

# Genome-wide consistent molecular markers associated with phenology, plant production and root traits in diverse rice (*Oryza sativa* L.) accessions under drought in rainfed target populations of the environment

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Drought is the most predominant constraint to rainfed rice production. Identifying molecular markers associated with drought resistance traits and deploying them in marker-assisted breeding will hasten the development of drought-resilient cultivars. A total of 49 diverse rice accessions, including traditional landraces, were evaluated for plant production and root traits under natural drought stress in rainfed target populations of environment (TPE) in six successive field trials from 2010 to 2015. Significant variation for phenology, plant production and root traits under drought was noticed among the accessions. Genotyping of the rice accessions using 599 polymorphic simple sequence repeat (SSR) markers showed considerable variation among them. STRUCTURE analysis grouped the 49 accessions into three subpopulations. Similarly, three clusters were observed in Neighbor joining tree

created using Nei's genetic distance. The subpopulation POP1 consisted mostly of landraces, while subpopulation POP3 consisted of advanced breeding lines and POP2 accessions from all groups. Genome-wide association mapping detected 61 markers consistently associated in two or more trials with phenology, plant production and root traits under drought in TPE. The markers PSM52 (Chr 3), RM6909 (Chr 4), RM242 (Chr9) and RM444 (Chr 9) were consistently associated with grain yield and root traits under drought. The markers PSM127 (Chr 3) and PSM133 (Chr 4) were consistently associated with yield, plant height and spikelet fertility. These markers with pleiotropic and consistent associations with yield and secondary traits under drought in TPE may be robust candidates for marker-assisted breeding for drought resistance in rice.

**Keywords:** Association mapping, drought resistance, molecular markers, rice.

RICE (*Oryza sativa* L.) is one of the most important crops providing food for more than half of the world's population and is grown in a wide range of environments<sup>1</sup>. According to United States Department of Agriculture (USDA), in 2012–13, globally rice was cultivated on area of 158 million hectares (m ha). Rainfed systems occupy about one-third of the area, with low yields ranging from 1.0 to 2.3 t ha<sup>-1</sup> compared to about 5.0 t ha<sup>-1</sup> in irrigated systems<sup>2</sup>. Drought is the most persistent threat to rice production in rainfed areas<sup>3</sup>, affecting 10 m ha and over 13 m ha of rainfed upland and lowland rice respectively<sup>4</sup>. Developing drought-resistant rice cultivars is critical for increasing productivity and income of rainfed rice farming<sup>5</sup>.

Yield improvement in water-limited environments can be achieved by selecting secondary traits contributing in drought resistance. In water-limited environments, roots are important in extracting water and nutrients from the soil aiding crop plants in maintaining their yield<sup>6</sup>. Large genetic variation exists for root traits in rice<sup>7–9</sup>. However, genetic improvement of root traits through conventional breeding is tedious mainly because of the difficulty in phenotyping them<sup>10</sup>. Mapping quantitative trait loci (QTLs) for root traits and their use in marker-assisted breeding (MAB) help in overcoming this bottleneck. Though several QTLs have been mapped for drought resistance, including root traits, it has not yet been possible to translate these results into successful MAB programmes in rice. Small phenotypic effect and large intervals of the QTLs detected using biparental mapping population limit their application in MAB<sup>11</sup>. Further, inconsistency of the QTLs across environments and genetic backgrounds limits their adoption in MAB. While providing valuable insights,

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the QTL approach is clearly not 'scalable' to investigate the genotypic and phenotypic variation of the 120,000 accessions available in public germplasm repositories<sup>12</sup>. Association mapping (AM) is a high-resolution method of identifying the association of markers and traits based on linkage disequilibrium. Mapping the markers related to drought resistance traits using diverse rice accessions will help in a better understanding of the genetic basis of drought resistance and to develop drought-resistant varieties. In rice, markers associated with disease resistance<sup>13</sup>, stigma and spikelet fertility characteristics<sup>14</sup>, flowering time<sup>15</sup>, yield and grain quality<sup>16</sup>, and agronomic traits<sup>17</sup> have been identified using AM.

In rainfed target populations of environment (TPE) of Asia, India for instance, rice farmers are still cultivating traditional landraces as subsistence farming practice<sup>18</sup>. In spite of low productivity, landraces possess excellent adaptation to local environmental changes and offer potential for genetic improvement, particularly for stress tolerance<sup>19,20</sup>. The genomes of domesticated rice, *Oryza sativa* L., contain a wealth of information that can explain the large morphological, physiological and ecological variation observed in the many varieties cultivated for food<sup>21</sup>. The innovative use of the *ex situ* conserved genetic diversity is vital to overcome future problems associated with narrowness of genetic base of modern cultivars. The present study was thus conducted to identify microsatellite markers associated with phenology, plant production and root traits under drought stress TPE using 49 rice accessions from 13 countries, including traditional landraces native to the target production environment, which are genetically diverse and have wide phenotypic variation for growth and yield traits. Thus the present study was conducted with the following objectives: (i) to study the extent of genetic variation in phenology, plant production and root traits under drought in rainfed TPE among diverse rice accessions across trials, and (ii) to detect genome-wide consistent microsatellite markers associated with phenology, plant production and root traits under drought stress predominant in rainfed TPE using AM in these diverse rice accessions.

## Materials and methods

### Field experiment

Field trials were conducted in the experimental fields of Tamil Nadu Agricultural University at the Agricultural Research Station (ARS), Paramakudi, India located in rainfed TPE, during six consecutive years using a set of 49 diverse rice accessions. The long-term average rainfall at this site is 476 mm. Seeds of the rice accessions were received from International Rice Research Institute (IRRI), Philippines; National Rice Research Institute, Cuttack, India and ARS, Paramakudi. The accessions

included the 20 *Oryza* SNP panel lines, improved varieties, advance breeding lines and locally adapted landraces (Table 1).

The rice accessions were evaluated for drought response consecutively in six experiments during 2010 (trial 1), 2011 (trial 2), 2012 (trial 3), 2013 (trial 4), 2014 (trial 5) and 2015 (trial 6) rainfed seasons. In all these trials, seeds were sown in dry soil pre-monsoon during September each year, at the rate of 80 kg ha<sup>-1</sup> in 0.4 m<sup>2</sup> plots replicated thrice. In trial 1, initial plant growth was poor; hence 25-day-old seedlings were transplanted to another field in unreplicated plot of 2.0 × 0.2 m<sup>2</sup>/accession. In all these trials, NPK fertilizers were applied at the rate of 50 : 25 : 25 kg ha<sup>-1</sup> respectively.

The rice accessions were also evaluated under irrigated conditions during the above six rainfed seasons as in rainfed condition to determine potential yield of the accessions. However, the rice plants could not be irrigated during 2013 because of the drying up of borewell in the experimental station due to extreme drought.

### Field measurements

In these trials, soil water table was recorded using peizometers of size 75 × 10 cm, fixed across the experimental plots. Data on days to 50% flowering, plant height, number of productive tillers, panicle length, number of grains per panicle and yield per plant were collected following standard evaluation system for rice<sup>22</sup>. All plants were harvested at maturity to determine grain yield and above-ground total biomass. In the trials conducted during 2010 and 2011, root traits were also measured as below. At maturity, the field was irrigated to field capacity to loosen the soil. A 50 cm deep trench was dug up from one side of the field and proceeded carefully towards the plants to be sampled. Observations on root traits were made from three plants per accession per replication. Upon sampling, the roots were cleaned initially with a fine jet of water and transferred to the laboratory in poly bags placed over ice. Maximum root length, nodal root thickness, deep nodal root dry weight and total root dry weight were determined. Maximum root length was measured from collar region to the tip of the longest root. The thickness of nodal root was measured in three roots per plant in each replication from 2 cm below the stem base using an ocularmeter in a stage microscope (model: CH20i BIMF, Olympus, India)<sup>23</sup>. Total root dry weight was determined by weighing all the nodal roots, while the deep root dry weight was observed in the longest nodal root after drying the roots at 70°C for two days.

### Genotyping

A total of 1403 rice microsatellite markers were selected at random covering the 12 rice linkage groups for

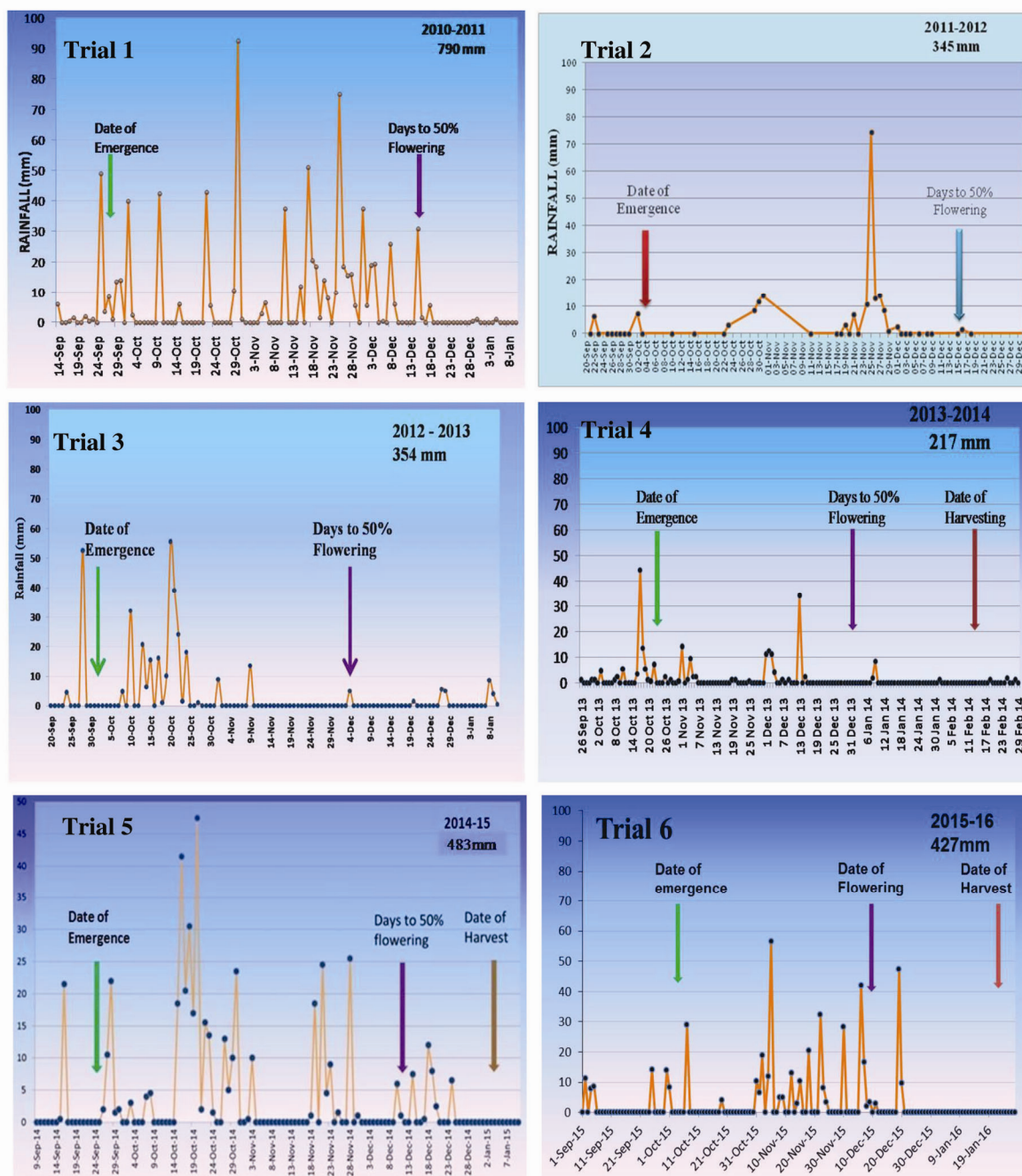
**Table 1.** Origin, ecotype and description of the 49 diverse rice accessions

Accession	Designation	Origin	Ecotype	Group	Trials tested
117275	Pokkali	India	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
–	Kallurundaikar	India	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
117266	Dular	India	<i>Aus</i>	LR	1, 2, 3, 4, 5, 6
117279	Tainung 67	Taiwan	<i>Japonica</i>	IV	1, 2, 3, 4, 5, 6
117281	Aswina	Bangladesh	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
40275	Blackgora	India	<i>Aus</i>	LR	1, 2, 3, 4, 5, 6
117273	N22	India	<i>Aus</i>	LR	1, 2, 3, 4, 5, 6
117276	Saducho	Korea	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
–	Kalinga III	India	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
50927	Bala	India	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
117267	FR13A	India	<i>Aus</i>	LR	1, 2, 3, 4, 5, 6
–	IR58821	Philippines	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	CO39	India	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
117264	Azucena	Philippines	<i>Japonica</i>	LR	1, 2, 3, 4, 5, 6
–	IR52561	Philippines	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
117265	Dom suffid	Iran	<i>Japonica</i>	LR	1, 2, 3, 4, 5, 6
117277	Shan-Huang Zhan-2 (SHZ2)	China	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
117270	M202	United States	<i>Japonica</i>	IV	1, 2, 3, 4, 5, 6
–	IR1552	Philippines	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
76300	Akiahikari	Japan	<i>Japonica</i>	LR	1, 2, 3, 4, 5, 6
–	IAC165	Brazil	<i>Japonica</i>	IV	1, 2, 3, 4, 5, 6
–	IRAT109	Côte d'Ivoire (West Africa)	<i>Japonica</i>	IV	1, 2, 3, 4, 5, 6
24274	Labelle	United States	<i>Japonica</i>	LR	1, 2, 3, 4, 5, 6
117280	Zhenshan	China	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
117271	Minghui	China	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
117274	Nipponbare	Japan	<i>Japonica</i>	IV	1, 2, 3, 4, 5, 6
117272	Moroberekan	Guinea	<i>Japonica</i>	LR	1, 2, 3, 4, 5, 6
–	KinandangPatong	Philippines	<i>Tropical Japonica</i>	IV	1, 2, 3, 4, 5, 6
117269	Li-jiang-Xin-Tuan-Hei-Gu (LTH)	China	<i>Japonica</i>	ABL	1, 2, 3
–	Nootripathu	India	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
–	Norungan	India	<i>Indica</i>	LR	1, 2, 3, 4, 5, 6
–	Anna 4	India	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
–	PMK 3	India	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
–	IR62266	Philippines	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	CT9993	Columbia	<i>Japonica</i>	ABL	1, 2, 3, 4, 5, 6
117268	IR64	Philippines	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
–	IR20	Philippines	<i>Indica</i>	IV	1, 2, 3, 4, 5, 6
–	NIL10	India	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	IR20/CT9993 NIL-212	India	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	IR20/CT9993 NIL-297	India	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	PM04022	India	<i>Indica</i>	ABL	1, 2, 3, 4, 5, 6
–	IR-83895 AYT56	Philippines	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	CPMBIR20AYT70	India	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	APO	Philippines	<i>Indica</i>	IV	2, 3, 4, 5, 6
–	IR-80013 PVS2	Philippines	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	IR-84894 PVS8	Philippines	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	IR-84887 PVS4	Philippines	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	R-RF-65PVS1	India	<i>Indica</i>	ABL	2, 3, 4, 5, 6
–	Rayada	Bangladesh	<i>Aus</i>	LR	4, 5, 6

\*ABL, Advance breeding line; IV, Improved variety; LR, Landrace.

genotyping. Genomic DNA was extracted from fresh leaves during seedling stage using CTAB procedure<sup>24</sup>. The DNA quality and quantity were assessed in 0.8% agarose gel and the final concentration was adjusted to 50 ng/μl. Polymerase chain reaction (PCR) amplification was performed with a reaction mix of 20 μl volume which contained 50 ng of template DNA along with 1 μm of each simple sequence repeat (SSR) primer (Sigma Aldrich, USA), 100 μM deoxy nucleotide, 1× *Taq* buffer,

and 0.02 U *Taq* polymerase (Bangalore Genei, India). PCR was performed in a Master Cycler Gradient (Eppendorf, Germany) with the initial denaturation at 94°C for 5 min followed by 36 cycles of amplification, where each cycle included 1 min at 94°C, 1 min at 55°C (depending on the annealing temperature of the markers), 1 min at 72°C and final extension step at 72°C for 5 min. The PCR products were separated in 3% agarose gel (BioWhittaker Molecular Applications, Vallensbaek Strand, Denmark)



**Figure 1.** Rainfall (mm) pattern and time of seedling emergence and 50% flowering in the six trials conducted under rainfed conditions in target populations of environment.

run in 0.5× tris-borate EDTA (TBE) buffer<sup>25</sup>. The sequence of the primers and amplification conditions of each primer set are available in public database<sup>26</sup>.

*Genetic diversity, phylogenetic analysis and population structure*

The summary statistics of the markers was determined using PowerMarker version 3.25 (ref. 27). The param-

eters determined were the number of alleles per locus, major allele frequency, gene diversity and polymorphism information content (PIC). Nei’s distance<sup>28</sup> was calculated and utilized for the unrooted phylogeny reconstruction using Neighbor joining method as implemented in PowerMarker, and MEGA 5.0 was used to visualize the tree<sup>29</sup>. Population structure of the rice accessions was determined using the STRUCTURE software version 2.3.4 (refs 30–32). The *K* value, which is the optimum

number of populations, was selected after ten independent runs with a burn-in period of 100,000 steps with 100,000 Monte Carlo Markov chain replicates. The range of genetic clusters was set from  $K = 1$  to 10. Each value of  $K$  was replicated 10 times<sup>33</sup>.

### Marker–trait associations

Association analysis between marker loci and phenotypic traits was performed in all trials using TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) software version 3.0 (ref. 34), after accounting for the gross level population structure ( $Q$ ) in GLM analysis. The  $Q + K$  (kinship) model was used in the MLM analysis with P3D algorithm<sup>35</sup>. It has been reported that the  $Q + K$  model reduces the false positive associations<sup>36</sup>. Hence, the results of MLM which uses the  $Q + K$  model are reported in the study. The marker  $P$  value was used to determine the significance of each marker trait association.

The 49 rice accessions tested in this study included 20 accessions of *Oryza* SNP panel. Data for grain yield, root dry weight and root length of these 20 rice lines from trials 1 and 2 of the present study were earlier reported in a multiple environment study across six countries<sup>37</sup>. Similarly, data for phenology and plant production traits of 17 accessions of *Oryza* SNP panel from trial 3 were also reported<sup>38</sup>. In the present study, marker–trait associations were performed using the genotypic data of 599 polymorphic SSR markers and the various traits measured in all the six trials using larger population of 49 diverse accessions.

## Results and discussion

### *Variation in phenology, plant production and root traits under drought in TPE*

The long-term average rainfall at the experimental site was 476 mm. The amount and distribution of rainfall varied between the trials conducted at TPE. The total rainfall received was 790, 345, 354, 217, 483 and 427 mm in trials 1, 2, 3, 4, 5 and 6 respectively (Figure 1). In trial 1, there was a dry spell of 24 days during grain-filling stage, while in trial 2, there was a dry period at the time of germination and at flowering stage. In trial 3, there was minimal rainfall for 22 days after emergence after which 28.3 mm rain was received till flowering, thus it had severe reproductive stage stress. Trial 4 recorded the lowest amount of rainfall, 217 mm during the cropping season among the trials. The crop experienced moderate stress at vegetative phase and severe drought stress at reproductive stage; hence there was a combination of vegetative and reproductive stage drought stress in this trial. Trials 5 and 6 received normal rainfall which was well distributed

throughout the cropping season. Thus, variation in rainfall distribution rather than total rainfall received led to the difference in the drought intensity among and within the trials, which affected the rice productivity<sup>39</sup>.

The 49 diverse rice accessions from different geographical locations used in this study were unique in that they include traditional landraces native to TPE. Landraces of domesticated species preserve useful genetic variation, yet untapped<sup>40</sup>. These 49 rice lines showed considerable variation in phenology, plant production and root traits under natural drought stress in the rainfed TPE. Variation for phenology and plant production traits under drought stress in TPE has been reported earlier among rice lines<sup>41–44</sup>. Table 2 presents the mean and range values of various traits in all trials. Average grain yield across the trials varied widely from 216 (trial 4) to 4007 kg/ha (trial 5). As discussed earlier, the combination of vegetative and reproductive stage drought in trial 4 led to poor plant development with fewer tillers and lower yield. Stress at different stages affects grain yield by decreasing the translocation of assimilates to the grain<sup>45</sup>. Reduction in grain yield under drought (i.e. rainfed) condition was 46.8% and 67.1% in trial 2 and 3 respectively, compared to irrigated condition. A 65% reduction in yield under drought compared to that of non-stress is considered severe drought in rice<sup>46</sup>. In trial 1, the poor initial growth of seedlings and additional transplanting shock to a different field were reflected by low biomass and yield of the accessions. Trials 5 and 6 had sufficient rainfall during the reproductive stage and hence had better grain yield than the other trials.

The ability of plants to absorb water from deeper layers of the soil might contribute to the yield under drought<sup>47</sup>. Root thickness under rainfed condition in the field ranged from 547 to 1180  $\mu\text{m}$  and 259 to 1051  $\mu\text{m}$  in trials 1 and 2 respectively. Root length ranged from 20.2 to 36.4 cm in trial 1. Positive relation of root traits with biomass and grain yield under drought stress has been reported earlier in rice<sup>48</sup>. Root length, and total as well as deep nodal root dry weights had a positive correlations with biomass ( $r = 0.65$ ,  $0.62$  and  $0.63$  respectively, with  $P < 0.001$ ) under rainfed condition in trial 1 (Supplementary Table 1). However, in trial 2, root dry weight showed negative correlation with grain yield ( $r = -0.35$ ,  $P < 0.05$ ), and biomass ( $r = -0.33$ ,  $P < 0.05$ ). Lack of significant positive relation between deep root growth and root thickness with shoot biomass was earlier reported in rice<sup>49,50</sup>. Root growth at soil depths below 30 cm may provide access to critical soil water reserves during drought in rainfed lowland rice<sup>8</sup>. The roots were mostly distributed in topsoil layers, since the average root length across the rice accessions hardly exceeded 30 cm in this study (Table 2). Under field drought conditions, root growth below 30 cm depth is rarely reported in rice<sup>51</sup>. Soil water deficit, as indicated by soil water depletion below 90 cm, and dry surface soil might have led to stomatal

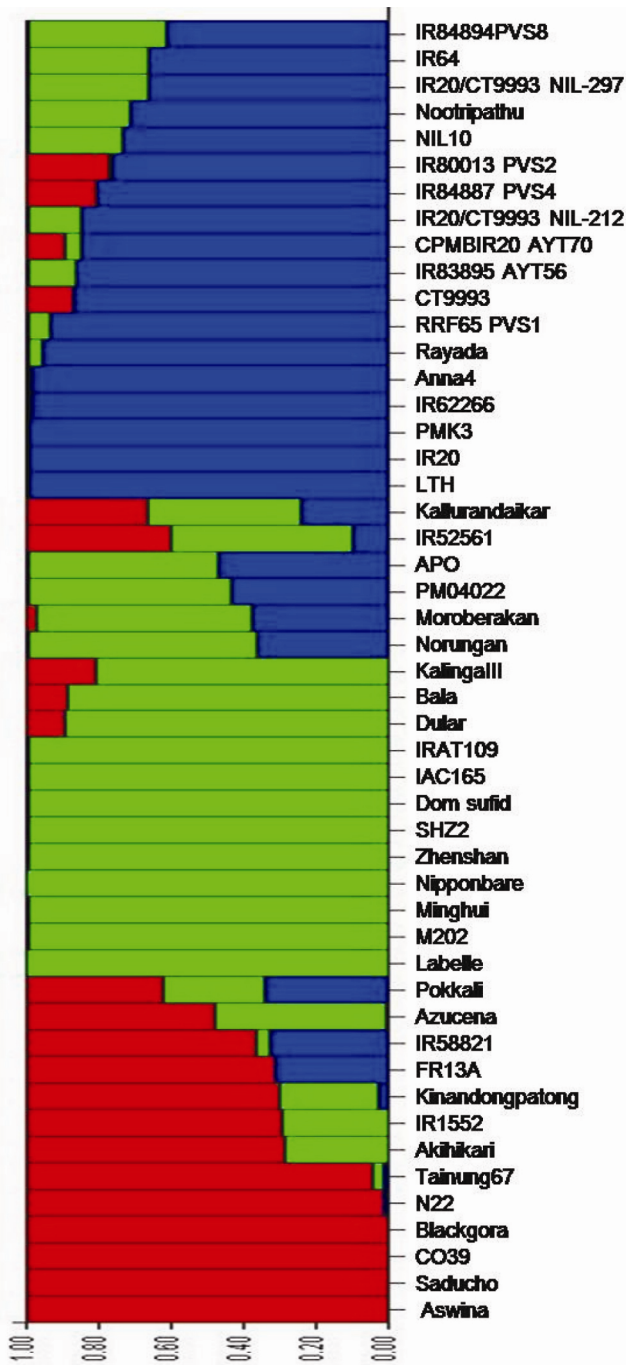
**Table 2.** Mean and range values of phenology, plant production and root traits for the 49 diverse rice lines under drought in target populations of environment for the six trials

	Mean ± SE						Range					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
DFF	79 ± 1.4	76 ± 1.1	100 ± 1	87 ± 1	75 ± 1	77 ± 1	65-98	64-91	64-112	74-100	55-92	63-90
PH	108 ± 3.1	82 ± 2.3	55 ± 1.3	45 ± 1.3	96 ± 2.06	114 ± 2.2	66-151	50-120	29-83	16-59	66.5-128	77-143
NT	5.4 ± 0.11	5.5 ± 0.2	5.5 ± 0.1	3 ± 0.2	4.6 ± 0.07	6.9 ± 0.13	3.2-7	2.2-9.2	4-7	1-6	3.7-6.2	5-9
NPT	5.3 ± 0.1	5.2 ± 0.3	4.5 ± 0.2	2 ± 0.1	4.0 ± 0.07	6.4 ± 0.12	2.9-7	1.1-9.8	2-6	1-5	3.1-5.8	4.4-8.2
PL	21 ± 0.4	20 ± 0.3	-	16 ± 0.3	21 ± 0.2	22 ± 0.3	15-29	14-24	-	9-19	17-25	18-29
NOG	92 ± 4.2	57 ± 3.3	57 ± 2.0	38 ± 1.9	69 ± 2.7	78 ± 2.7	33-180	7-143	34-87	7-72	30-126	39-136
SF	84.6 ± 1.7	67.0 ± 2.9	81.7 ± 1	73 ± 2.4	84.2 ± 1.6	76 ± 1.6	45-98	11-94	59-91	25-95	53-99	40-92
GY	787 ± 53	1392 ± 135	926 ± 36	216 ± 50	4007 ± 155	2749 ± 139	625-1937	229-5833	338-1620	43-2441	430-5970	775-4446
TBM	2083 ± 125	5831 ± 463	4063 ± 247	1133 ± 158	11739 ± 344	7590 ± 214	375-4375	2500-22292	1696-10101	58-6348	5908-17611	4045-11859
HI	0.38 ± 0.02	0.24 ± 0.01	0.33 ± 0.02	0.29 ± 0.02	0.34 ± 0.01	0.36 ± 0.01	0.1-0.8	0.06-0.38	0.08-0.59	0.04-0.70	0.06-0.58	0.17-0.53
RT	861 ± 19	613.1 ± 24	-	-	-	-	547-1180	259-1051	-	-	-	-
RDW	4.6 ± 0.3	6.20 ± 0.5	-	-	-	-	1.4-10.3	1.9-17.6	-	-	-	-
RL	27.8 ± 0.6	25.4 ± 0.37	-	-	-	-	20.2-36.4	20.4-31	-	-	-	-
DRDW	1.54 ± 0.09	0.92 ± 0.04	-	-	-	-	0.5-3.4	0.4-1.8	-	-	-	-

DFF, Days to 50% flowering; PH, Plant height (cm); NT, Number of tillers; NPT, Number of productive tillers; PL, Panicle length (cm); NOG, Number of grains/panicle; SF, Spikelet fertility (%); GY, Grain yield (kg/ha); TBM, Total biomass (kg/ha); HI, Harvest index; RT, Root thickness (µm); RDW, Total root dry weight (g/plant); RL, Root length (cm) and DRDW: Deep root dry weight (g/plant).

**Table 3.** Number of alleles per locus, gene diversity, polymorphism information content (PIC) and major allele frequency (MAF) among the 49 diverse rice accessions using 599 polymorphic simple sequence repeat markers

	MAF	Number of alleles	Gene diversity	PIC
Mean	0.6814	4.7362	0.4390	0.4034
Minimum	0.2245	2.0000	0.0400	0.0392
Maximum	0.9796	14.0000	0.8571	0.8443



**Figure 2.** Population structure of the 49 diverse rice accessions using 599 polymorphic SSR markers ( $K = 3$ ). Bar colours represent different subgroups based on the  $K$  value and bar length indicates probability of accessions belonging to a specific subgroup.

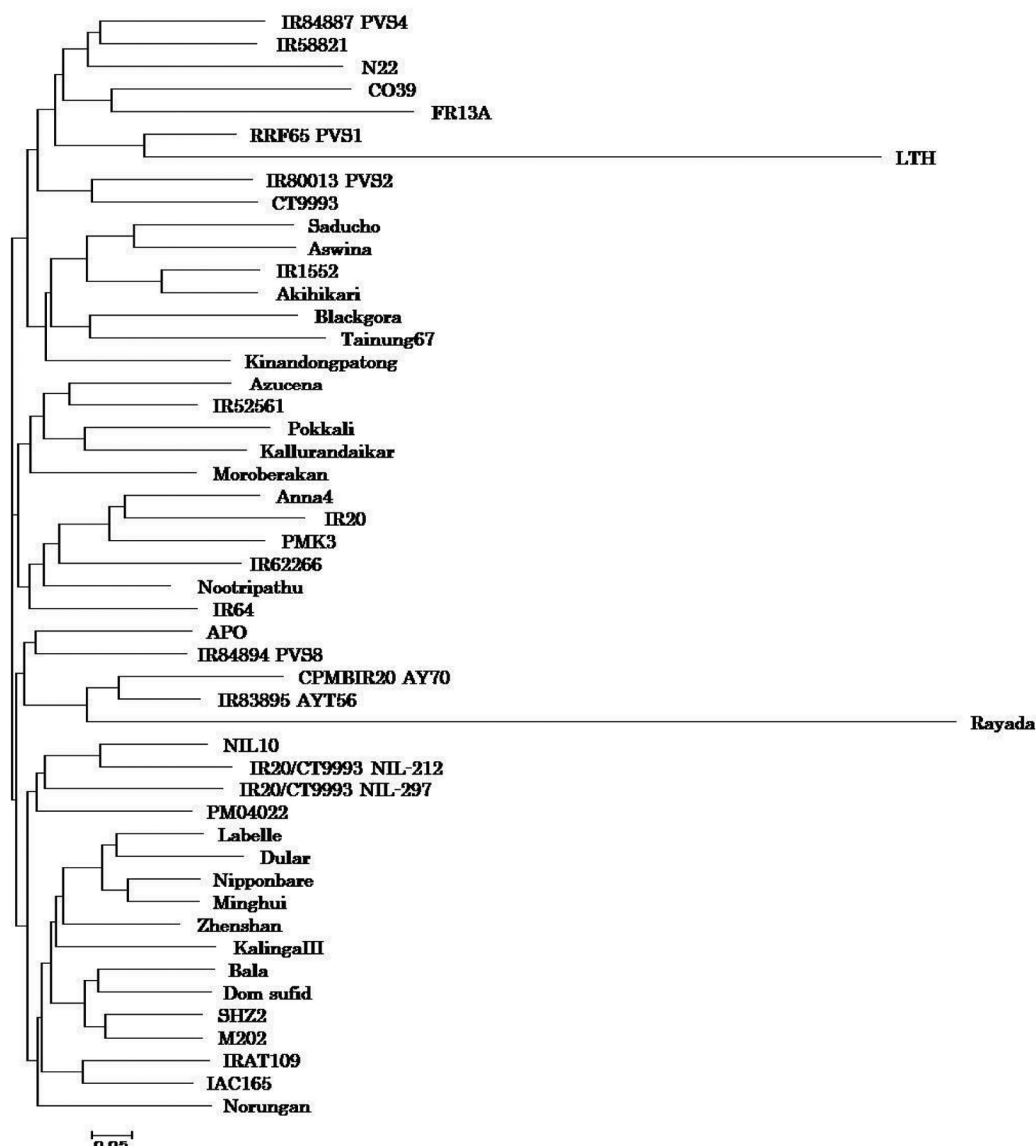
closure through root ABA signal with consequent reduction in photosynthesis, biomass and yield in trial 2. The positive association of root traits with biomass in trial 1 may be related to initial seedling establishment and growth rather than drought resistance, since the stress was mild in this trial. Difficulty to phenotype roots intact in field conditions, especially under drought stress is still a bottleneck in determining the real contribution of root system traits to yield under water-limited environments in rice.

#### Genetic diversity

Considerable genetic diversity was observed among the 49 rice accessions used in this study (Table 3). A total of 1403 SSR markers covering all the 12 chromosomes were screened with the 49 rice accessions and 599 markers (40.9%) were found to be polymorphic. From these 49 rice accessions, a total of 2837 alleles were detected using the 599 polymorphic markers (Supplementary Table 2). A minimum of two to a maximum of 14 alleles were detected among the accessions per marker, with an average of 4.7 alleles. This range is higher compared to 1–11 reported earlier<sup>52</sup> and comparable with that of a recent study involving rice accessions from Malaysia<sup>53</sup>. The PIC varied from 0.0392 to 0.8443, with an average of 0.4034. The mean PIC value of 0.40 in this study is also higher compared to earlier reports for rice<sup>54,55</sup>. Out of the 599 polymorphic markers, 220 markers (36.6%) were highly informative ( $PIC \geq 0.5$ ), 219 markers (36.5%) were reasonably informative ( $PIC = 0.25-0.5$ ) and 160 markers (26.6%) were less informative ( $PIC \leq 0.25$ ). The gene diversity ranged from 0.0400 to 0.8571, with an average of 0.4390. The wide range of genetic diversity within this small set of 49 accessions will serve as a valuable resource to mine genes for drought resilience in rice.

#### Population structure

The structure of the population is important in association mapping studies. It can be a cause of type-I error<sup>56</sup>. However due to their natural and artificial selection, rice cultivars seem to be highly structured<sup>57</sup>. Unravelling the genetic basis of adaptation of local landraces to target



**Figure 3.** Neighbor joining tree of the 49 diverse rice accessions based on Nei's genetic distance.

production environments is essential for world food security. Here, we also studied certain underexploited rice landraces, viz. Nootripathu, Norungan and Kallurundaikar, traditionally grown under rainfed conditions in Tamil Nadu, India in subsistence farming systems. Rice landraces are a repertoire of genes of agronomic importance<sup>17</sup>, such as tolerance to submergence<sup>58</sup>, salinity<sup>59</sup> and drought<sup>60</sup>. STRUCTURE analysis grouped the 49 accessions into three subpopulations (Figure 2). The subpopulation POP1 had six accessions, mostly landraces. These landraces have the capacity to tolerate biotic and abiotic stresses. This suggests an evolutionarily conserved adaptive response to abiotic stresses in these accessions. Thus identifying the genetic basis of the stress tolerance will provide important insights for breeding resilient rice varieties for sustainable agriculture<sup>17</sup>. POP2 had 12

accessions which included mostly improved lines. POP3 had 12 accessions which included mostly advanced breeding lines of *indica* ecotype and the rest were admixtures. Three clusters were observed in the Neighbor joining tree constructed based on Nei's genetic distance using PowerMarker (Figure 3).

#### Marker-trait association

Based on MLM analysis, a total of 192, 250, 172, 137, 124 and 134 marker-trait (QTL) associations ( $P < 0.005$ ) distributed throughout the rice genome for the various traits under drought in TPE were detected in trials 1–6 respectively (Supplementary Table 3). In trial 1, the markers PSM43 (Chr 1) and RM6275 (Chr 2) were



**Table 4.** Consistent marker–trait associations across trials under drought in TPE in the 49 diverse rice lines

Trait	Marker	Chromosome	Trial	Trait	Marker	Chromosome	Trial
DFF	PSM144	7	3, 4	NPT	RM5404	2	2, 5
	PSM194	4	3, 4		PSM35	1	5, 6
	PSM322	9	2, 3	PL	RM579	1	4, 6
	PSM419	12	3, 5		PSM107	4	4, 5
	RM1054	5	1, 3, 4		PSM108	4	1, 5
	RM3815	3	3, 5		PSM120	2	1, 6
	RM5221	4	2, 3		RM1234	2	2, 5
	RM233B	2	3, 6	RM5349	11	1, 2	
	RM3825	1	2, 6	RM6748	4	1, 2	
	RM586	6	1, 6	NOG	RM1234	2	3, 4
RM6275	2	2, 6	RM1155		4	5, 6	
PH	PSM116	2	2, 3	SF	PSM116	2	1, 5, 6
	PSM120	2	1, 2, 5, 6		PSM126	3	1, 6
	PSM127	3	5, 6		PSM157	9	2, 6
	PSM133A	4	1, 2, 5, 6		PSM322	9	5, 6
	PSM142a	7	1, 2, 5		RM1089	5	2, 4, 5
	PSM190	12	3, 4		RM1135	7	2, 4, 5
	PSM3	6	1, 2, 5, 6		RM12350	2	4, 5
	PSM322	9	2, 5, 6		RM170	6	3, 4
	PSM366	11	3, 4		RM5548	10	2, 6
	RM192	7	2, 6		RM8236	1	2, 5
	RM263A	2	5, 6	GY	PSM127	3	5, 6
	RM81	3	5, 6		PSM133A	4	1, 5
	RM1089	5	2, 5, 6		PSM197	4	1, 4
	RM1167	1	3, 5	TBM	RM1694	2	2, 3
	RM22	3	3, 4		OSR2	2	3, 4
	RM508	6	3, 4, 5		PSM139	7	5, 6
NT	PSM188	12	2, 5		PSM405	10	3, 4
	PSM368	1	1, 3		PSM432	7	1, 3, 4
	RM36	3	3, 6	HI	RM1108	1	2, 3
	RM1134	7	2, 3		PSM139	7	4, 5
	RM1155	4	2, 3		PSM156	9	4, 5
	RM1019	8	3, 6		PSM164	10	4, 5
	RM3131	3	2, 3		RM1167	1	5, 6
	RM521	2	2, 3				

Abbreviations for traits as in Table 2.

associated with total root dry weight, deep root dry weight and root length. In trial 2, PSM153 (Chr 8) was associated with total root dry weight and root length. PSM153 was also associated with grain yield in trial 5. Several marker regions showed pleiotropic effect for grain yield and root traits. For instance, in trial 1, the marker RM7051 (Chr 4) was associated with grain yield and deep as well as total root dry weights. In trial 2, the markers PSM52 (Chr 3), RM242 (Chr 9) and RM6909 (Chr 4) were associated with grain yield and root thickness, while marker RM444 (Chr 9) was associated with grain yield and root length. Several of the marker–trait associations are consistent with earlier reports for drought resistance traits in rice detected using traditional QTL mapping with bi-parental populations. For instance, in the present study marker RM242 (Chr 9) was associated with grain yield, root thickness, plant height, number of tillers, number of productive tillers and total biomass. This marker was earlier reported

to be associated with meta QTLs for grain yield under drought<sup>61</sup> and root length<sup>62,63</sup> as well as penetrated root thickness<sup>64</sup> in rice. The root trait QTLs at this marker have been used in MAB to introgress deep root traits from Azucena<sup>65</sup> and CT9993<sup>66</sup> into elite rice cultivars. Similarly, the marker RM6748 on Chr 4 associated with grain yield and biomass in trial 6, and panicle length in trials 1 and 2, is located within the QTL region for grain yield under drought<sup>48</sup> and basal root thickness<sup>64</sup> in CT9993/IR62266 rice doubled haploid lines. This region is also used in MAB to introgress deep and thick root traits from CT9993 into elite rice cultivars<sup>66</sup>. Hence, genome-wide association mapping (GWAS) may help to identify markers consistently associated with grain yield and root traits (e.g. RM242 and RM444 on Chr 9) using diverse accessions, thus saving resources and time needed for conventional QTL mapping effort with narrow genetic variation using a large number of biparental breeding lines.

*Consistent marker–trait associations*

A total of 61 markers were consistently linked to various traits across trials (Table 4). Among these, 14 markers were consistently associated with more than one trait in two or more trials. The marker, PSM322 (Chr 9) was consistently associated with days to 50% flowering in trials 2 and 3, plant height in trials 2, 5 and 6, and spikelet fertility in trials 5 and 6. The marker PSM120 (Chr 2) was associated with plant height in trials 1, 2, 5 and 6, and spikelet fertility in trials 1, 5 and 6. Further, some markers showed co-location with plant height and grain yield in this study. The marker PSM133 (Chr 4) was associated with plant height in four trials (1, 2, 5, 6) and grain yield in two trials (1, 5). Near this region lies the improved water use efficiency locus with QTLs for leaf water status, stomatal conductance and carbon isotope discrimination<sup>67</sup>. Another marker, RM1694 (Chr 2) associated with grain yield in trials 2 and 3 is located near to the marker PSM120. This region lies within the epistatic QTL reported for grain yield (qDTY2.3)<sup>68</sup>. The marker PSM 127 (Chr 3) was consistently associated with grain yield and plant height in trials 5 and 6. The marker PSM197 (Chr 4) was associated with grain yield in trials 1 and 4, and spikelet fertility in trial 5. The marker RM22 (Chr 3) was associated with plant height in trials 3 and 4. This region has been reported to be associated with days to flowering, biomass, plant height and harvest index in a N22 × Swarna population<sup>69</sup> and a metaQTL for yield (mQTL3.1)<sup>70</sup>. Thus in this study, consistent marker–trait associations were detected for grain yield and secondary traits under drought in TPE.

**Conclusion**

To sum up, considerable variation in phenology, production and root traits observed under drought in TPE among the accessions may help further increase yield in water-limited environments in rice. Use of diverse rice accessions, especially traditional landraces native to the target production environment is the uniqueness of this study and offers potential to mine valuable alleles from the untapped germplasm. Grouping of landraces into one subpopulation in STRUCTURE analysis, despite the wide genetic diversity and ecotype among the accessions, points to a common evolutionarily conserved response to environmental stresses such as drought, submergence and salinity in rice. Markers PSM52 (Chr 3), RM242 (Chr 9), RM6909 (Chr 4) and RM444 (Chr 9) are consistently associated with both grain yield and root traits under drought in TPE. These genomic regions may be good candidates for MAB, and to better understand the genetics and physiology of drought resistance through map-based cloning and functional genomics studies. GWAS thus helps mine robust alleles for drought resistance using diverse rice germplasm.

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