parents, were evaluated for drought and yield traits under aerobic conditions. Among these, IM 65, IM71, IM84, IM108, IM122, IM151 and IM181 showed superior field traits. Furthermore, the genomic regions of IM65 (drought tolerant) and IM84 (drought susceptible) were graphically genotyped with the help of 45 SSR markers. Identification of DNA fragments responsible for drought tolerance may further help in developing introgression lines with an IR50 background and with Moroberekan-like drought tolerance.

Keywords: *indica* rice, *japonica* rice, RILs, SSR markers, QTL
Abbreviations: ANOVA, analysis of variance; **GCV**, genotypic coefficient of variation; **GA**, genetic advance; **PCV**, phenotypic coefficient of variance; **QTL**, quantitative trait locus; **RILs**, recombinant inbred lines; **SSR**, simple sequence repeat

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for most people in Asia and is a major caloric dietary source. It supplies 23% of global per capita energy and 16% of per capita protein. As population and economy expand, the demand for consumption of rice will increase (Khush 1996). By 2030, global rice demand is projected at 800 million tons, which is 200 million tons higher than the production in 2000 (FAOSTAT 2003). To meet this growing demand it is required to increase the productivity levels of rice by combating biotic and abiotic stresses and other constraints. Among abiotic stresses, moisture stress is the 5th major problem limiting rice production in many regions of Asia. Rice is the single biggest user of water, requiring 2 to 3 times more water per unit of grain produced than crops such as wheat and maize (Wassmann *et al*. 2009).

Drought is one of the primary constraints depressing the yield and destabilizing rice production. Plants are most susceptible to water stress at the reproductive stage. Dramatic reduction of grain yield occurs when stress coincides with the irreversible reproductive processes, making the genetic analysis of drought resistance at the reproductive stage crucially important (Cruz and O'Toole 1984; Price and Courtois 1999; Boonjung and Fukai 2000; Pantuwan *et al.* 2002). Early flowering genotypes can escape from late season drought and this is simple, but often the most effective way of increasing yield under terminal drought. Replacing late maturing cultivars with medium maturing cultivars that have good yield potential in rainfed lowlands, provides a better chance of escaping late season drought (Ouk *et al.* 2007). Earlier flowering time may be especially useful for upper positions in a toposequence, because standing water often disappears earlier than in lower positions (Homma *et al.* 2003). Sets of upland experiments with diverse germplasm (*i.e.*, *indica*, *aus* and *japonica* subspecies) also show the advantages of earlier flowering over late flowering in terms of higher spikelet fertility, higher HI (HI) and higher yield (Lafitte and Courtois 2002).

Five transgressive variants (advanced breeding lines from BC_2F5 and BC_2F_6 generation) were derived from a cross between the wild relative, *O. rufipogon* Griff. and *O. sativa* L. subsp. *indica* cv. MR219, a popular high yielding Malaysian rice cultivar were evaluated for the pericarp colour of the grains along with yield potential and to validate quantitative trait loci (QTLs) for agronomic traits (Bhuiyan *et al.* 2011). In contrast, Naresh Babu *et al.* (2011) used drought susceptibility index (DSI) and relative yield (RY) values to describe yield stability and relative yield among genotypes.

Tropical *japonica* upland rice cultivars have thicker and deeper roots than *indica* cultivars (Courtois *et al.* 1996) whereas *indica* rice varieties have more ability to maintain osmo regulation than *japonica* rice (Robin *et al.* 2004). Hence in present study two genotypes *viz.*, IR50 and Moroberekan were chosen. IR50 is an early-medium maturing agronomically superior *indica* semi-dwarf variety with high yield potential and good plant type. It is susceptible to most of the biotic (blast, bacterial blight, etc.) and abiotic stresses (drought-owing to shallow root system, cold, etc.). Morokerekan is a tall West African upland genotype, with sturdy stem and greater fertile panicle with bold grains, exhibiting resistance to blast and tolerance to drought. These contrasting characters in the two genotypes provide an opportunity to transgress *indica* and *japonica* alleles that could generate a range of recombinants which can provide genotypes with high tolerance to biotic and abiotic stresses and good yield.

The concept of graphical genotypes was first given by

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Young and Tanksley (1989). They said that graphical genotype portrays the parental origin and allelic composition throughout the genome. In this the primary goal would be to transform restriction fragment length polymorphism marker data, obtained in a numerical form, into an easily interpretable and accurate graphical image.

One hundred and eight polymorphic markers were used to genotype five hundreds BC_4 lines. One hundred and forty putative near-isogenic introgression lines were obtained by graphical genotyping. For the whole genome of rice, eighty eight introgression lines were consequently selected to construct a contiguous introgression line population (Mu *et al*. 2004).

Girish *et al.* (2006) carried out genetic variability, correlation, path coefficient analysis and test of normality was conducted in an F8 recombinant inbred aerobic rice population developed by single seed descent method to evaluate its potential as a mapping population. Shapiro- Wilks ''W test of normality'' indicated that the population was skewed towards female parent IR50 for some traits and for some others towards Moroberekan, the male parent. Most of the characters that showed skewness were platykurtic with a kurtosis value of less than 3. The subset of same population was used for present study.

A linkage map of 1475.7 cM was constructed by Angaji *et al.* (2009) using 135 polymorphic simple sequence repeats (SSR) and 1 indel marker and five putative quantitative trait loci (QTL) were detected in BC_2F_2 lines with IR64 as the recurrent parent and Khao Hlan as the donor parent. Graphical genotyping of the lines with highest and lowest survival in flooding conditions verified the detected QTLs that control tolerance and some QTLs co-localize with previously identified QTLs for traits relevant to tolerance to flood.

The graphical form of presentation of a linkage map has a number of advantages over numerical genotypes or linkage maps, as the graphical genotype from a marker would show the genomic constitution and parental derivation for all points in the genome. Thus, graphical genotypes will help in performing whole genome selection to breed for phylogenic characteristics in plants and animals (Young and Tanksley 1989).

MATERIALS AND METHODS

The study included RILs developed from a cross between IR50 and Moroberekan at the Department of Genetics and Plant Breeding by Dr. Shailaja Hittalmani in field experiments conducted at K block (GKVK, University of Agricultural Sciences, Bengaluru, India) of the Department of Genetics and Plant Breeding and 41

Table 1 List of SSR markers used for graphical genotyping.

Primer	Forward sequence	Reverse sequence	Repeated motif	Product size (bp)	Chro
RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC	$(AG)_{16}$	163	2
RM9	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC	$(GA)15GT(GA)_2$	136	1
RM10	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC	$(GA)_{15}$	159	7
RM29	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG	(GA)7	250	2
RM71	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG	$(ATT)_{10}T(ATT)_4$	149	2
RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	$(TAT)_5C(ATT)_{15}$	166	8
RM80	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG	$(TCT)_{25}$	142	8
RM101	GTGAATGGTCAAGTGACTTAGGTGGC	ACACAACATGTTCCCTCCCATGC	$(CT)_{37}$	324	12
RM104	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC	$(GA)_9$	222	1
RM112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCAGTGGACGGTGAC	$(GAA)_5$	128	2
RM126	CGCGTCCGCGATAAACACAGGG	TCGCACAGGTGAGGCCATGTCG	$(GA)_7$	171	8
RM130	TGTTGCTTGCCCTCACGCGAAG	GGTCGCGTGCTTGGTTTGGTTC	$(GA)_{10}$	85	3
RM138	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG	$(GT)_{14}$	233	2
RM144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	$(ATT)_{11}$	237	11
RM145	CCGGTAGGCGCCCTGCAGTTTC	CAAGGACCCCATCCTCGGCGTC	-----	------	2
RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	$(GGC)_{10}$	151	8
RM156	GCCGCACCCTCACTCCCTCCTC	TCTTGCCGGAGCGCTTGAGGTG	$(CGG)_8$	160	3
RM163	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	$(GGAGA)_4(GA)_{11}C(GA)_{20}$	124	5
RM164	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	$(GT)_{16}TT(GT)_4$	246	5
RM170	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC	$(CCT)_7$	121	6
RM197	GATCCGTTTTTGCTGTGCCC	CCTCCTCTCCGCCGATCCTG	$(ACC)_7$	106	6
RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	$(GT)_{30}$ $(AT)_8$	158	9
RM204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	$(CT)_{44}$	169	6
RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	$(CT)_{21}$	147	11
RM209	ATATGAGTTGCTGTCGTGCG	CAACTTGCATCCTCCCCTCC	$(CT)_{18}$	134	11
RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	$(CT)_{24}$	136	1
RM214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	$(CT)_{14}$	112	7
RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	$(AAG)_8(AG)_{13}$	157	11
RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	$(TC)_{11}(CT)_5C_3(CT)_5$	116	11
RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	$(CT)_{36}$	156	7
RM242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	$(CT)_{26}$	225	9
RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC	$(CT)_{29}$	147	3
RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	$(CT)_{24}$	147	9
RM264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	$(GA)_{27}$	178	8
RM278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC	$(GC)_{17}$	141	9
RM281	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG	$(GA)_{21}$	138	8
RM282	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG	$(GA)_{15}$	136	3
RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	$(GT)_{30}(AT)_8$	156	1
RM315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	$(AT)_4(GT)_{10}$	133	1
RM318	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC	$(GT)_{15}$	140	2
RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	$(CAT)_{21}$	175	2
RM331	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG	$[(CTT)_4GTT]_2(CTT)_{11}$	176	8
RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	$(CTT)_8$	111	8
RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	$(AG)_{10}$	247	3
RM526	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	$(TAAT)_5$	240	2

selected early duration genotypes were provided for this experiment were evaluated under moisture stress during kharif season of 2008 in a randomized complete block design (RCBD) with two replications. Low moisture stress was induced by withholding irrigation for 10 days during 13 to 23 September *i.e.*, peak reproductive growth stage. Observations were recorded regarding plant height, tillers/plant, days to 50% flowering, panicles/plant, panicle length, fertile seeds/plant, percent sterility, grain length, grain breadth, length and breadth ratio, test weight, grain yield/plant, biomass/plant HI. Analysis of variance (ANOVA) was conducted and the genotypes were evaluated for genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, Genetic Advance as per mean (GA) and correlation coefficients were computed to find the anticipation amongst characters.

Genomic DNA was extracted from leaf tissues of 25-days-old seedlings of the 41 recombinant inbred lines (RILs) and two parents by modified cetyl trimethyl ammonium bromide method. The DNA was quantified at 260 nm using a UV spectrophotometer. After determining polymorphism between parents, 45 SSR markers were used for genotyping the genomic regions of selected genotypes. These SSR markers are already mapped and the mapping positions of these markers were downloaded from the Gramene website (www.gramene.org/). The PCR reaction mixture contained 40 ng of template DNA, 0.2 mM of each primer pair (Sigma Aldrich), 1 mM dNTPs, 10X PCR buffer (1X = 10 mM Tris Hcl pH 8.8 at 25°C, 1.5 mM KCl and 0.1% Triton-X 100), 1 unit of *Taq* polymerase (Bangalore Genei) and deionized water to get a total reaction volume of 20 µl. The PCR profile involved an initial denaturation of 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min and a final extension for 5 min at 72°C. The amplified PCR products were resolved on 3% agarose gels in 1X TAE buffer. Graphical genotyping was done for the selected superior RILs along with parents using computer software 'GGT32' (http://www.spg.wau.nl/pv/pub/GGT) using molecular marker data generated from these 45 SSR markers (**Table 1**) and graphical genotypes were obtained.

RESULTS AND DISCUSSION

In rice, grain yield is primarily determined by three component traits: number of panicles, grains per panicle and grain weight (Xing and Zhang 2010). However, several other traits can also affect grain yield, including plant height, days to heading, tiller angles, and grain shattering. Yue *et al.* (2006) detected 27 QTLs for nine fitness and yield traits using a RIL population. In the present investigation the analysis of variance indicated significant differences among the mean of different genotypes for plant height, number of tillers, days to 50% flowering, days to maturity, number of panicles/plant, panicle length, fertile seeds/plant, % sterility, grain length, length and breadth ratio and grain yield (**Table 2**). High phenotypic coefficient of variability was observed for panicle number, % sterility, grain yield/plant, biomass/plant and HI where as high genotypic coefficient of variability was observed for grain yield/plant and % sterility. Moderate phenotypic coefficient of variability was observed for plant height, days to 50% flowering, panicle length, fertile seed/ plant, length and breadth ratio and test weight and characters such as plant height, days to 50% flowering, panicle number, biomass and fertile seeds/plant moderate genotypic coefficient of variability was estimated. PCV and GCV value was narrow for all except panicle number, grain yield/plant, biomass and HI. This indicates a low level of environmental influence in the expression of these traits under stress (**Table 3**).

High heritability was noticed for plant height, days to 50% flowering, days to maturity and moderate heritability recorded for number of tillers, panicle length, fertile seeds/plant, grain breadth, length and breadth ratio and total grain yield/plant and remaining traits showed low heritability.

Genetic advance as % mean was high for plant height, days to 50% flowering and total seed weight/plant where as number of tillers, panicle length grain length, grain breadth, length and breadth ratio, test weight and HI was reported to

Table 2 Analysis of variance for yield and its associated traits in early and mid duration RILs of IR50/Moroberekan under reproductive stage stress condition during *kharif* 2008.

Source of variation	Df	Mean sum of squares														
		PHT	NT	DAF	DAM	PN	PLT	FS	PS	GL	GB	LBR	TW	GY	BMS	HI
Replications	1	4.11	0.2	0.3	0.3	2.17	0.7	0.87	2.7	0.10	0.01	0.06	4.0	6.6	0.3	0.0
Treatments	42	363.3 **	8.3* *	165. 5**	165.5 **	11.9 *	6.5*	321.7 *	61.6*	0.28 **	0.08	0.2**	4.5	90.1 7*	869.6	0.01
Error	42	47.9	3.5	1.05	1.05	7.12	3.5	156.9	15.01	0.24	0.03	0.09	2.7	48.5	624.0	0.01

	PHT	NT	DAF	DAM	PN	PLT	FS	PS	GL	GB	LBR	TW	GY	BMS	HI
SEm	4.90	1.34	0.73	0.73	1.89	1.33	8.86	2.74	0.35	0.13	0.22	1.17	4.93	17.66	0.08
CD at 1%	18.7	5.1	2.8	2.8	7.2	5.1	33.9	10.5	1.3	0.5	0.9	4.5	18.8	67.5	0.3
CD at 5%	14.0	3.8	2.1	2.1	5.4	3.8	25.3	7.8	1.0	0.4	0.6	3.4	14.1	50.5	0.2

* Significant at 5%; ** Significant at 1%; PHT = plant height (cm); NT = number of tillers per plant ; DAF = days to 50% flowering; DAM = days to maturity; PN = number of panicles per plant PLT = Panicle length (cm); FS = Fertile seeds per plant; PF = per cent fertility; GL = grain length (cm); GB =grain breadth; LBR = grain length to breadth ratio; TW = test weight (g); GY = grain yield (g); BMS = Biomass per plant (g); HI = Harvest index

Table 3 Estimates of genetic parameters for different traits in early and mid duration RILs of IR50/ Moroberekan under reproductive stage stress during *kharif* 2008.

Character	Minimum	Maximum	Mean	PCV	GCV	Heritability BS %	GAPM %
Plant height (cm)	66.67	137.5	97.42	14.72	12.89	76.67	23.11
Number of tillers	7	39	26.38	9.27	5.86	40.02	7.60
days to 50% flowering (days)	68	103	79.22	11.52	11.44	98.73	23.29
Days to maturity (days)	103	138	114.22	7.99	7.94	98.73	16.15
Panicle number	8	23	11.73	26.32	13.25	25.36	13.69
Panicle length (cm)	11.67	27	19.15	11.71	6.44	30.24	7.26
Fertile seeds per panicle	56.5	132	88.68	17.44	10.23	34.43	12.30
Per cent sterility (%)	5.25	39	19.56	31.64	24.68	60.83	39.44
Grain length (mm)	3	7.9	7.05	7.33	1.94	7.04	1.06
Grain breadth (mm)	2	3.2	2.46	9.90	6.55	43.72	8.88
Length/breadth ratio	1.1538	3.6	2.89	13.58	8.08	35.40	9.85
Test weight (g)	10.92	21.1	16.56	11.55	5.74	24.70	5.88
Total seed weight per plant (g)	10	58	20.62	40.37	22.10	29.97	24.80
Biomass (g)	22.5	164.5	83.54	32.71	13.26	16.44	11.01
Harvest index	0.0998	0.792	0.26	42.35	8.45	3.98	3.46

PCV = phenotypic coefficient of variation; GCV = genotypic coefficient of variation; BS = broad sense; GAPM = genetic advance as per mean

Table 4 Phenotypic correlation coefficient among 15 traits studied under reproductive stage stress condition in early duration RILs of IR50/Moroberekan during *kharif* 2008.

	PHT	NT	DAF	DAM	PN	PLT	FS	PS	GL	GB	LBR	TW	GY	BMS	HI
PHT	1	-0.089	0.058	0.058	-0.038	-0.042	-0.15	-0.013	-0.066	0.061	-0.038	-0.034	-0.13	0.157	-0.246
NT		1	0.048	0.048	0.228	0.039	0.300*	0.084	0.079	0.091	-0.048	0.19	-0.083	-0.013	-0.002
DAF			1	0.72**	0.39**	0.025	0.171	-0.081	0.078	-0.359*	0.302*	-0.073	0.306*	0.079	0.188
DAM				1	0.39**	0.025	0.171	-0.081	0.078	-0.359*	0.302*	-0.073	0.306*	0.079	0.188
PN					1	0.053	0.351*	-0.047	0.001	-0.121	0.088	-0.092	0.368*	0.216	0.145
PLT						1	0.351*	0.278	0.079	-0.078	0.11	-0.106	-0.063	-0.006	-0.063
FS							1	0.337*	-0.105	0.035	-0.083	-0.126	0.008	0.228	-0.131
PS								1	-0.023	0.183	-0.143	-0.046	-0.036	-0.035	-0.063
GL									1	-0.303*	0.724**	-0.037	0.022	-0.017	0.06
GB										1	-0.868**	-0.011	-0.116	-0.044	-0.1
LBR											1	-0.011	0.131	0.028	0.133
TW												1	-0.025	0.02	-0.05
GY													1	0.3*	0.722**
BMS														1	-0.317*
HI															1

* Significant at 5%; ** Significant at 1%; PHT = plant height (cm); NT = number of tillers per plant ; DAF = days to 50% flowering; DAM = days to maturity; PN = number of panicles per plant PLT = panicle length (cm); FS = fertile seeds per plant; PF = per cent fertility; GL = grain length (cm); GB = grain breadth; LBR = grain length to breadth ratio; TW = test weight (g); GY = grain yield (g); BMS = biomass per plant (g); HI = harvest index

be moderate. Low Genetic advance as per cent mean was observed for remaining characters.

The significant positive association existed between days to 50% flowering and panicle number. Association of the panicle number was positive with fertile seeds/plant, grain yield/plant. The panicle neck diameter was measured in a rice population of 187 lines from the cross "'ZS97B'/ 'IRAT'" under water regimes by Liu *et al*. (2008). Panicle neck diameter was found to be significantly correlated with many agronomic traits, especially with the panicle size. It has been noticed that grain length was positively and significantly associated with length and breadth ratio but grain breadth is negatively associated with same character (**Table 4**). The significant and negative relationship was observed between grain length and grain breadth. Thought the grain yield under stress is primary trait for selection for drought prone environments, secondary traits that are associated with yield under stress can provide additional information (Lafitte *et al.* 2003). Akinwale *et al.* (2011) estimated the phenotypic and genotypic coefficients of variation, broad sense heritability, genetic gain and correlations in rice. They found that grain yield exhibited significantly positive correlation with the number of tillers per plant panicle weight and number of grains per panicle. Analysis of variance (ANOVA) of five transgressive variants (advanced breeding lines from BC_2F_5 and BC_2F_6 generation) were derived from a cross between the wild relative, *O. rufipogon* Griff. and *O. sativa* L. subsp. *indica* cv. MR219, a popular high yielding Malaysian rice cultivar showed that the seasonal factors influenced different agronomic traits. Variant G33 produced significantly ($P < 0.05$) higher yield (5.20 t/ha) than the control, MR219 (4.53 t/ha) (Bhuiyan *et al.* 2011).

In the present experiment days to 50% flowering (0.306), days to maturity (0.306), number of panicles/plant (0.368), fertile seeds/plant (0.008), grain length (0.22) and length and breadth ratio (0.131) showed a positive association with grain yield. A negative association was noticed between yield and plant height (0.13), number of tillers (0.083), panicle length (0.063), % sterility (0.036), grain breadth (0.116) and test weight (0.025). Grain yield was also positively and significantly associated with biomass/ plant (0.300) and HI (0.722) whereas biomass was noticed to be significantly and negatively associated with HI (0.317). Two independent groups detected a QTL, OsSPL14 (IPA1, WFP) that affects plant architecture and yield in rice (Jiao *et al.* 2010; Miura *et al.* 2010).

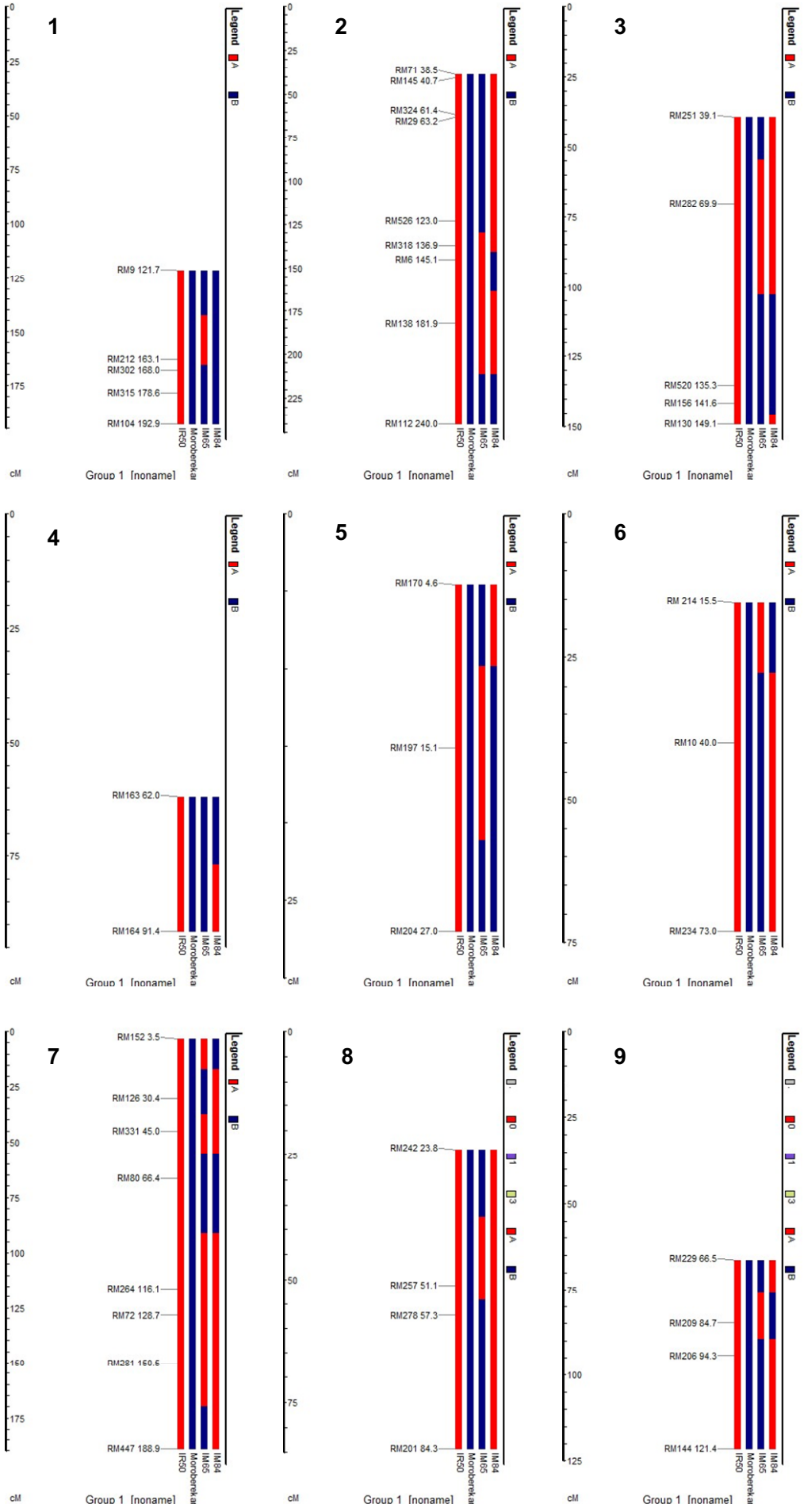
Early flowering genotypes can escape from reproductive stage drought (Pantuwaqn *et al.* 2002; Ouk *et al.* 2007). Thus a positive association has been observed between days to 50% flowering and grain yield indicating that during the reproductive stage stress, plants try to complete their life cycle by flowering early with some reduction in yield. Rice heading date typifies a quantitative trait controlled by many genes, which are expressed or suppressed due to a close interaction with environmental factors such as day length and temperature (Tsuji *et al*. 2008). Xue *et al*. (2008) detected the Ghd7 QTL, which elicits substantial effects on heading date, number of grains/panicle and plant height. Using a BC_2F_2 population, Matsubara *et al*. (2008) identified two new QTLs, Hd16 and Hd17, involved in heading date differences between *japonica* rice cultivars.

A field experiment was conducted to study the response of rice genotypes under aerobic conditions. Variance studies revealed significant differences among the genotypes for the traits, days to flowering, plant height, HI, grain yield, panicle number, straw yield, panicle length, test weight and biomass. Higher values of heritability and genetic advance were observed for plant height and days to flowering. Grain yield per plot showed positive association with HI and total biomass. Correlation and path analysis revealed an ideal plant type of genotype under aerobic conditions should have high HI and biomass (Keshava Murthy *et al.* 2011).

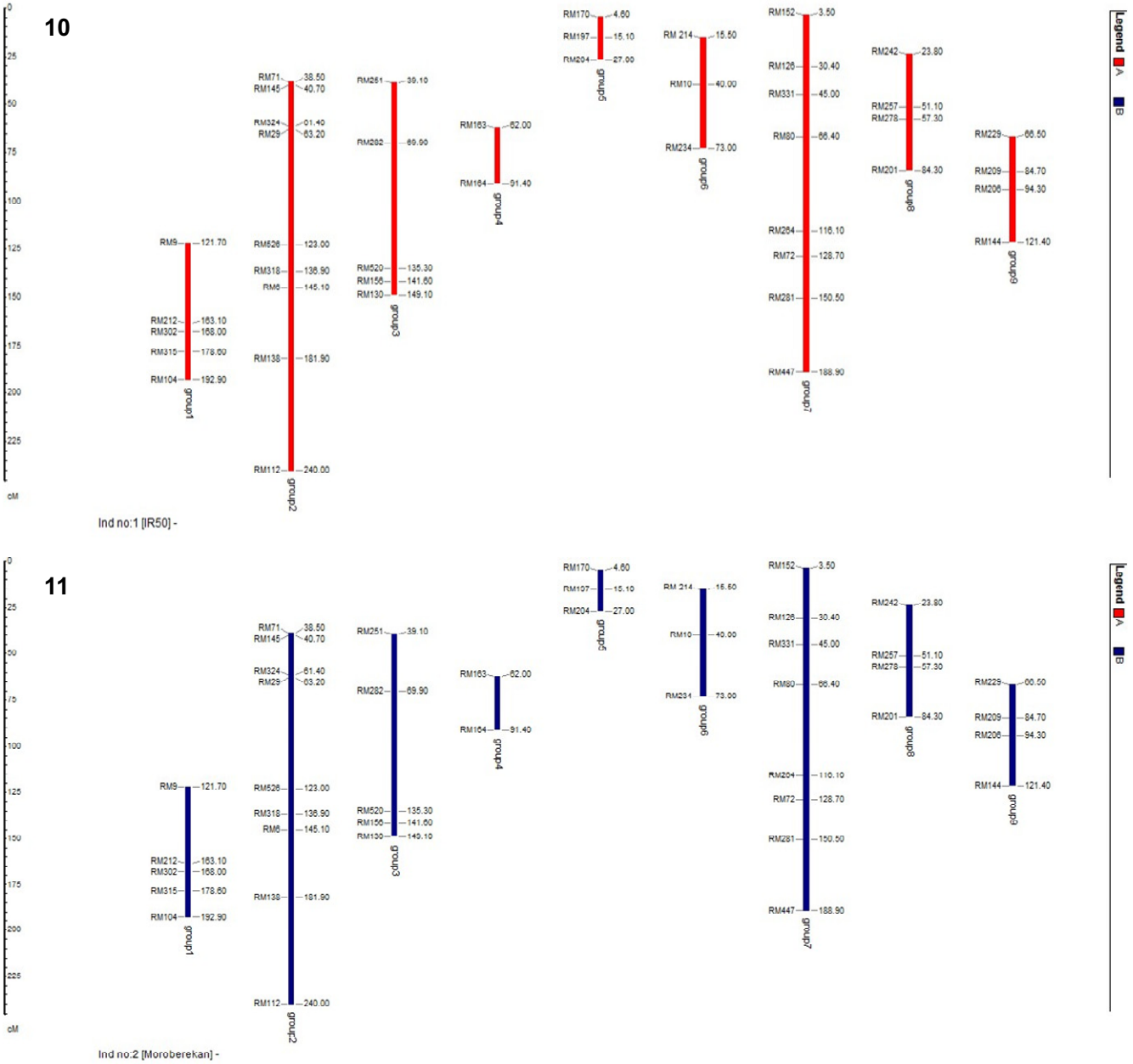
Based on a field evaluation, two genotypes *viz* IM65 and IM84 were chosen for graphical genotyping. Here chromosome 1, 2, 3, 5, 6, 7, 8, 9, 11 were represented since the SSR markers used for the study did not cover chromosome 4 and 10 whereas there was only one marker found on chromosome 12. Therefore, only 9 chromosomes have been included in the study. In graphical representation red color indicate the genome fragment donated by first parent *i.e.*, IR50 and blue color indicates the genome fragment derived from second parent *i.e.*, Moroberekan. Graphical representation of individual chromosome of two progenies has been compared with both the parents (**Fig. 1-9**), graphical picture of two parents (**Fig. 10, 11**), including individual graphical representation of genome content of IM65 (**Fig. 12**) and IM84 (**Fig. 13**).

In IM65, 13 bands were generated as that of banding pattern of IR50 and 30 bands were generated as that of banding pattern of Moroberekan. Thus 38.7% of the genome content is from IR50 whereas the remaining 61.3% of the genome was donated by Moroberekan. Among the 44 bands of IM84, 27 were similar to those of IR50 *i.e.*, 60.7% was similar to the female parent and 17 bands were as that of the male parent Moroberekan *i.e.*, 39.3% of genome content was donated from Moroberekan (**Tables 5, 6**).

Zheng *et al.* (2008) identified 32 putative quantitative trait loci (QTLs) in doubled haploid rice lines of *indica* and *japonica* cross for four seedling traits: average of three adventitious root lengths (ARL), shoot height (SH), shoot biomass (SW), and root to shoot dry weight ratio (RSR) with the use of 208 restriction fragment length polymorphism (RFLP) and 76 microsatellite (SSR) markers. Markers associated with economically important traits will help in moni-



Comparison of graphical representation of IR50, Moroberekan, IM65 and IM84 for chromosome No. 1 (**Fig. 1**), No. 2 (**Fig. 2**), No. 3 (**Fig. 3**), No. 5 (**Fig. 4**), No. 6 (**Fig. 5**), No. 7 (**Fig. 6**), No. 8 (**Fig. 7**), No. 9 (**Fig. 8**), No. 11 (**Fig. 9**). IN all cases, IR50, female parent; Moroberekan, male parent; IM65, IM84, RILs.



Graphical representation of IR50 (female parent) (**Fig. 10**) and Moroberekan (male parent) (**Fig. 11**).

toring effective transformation of such QTLs to elite lines.

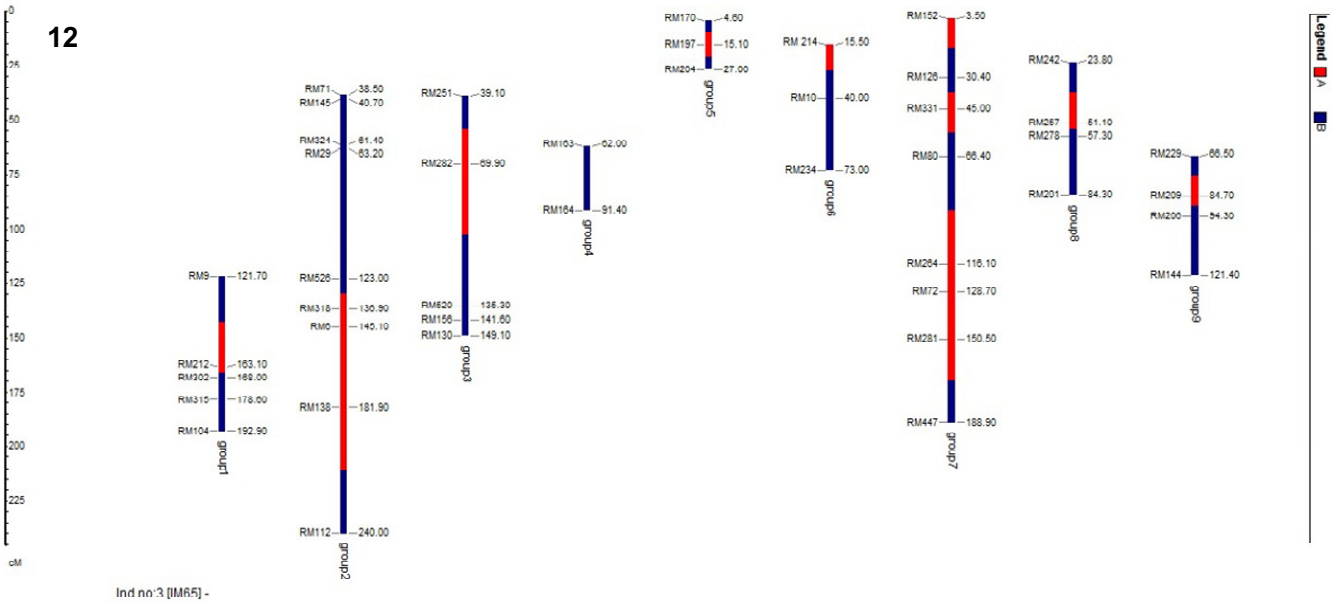
Kanagaraj *et al*. (2010) carried out BSA to identify markers linked to drought resistance using 23 RILs of IR20/ Nootripathu, two *indica* ecotypes with an extreme drought response. Three primers, RM212, RM302 and RM3825 were found to co-segregated with RILs constituting the resistant bulk.

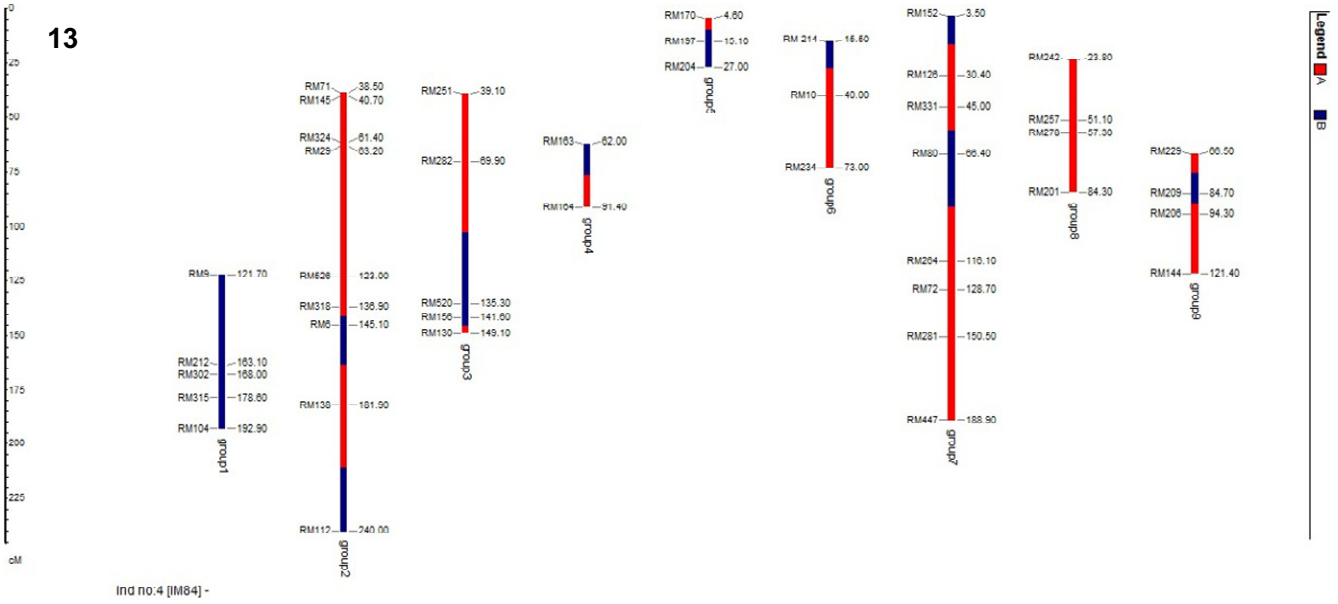
In the present investigation it was found that IM65 is highly drought tolerant whereas IM84 is a susceptible to drought. The performance of these two genotypes differed even though they were derived from the same parents. To know the actual amount of genome content donated by each parent to each progeny graphical genotyping was done.

It was inferred that IM65 obtained the maximum % of the genome from the male parent Moroberekan whereas IR50 donated a high amount of genome fragment to IM84. In the field evaluation IM65 was found to be drought resistant while IM84 was drought susceptible. This helped to confirm field results in the laboratory and *vice versa*. Since the number of genotypes and number of primers used in the study were less than the actual number of markers required for assessing which is the particular fragment responsible for drought tolerance.

Based on a linkage group of 213 SSR markers, Liu *et al.* (2008) found that the chromosomal region on chromosome 4 (RM241-RM349) hosted QTLs for seven panicle traits, including panicle number, panicle length, primary branch number, secondary branch number, spikelet number/panicle, spikelet density and grain number/panicle. They also inferred that the effect of genes located on this chromosomal region most probably served as the cause of the high positive correlation between panicle neck diameter and traits of panicle size, and the negative correlation between panicle neck diameter and panicle number.

In the present study, IM84 was found to be susceptible to drought stress but still it has given medium yield in the field condition. It indicates that it is has the potential for good yield as that of female parent IR50 and the yield has been reduced due to drought stress whereas IM65 is drought resistant similar to the male parent but has good yield. Furthermore, detailed graphical genotyping with more primers of at least one marker lying at every 1cM distance, can reveal which chromosomal fragments were donated by Moroberekan to IM65 that are responsible for giving drought tolerance to IM65 and that are absent in IM84. Further, the site-specific introgression of these fragments into IM84 or any other drought susceptible but good yielding agronomically superior genotype may give another superior





Graphical representation of IM65 (RIL) (**Fig. 12**) and IM84 (RIL) (**Fig. 13**).

Table 5 Genome content donated by each parent to drought-tolerant genotype IM65 in percentage and cM.

	%	cM
IR50	38.7	307.3
Moroberekan	61.3	487.5
Total genome covered	100	794

Table 6 Genome content donated by each parent to drought-susceptible genotype IM84 in percentage and cM.

	%	cM
IR50	60.7	482.6
Moroberekan	39.3	312.2
Total genome covered	100	794

line with drought resistance.

Graphical genotyping could help to identify:

I. The parental origin and proportion of chromosomal segment contributed by each parent.

II. The presence of sufficient molecular difference that leads to varieties.

III. A specific regions of the genome (chromosome segment) harboring genes responsible for desirable traits (Semagn *et al.* 2006).

CONCLUSION

From the present investigation it can be inferred that selection for early genotypes in the reproductive stage during drought can give early maturing drought-tolerant genotypes. The graphical representation of these superior lines will help for site specific introgression of chromosomal fragments governing drought tolerance.

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