Biparental Mapping Populations to Validate Markers in Linkage Disequilibrium with Drought Adaptive Traits in Rice (*Oryza sativa* L.)

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Abstract

Association mapping is a powerful strategy to discover markers simultaneously to diverse drought adaptive traits. However, these markers need to be validated before deploying in marker-assisted breeding for crop improvement. Rice genotypes used as parents for specific mapping populations were procured from various centres in India and were extensively phenotyped to capture genetic variability. Specific pairs of contrasts representing diversity in root and WUE traits were identified. Markers associated with these traits discovered at UASB and reported elsewhere in the literature are being analysed for polymorphism among the selected parental lines.

Rice is an important cereal crop, consumed by over half of the world human population as a staple diet including India. Cultivation of rice requires nearly 5000 litres of water to produce 1kg of rice grains. With the increasing demand from other service sectors, water availability for agriculture in general and rice cultivation in particular is decreasing steadily at an alarming rate (Andersen, 2003). Therefore, strategies such as semi irrigated aerobic cultivation have emerged as potential water saving practices. To harness the water saving advantages of this aerobic practice, it is imperative that the water requirement of rice is reduced by genetic enhancement.

A trait based approach is being strongly proposed as the most appropriate genetic enhancement method to achieve this. Traits associated with water mining, water conservation and efficient use of water for biomass production are the most relevant traits that deserve exploitation. Significant efforts have been made to assess genetic variability in root traits and WUE (Sheshshayee *et al.*, 2003; Impa *et al.*, 2005). Introgression of these complex traits is best achieved by the adoption of marker assisted breeding. Although markers have been identified, validation of such markers are still in the preliminary stage and are debatable (Pennisi, 2008).

Association mapping is a powerful approach that can simultaneously discover markers in linkage disequilibrium (LD) with several diverse traits also being able to identify specific trait donor genotypes. The simple and perhaps most effective approach for marker validation is to use specific bi-parental mapping populations.

An elaborate association mapping effort at the Department of Crop Physiology, UAS, Bengaluru, leading to the discovery of markers in LD with several relevant traits (Raju *et al.*, 2016) formed the basis of this study. Bi parental mapping populations developed by breeders in various institutes like Indian Agricultural Research Institute (IARI), Tamil Nadu Agricultural University (TNAU), Indian Institute of Rice Research (IIRR) and University of Agricultural Sciences (UAS), Bengaluru were accessed to phenotype for the target traits.

Diverse rice genotypes obtained from these centres (Table I) were extensively phenotyped for root traits, WUE (based on $\Delta^{13}C$) (Farquhar *et al.*, 1989) and other associated physiological traits.

Phenotyping for root traits was carried out in specially designed root structures measuring 5 ft tall, 10 ft wide and 60 ft long, built using cement bricks (Sheshshayee *et. al.,* 2011)**.** The major advantage of this technique lies in the fact that plants are grown with the recommended spacing and hence a specific plant density can be maintained, hence leading to the

TABLE I

List of genotypes accessed for screening.

UAS, Bengaluru	TNAU, Coimbatore	IARI, New Delhi		IIRR, Hyderabad
IR-64 (GKVK)	*Dr. Raveendran*	*Dr. A. K. Singh*		*Dr. Sarala*
AC 39020	White Ponni	FP 1	FP 16	Swarna
IET 16348	APO	FP 2	FP 17	Jalmagna
IET 15963	N 22	FP 3	FP 18	Madhukar
KMP 175	Kallurandaikar	FP 4	FP 19	
JBT 36/14	IR 64	FP 5	FP 20	
Thanu		FP 6	FP 21	
	Dr. Robin	FP 7	FP 22	
	Nootripathu	FP 8	FP 24	
	Norungan	FP 9	FP 25	
	IR 50	FP 10	FP 26	
	IR 20	FP 11	FP 27	
		FP 12	FP 28	
		FP 13	FP 29	
		FP 14	FP 30	
		FP 15		

TABLE II

Estimates of mean, range and genetic parameters of lines

Traits	Mean	Range		PCV	GCV	h^2
		Max	Min			
SL	77.7*	101.6	48.5	0.19	0.18	0.90
RL	36.3*	52.5	27.5	0.16	0.13	0.64
TN	21.6	44.0	8.6	0.33	0.32	0.91
SW	31.1*	65.7	5.8	0.45	0.41	0.85
RW	7.3	26.9	2.2	0.61	0.47	0.59
LW	11.4	23.2	2.4	0.47	0.32	0.46
TDM	44.4*	96.6	11.7	0.45	0.41	0.85
$\Delta^{13}C$	20.1*	21.9	17.2	0.04	0.03	0.64
SLA	202.4	309.9	138.4	0.17	0.14	0.73
TLA	2555.3	5203.7	884.4	0.47	0.32	0.47
R: S	0.28	0.69	0.05	0.57	-	0.2

*Significant at P ≤ 0.05. SL- Shoot Length, RL- Root Length, TN- Number of Tillers, SW- Shoot Weight, RW- Root Weight, LW- Leaf Weight, TDM- Total Dry matter, $\Delta^{13}C$ - Carbon Isotope Discrimination, SLA- Specific Leaf Area, TLA- Total Leaf Area, R: S- Root to Shoot ratio

phenotypic expression like in the field situation. The Carbon Isotope Discrimination (Δ^{13}C) was determined as a surrogate for WUE among the genotypes.

A significant genetic variability in root traits, WUE and other growth parameters was noticed among the genotypes (Table II). A continuous variability in these traits illustrating quantitative inheritance of these drought adaptive traits. Root weight varied from 2.25 to 26.99g while Δ^{13}C ranged from 17.22 to 21.89 per cent representing a significant variability.

High phenotypic coefficient variance (PCV) and genotypic coefficient variance (GCV) was observed in root weight, leaf weight, shoot weight and total dry matter. Low PCV and GCV were observed in Δ^{13}C, root length, shoot length and specific leaf area. Difference between PCV and GCV was observed to be high in root weight, leaf weight and total leaf area indicating the influence of environment on these traits. Heritability was observed to be high in shoot length, shoot weight, number of tillers and total dry matter, whereas, moderate heritability was observed in root weight, Δ^{13}C and specific leaf area.

Based on these phenotypic observations, contrasting genotypes have been selected for which the mapping populations were already developed. Based on these observations, Thanu×IET15963, Madhukar×Swarna and Kallurandaikar×IR 64 were selected (Table III).

Genomic DNA was extracted from young leaves of these contrasting parental lines using CTAB (cetyltrimethylammonium bromide) method. Polymorphism of the 82 associated SSR markers were screened among the selected parental lines by PCR amplification (Fig.1) (Raju *et al.*, 2016). Of these, 18 markers (8 for roots, 2 for "^{13}C and 8 for related traits) were identified for validation. It is envisaged to perform selective genotyping of groups of specific RILs representing contrasting variations in the traits of interest.
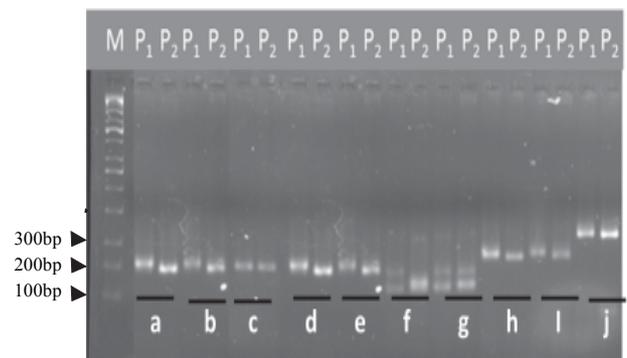
Fig. 1. Representative gel picture showing polymorphism between contrasting lines. P_1-IR64, P_2-Kallurandaikar, a-RM493, b-RM4455, c-RM4, d-RM239, e-RM211, f-RM518, g-RM227, h-RM167, i-RM1113, j-RM586

The diversity in target drought adaptive traits among rice genotypes was analysed. The novelty of this approach is in the identification of most contrasting pairs of genotypes for which bi parental mapping populations are available. Out of the 82 markers discovered to be in LD, 18 were found to be polymorphic. These markers will be examined further to validate by selective genotyping of phenotypic groups of the respective mapping population.

TABLE III

Mean values of selected genotypes for drought adaptive traits

Traits	IR 64	Kallurandaikar	Madhukar	Swarna	Thanu	IET 15963
Δ^{13}C (%)	20.67	19.53	21.45	21.89	21.06	22.25
Root Length (cm)	34.00	36.00	32.00	39.67	49.00	40.00
Root Weight (g)	4.11	8.29	3.31	7.35	2.9	4.4
Days to 50% flowering	107	105	99	114	106	92
RIL-F_8(n)		160		230		260

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