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Genetic structure and molecular markers-trait association for physiological traits related to seed vigour in rice

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ABSTRACT

Good quality seed is an important factor for a good crop and harvest. Vigour is the key performing trait of quality seed. Many physiological traits influence seed vigour in rice. Association of molecular markers with eleven physiological traits were investigated in a representative population. A representative population was constituted by including genotypes from all the phenotypic groups of the 11 physiological traits from the shortlisted population of 120 genotypes. The genotypic accessions with rich in multiple physiological traits were identified from the representative population. STRUCTURE, GenAlEx and Darwin software were used to classify the population. Generalized Linear Model (GLM) and Mixed Linear Models (MLM) were used in the marker-trait association analysis using the TASSEL software. Wide variations among the genotypic accessions were observed for the physiological traits estimated from the panel population. Linkage disequilibrium was detected for the 11 physiological traits in the panel population. STRUCTURE software classified the population into two genetic structure groups. Higher values of gene diversity and polymorphic information content (PIC) were estimated from the population based on genotyping using 50 simple sequence repeat markers. Genotypes present within groups and sub-groups showed similarity for their physiological traits. Strong association of the markers with physiological parameters namely RM167 for seed vigour index-I; RM7364 and RM235 for seed vigour index-II; RM440, RM223 for seedling dry weight; RM 256,RM25181, RM6547, RM328, RM201 for rate of root growth; RM 20, RM13335, RM216 for rate of shoot growth while RM20A and RM201 for absolute growth rate were detected by both GLM and MLM analyses. Markers detected in this association analysis may be potential use for future seed vigour improvement in rice.

1. Introduction

Rice is the main cereal for carbohydrate source across all Asian developing countries which provide about 23% of global caloric intake (Li et al., 2011; Pandit et al., 2021). To meet the challenging and eventual food demand, a quantum increase in agricultural productivity is very much essential for which production and distribution of high quality seed is becoming increasingly important. As one of the efficient and economic input for agricultural development, seed of high quality could alone cause surge in productivity up to 25–30% (Hasanuzzaman, 2015). Good seed vigour not only ensures seed germination, growth whereas establishment of seedling also by combating adverse climatic condition (Rajjou et al., 2012; Ventura et al., 2012). Therefore, improving the seed vigour remains one of the prime objective of rice breeding as it also decides the yield and ameliorates the crop resilience against biotic and abiotic restraints (Finch-Savage and Bassel, 2016; Diwan et al., 2013). In addition, seed vigour plays an important role in early establishment of crop in the field by competing against weeds in case of direct seeding which is foreseen as future cultivation strategy of rice (Yamauchi and Winn, 1996; Dingkuhn et al., 1999; Rao et al.,

Abbreviations: AGR, absolute growth rate; GI, germination index; MGT, mean germination time; RGR, relative growth rate; RI, rate of imbibition; RRG, rate of root growth; SDW, seedling dry weight; SG, seed germination; RSG, rate of shoot growth; SVI-I, seed vigour index I; SVI-II, seed vigour index II..

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Table 1
Mean values of eleven physiological traits estimated from the panel population.

SL. NO.	Genotypes(Accession no./ Vernacular name)	RI(ml/h)	GI	SG (%)	SDW (g)	SVI-I	SVI-II	RRG(cm/day)	RSG(cm/day)	RGR(g/day)	AGR(cm/day)	MGT (day)
1	AC10608	0.005	1.96	38	0.005	320.56	0.175	0.440	0.850	0.121	1.29	5.02
2	AC10187	0.006	4.29	80	0.005	1060.20	0.412	1.917	0.667	0.157	2.58	4.86
3	AC10162	0.007	1.28	28	0.003	256.24	0.071	1.067	0.890	0.073	1.96	5.50
4	AC7282	0.007	0.92	20	0.004	166.32	0.074	0.927	0.990	0.075	1.92	5.50
5	AC7269	0.004	0.83	18	0.011	113.00	0.194	0.570	0.640	0.244	1.21	5.45
6	AC7134	0.006	0.92	20	0.008	142.72	0.140	0.687	0.690	0.233	1.38	5.50
7	AC7008	0.007	0.73	16	0.006	129.76	0.088	0.660	0.700	0.191	1.36	5.50
8	AC9093	0.006	2.62	44	0.030	446.16	1.298	0.457	0.573	0.150	1.03	3.27
9	AC9090	0.006	2.17	38	0.028	451.36	1.050	0.477	0.797	0.147	1.27	3.47
10	AC9076A	0.007	0.62	12	0.015	173.76	0.180	0.543	0.133	0.195	0.68	4.00
11	AC9065	0.007	2.73	46	0.024	498.24	1.094	0.270	0.883	0.121	1.15	3.31
12	AC9063	0.005	1.83	34	0.025	326.12	0.856	0.367	1.190	0.126	1.56	3.80
13	AC9058	0.006	2.09	38	0.025	332.96	0.962	0.237	1.170	0.115	1.41	3.63
14	AC9053A	0.005	0.78	16	0.005	105.28	0.080	0.140	0.857	0.013	1.00	4.25
15	AC9050	0.007	1.80	34	0.017	261.08	0.564	0.217	0.257	0.151	0.47	3.83
16	AC9005	0.008	4.33	48.3	0.012	849.97	0.563	0.803	1.109	0.077	1.91	3.41
17	AC20389	0.006	5.71	80	0.012	999.32	0.908	0.513	0.707	0.109	1.22	3.58
18	AC20371	0.007	7.42	94	0.013	1328.72	1.216	0.333	1.153	0.107	1.49	3.32
19	AC20423	0.007	1.73	32	0.010	475.64	0.204	1.090	0.590	0.142	1.68	4.69
20	AC20362	0.006	6.18	84	0.011	1129.04	0.886	0.377	0.810	0.100	1.19	3.50
21	AC20328	0.007	6.22	86	0.014	1255.68	1.161	0.540	1.120	0.100	1.66	3.56
22	AC20317	0.006	5.02	68	0.011	569.16	0.442	0.347	0.150	0.146	0.50	3.47
23	AC20282	0.01	4.51	70	0.011	1149.28	0.789	0.883	1.150	0.094	2.03	3.99
24	AC20246	0.007	3.21	48	0.010	660.96	0.452	0.403	0.893	0.123	1.30	3.76
25	AC20347	0.008	1.63	28	0.008	310.60	0.138	0.563	0.693	0.130	1.26	4.47
26	Palina dhan-1	0.003	0.97	16	0.010	102.88	0.038	0.267	0.660	0.075	0.93	4.25
27	Chatuimuchi	0.005	5.90	84	0.008	608.80	0.329	0.663	0.527	0.142	1.19	3.76
28	Uttarbanga local-9	0.006	0.62	10	0.004	56.92	0.048	0.200	0.107	0.159	0.31	4.25
29	Gochi	0.005	1.67	26	0.012	171.96	0.120	0.250	0.447	0.159	0.70	4.07
30	Sugandha-2	0.003	6.00	84	0.008	540.80	0.208	0.457	0.433	0.029	0.89	3.66
31	Jhingesal	0.004	2.14	34	0.006	370.44	0.169	0.610	0.937	0.182	1.55	4.13
32	PK-19 Cheruvirippu	0.007	3.24	56	0.010	411.04	0.149	0.300	0.503	0.084	0.80	4.39
33	PK-28 Mahamaga	0.007	2.35	42	0.010	250.08	0.066	0.443	0.617	0.100	1.06	4.60
34	PK- 25 Jaya	0.006	3.63	64	0.012	442.00	0.300	0.527	0.507	0.273	1.03	4.49
35	PK-33 D1	0.005	1.73	34	0.012	185.36	0.130	0.357	0.483	0.241	0.84	5.08
36	Pk-21	0.006	5.84	100	0.008	1151.00	0.400	0.787	1.613	0.059	2.40	4.34
37	PK-22 Gandhakasala	0.004	3.34	58	0.008	397.76	0.152	0.720	0.670	0.091	1.39	4.41
38	PK- 23 Sreyas	0.005	1.79	32	0.004	235.36	0.127	0.667	0.707	0.064	1.37	4.55
39	Gondiachampeisiali	0.007	3.72	66	0.014	1010.48	0.647	0.513	0.910	0.084	1.42	4.52
40	Chinamal	0.005	2.50	46	0.004	637.40	0.411	0.583	0.533	0.199	1.12	4.71
41	Magra	0.005	1.62	30	0.008	390.36	0.138	1.213	0.860	0.180	2.07	4.75
42	Landi	0.006	2.70	46	0.010	344.17	0.174	0.547	0.665	0.154	1.21	4.70
43	Lal gundi	0.006	2.04	36	0.010	334.88	0.054	0.243	0.600	0.143	0.84	4.48
44	Balisaralaktimachi-K	0.007	2.33	40	0.012	579.96	0.244	1.193	0.977	0.141	2.17	4.40
45	Laxmibilash	0.004	1.11	22	0.012	257.00	0.096	0.273	0.377	0.259	0.65	–
46	Kaniar	0.007	3.80	66	0.006	918.28	0.619	0.407	0.843	0.051	1.25	4.45
47	Kanak champa	0.006	1.09	24	0.012	277.68	0.204	0.733	0.457	0.308	1.19	5.58
48	Magura-s	0.005	1.58	32	0.024	400.40	0.136	0.647	1.350	0.246	2.00	5.30
	CD(5% df)	0. 034	0.891	15.6	0.049	35.80	0.23	0.29	0.48	0.049	0.36	0.46
	CV(%)	9.56	11.52	4.42	12.6	10.36	13.6	15.54	13.71	13.42	11.48	4.591

RI:Rate of imbibition,GI:Germinationindex,SG:Seed germination,SDW:Seedling dry weight,SVI-I:Seed vigour index I,SVI-II:Seed vigour index II, RRG:Rate of root growth, RSG:Rate of shoot growth, RGR:Relative growth rate,AGR:Absolute growth rate,MGT:Mean germination time.

2007).

Seed vigour governs by various physical, physiological and biochemical traits which are controlled by many quantitative trait loci (QTL) located on different chromosomes. Information on the genomic regions controlling these associated traits is very important for seed vigour improvement in rice. Here, the QTL mapping has emerged as an important aid to study the heritability of such complex trait (Regan et al., 1992; Redona and Mackill 1996; Cui et al., 2002; Miura et al., 2002; Zhang et al., 2005a, 2005b; Fujino et al., 2008; Cairns et al., 2009; Wang et al., 2010; Dang et al., 2014; Xie et al., 2014; Liu et al., 2014; Jiang et al., 2017; Zhang et al., 2017). These studies have reported correlation of seed vigour with several physiological and physical attributes namely, thousand seed weight, seedling dry weight, length of root, length of shoot, germination rate, length of radicle, activity of root, length of coleoptile, length of mesocotyl days to achieve 50% germination, germination index and germiation potential. Also the recent report of Sahoo et al., 2020 has analyzed the marker-trait association of biochemical traits related to seed vigour in rice.

In previous study, QTLs controlling root length related to seed vigour in rice were reported by different teams in mapping studies (Redona and Mackill, 1996; Cui et al., 2002; Zhang et al., 2005a, 2005b; Lee et al., 2012; Dang et al., 2014; Xie et al., 2014; Zhang et al., 2017; Yang et al., 2010; Dang et al., 2014; Zhang et al., 2017; Yang et al., 2019; Yang et al., 2020). Shoot length was reported to be controlled by as many as 117 QTLs and mapped on different chromosomes (Huang et al., 2004; Zhang et al., 2005a; Zhou et al., 2007; Yang et al., 2010; Dang et al., 2014; Zhang et al., 2017; Singh et al., 2017; Yang et al., 2019; Chen et al., 2019; Yang et al., 2020). Root dry weight is regulated by the QTLs *qRDW1*, *qRDW5-1–1*, *qRDW5-2–2*, *qRDW10* and *qRDW3.1* reported on the chromosome 1, 3, 5 and 10 (Cui et al., 2002; Singh et al., 2017).

Shoot dry weight was reported to be controlled by 16 QTLs and located on chromosome 1, 3, 5, 6, 9 and 12 (Cui et al., 2002; Dang et al., 2014; Singh et al., 2017). However, a total of 77 QTLs were detected related to the total seedling dry weight and located on the chromosomes

Table 2
Statistical parameters for various physiological parameters estimated from the 48 mini core rice population related to seed vigour.

Parameters	PCV %	GCV %	h2%	Genetic advance	CD (5%)	CV (%)
RI	22.48	20.24	81.03	0.22	0.034	9.56
GI	78.11	76.11	96.93	2.84	0.891	11.52
SG(%)	69.77	68.27	95.75	50.64	15.6	4.42
SDW(g)	54.78	52.34	91.28	0.006	0.49	12.6
SVI-I	69.76	67.82	94.49	673.92	35.80	10.36
SVI-II	75.59	74.32	96.66	0.28	0.23	13.6
RRG(cm/day)	59.16	58.77	98.68	1.18	0.29	15.54
RSG(cm/day)	18.92	18.16	92.15	0.29	0.48	13.71
RGR(g/day)	53.70	52.05	93.92	0.14	0.049	13.42
AGR(cm/day)	31.54	31.12	97.37	1.13	0.36	11.48
MGT(day)	6.52	5.01	59.16	0.42	0.46	4.591

RI: Rate of imbibitions, GI: Germination index, SG:Seed germination, SDW: Seedling dry weight,
SVI-I: Seed vigour index I, SVI-II: Seed vigour index II, RRG: Rate of root growth, RSG: Rate of shoot growth, RGR: Relative growth rate, AGR: Absolute growth rate, MGT: Mean germination time.

1, 2, 3, 4, 5, 6, 8, 9, 10 and 12 (Cui et al., 2002; Zhang et al., 2005b, 2017; Zhou et al., 2007; Yang et al., 2010; Cheng et al., 2013; Singh et al., 2017; Chen et al., 2019). The germination rate was controlled by the QTLs present on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 11, (Cui et al., 2002; Zhang et al., 2005a, 2005b; Wang et al., 2010; Diwan et al., 2013; Liu et al., 2014; Sabouri et al., 2012; Yang et al., 2019). The germination index (GI) was found to be regulated by 13 QTLs on the chromosomes 1, 3, 7, 8, 10 and 11 at 3 different stages of harvesting (Wang et al., 2010; Liu et al., 2014; Yang et al., 2019). A set of 7 QTLs were associated with time of 50% germination (T50) and mapped on the chromosomes 1, 3 and 7 at 3 different stages of harvesting (Liu et al., 2014). A total of 41 QTLs were observed to influence germination percentage (Wang et al., 2010; Sabouri et al., 2012; Liu et al., 2014; Xie et al., 2014; Yang et al., 2019; Najeeb et al., 2020).

The reported QTLs were analyzed from bi-parental segregating populations with use of simple sequence repeat markers (SSR). However, few reports of QTLs mapped were based on association mapping (Dang et al., 2014; Zhao et al., 2019; Chen et al., 2019; Sahoo et al., 2020). The QTLs identified by using bi-parental segregating population usually show a low resolution (Flint-Garcia et al., 2005; Pradhan et al., 2016a). Association mapping in recent is adapted for QTL analysis to explore large number of elite alleles in plants due to higher resolution and detection of multiple alleles in the natural variation available in a population (Anandan et al., 2016; Pandit et al., 2017; Pradhan et al., 2019a; Pawar et al., 2021).

In this investigation, marker-trait association of 11 physiological traits related to seed vigour in rice was mainly focused using a small panel population (48 genotypic accessions selected from 120 diverse genotypic accessions). The population was studied for diversity, population genetic structure and marker trait association by using 50 SSR markers.

2. Material and methods

2.1. Plant materials

A set of 120 diverse genotypic accessions were collected from the Gene Bank of ICAR-National Rice Research Institute (NRRI), Cuttack. These genotypic accessions were raised in the experimental field of the

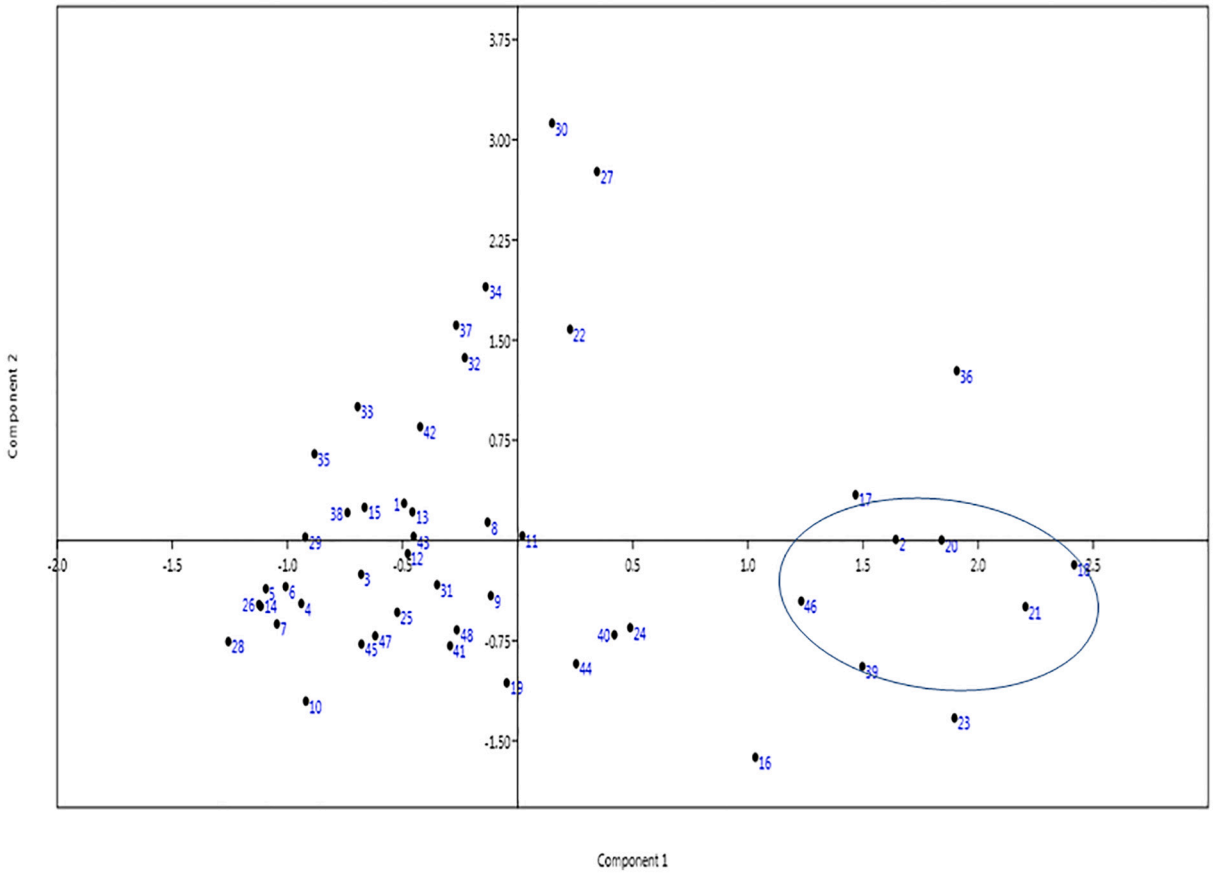

Fig. 1. Genotype-by-trait biplot diagram showing 48 germplasm lines in the two PCs for the eleven physiological traits.

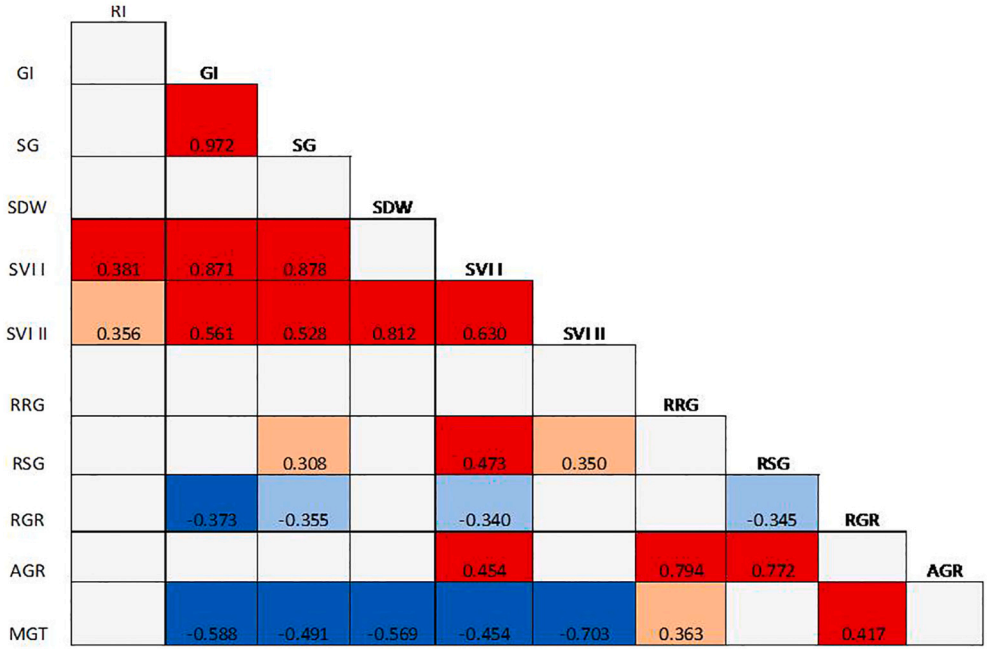

Fig. 2. Heat map showing Pearson's correlation coefficients for physiological traits in rice.

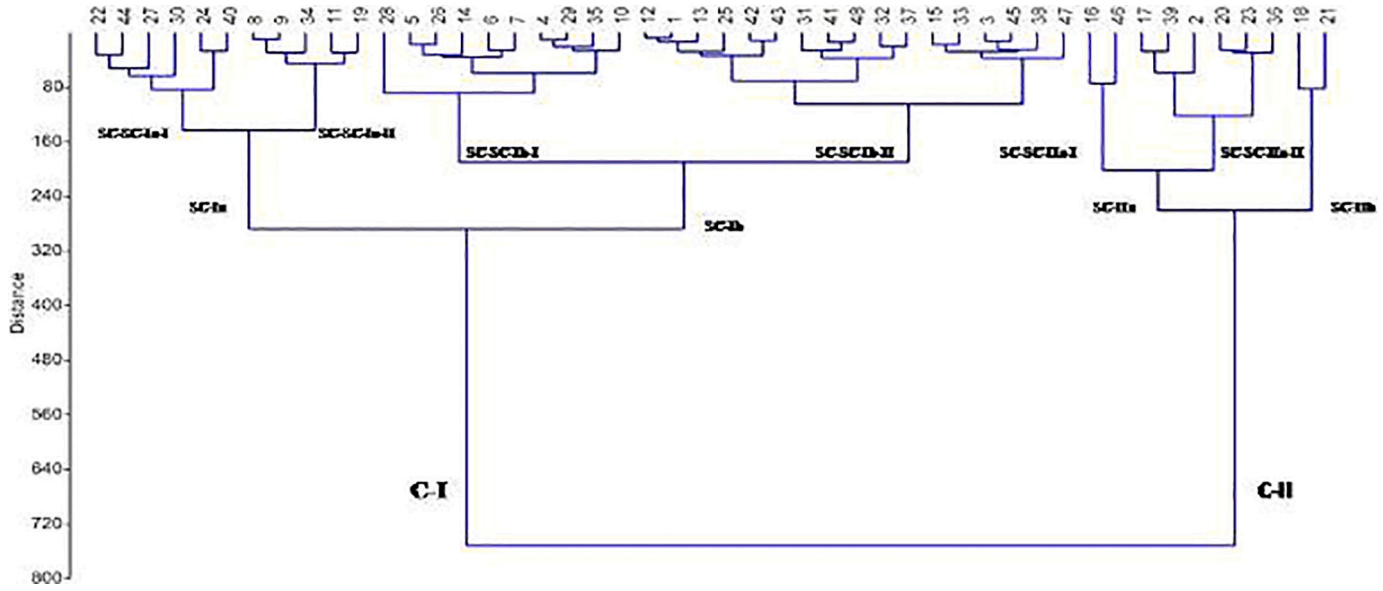

Fig. 3. Cluster Analysis of 48 genotypes based on physical and physiological traits. The name of each genotype is listed in the Table 1.

Institute (20.45 °N, 85.93 °E, 23.5 m) where average temperature of 31.7 °C, humidity of 67% and rainfall of 74 mm observed during the wet season, 2018–19. Soil type of the field was sandy clay loam and pH was 6.6. The plot size for each treatment was 3.15 m^2. These genotypic accessions were collected from five states of India viz., Assam, Madhya Pradesh, Kerala, Odisha and Manipur known for rich diversity of rice. Genotypic accessions from Jeypore tract of Odisha, the secondary centre of origin of rice were also included in the study. The fresh seeds harvested were used for the estimation of all the physiological traits after a storage period of 90 days. A panel population of 48 genotypes (highlighted in Supplementary Table 1) selected from 120 diverse genotypic accessions were used to analyse the genetic structure and it was investigated the association of molecular markers with the studied physiological traits.

2.2. Phenotyping for the physiological traits

Seed physiological traits such as rate of imbibition (RI), seed germination (SG), germination index (GI), seedling dry weight seed (SDW), vigour index I (SVI-I), seed vigour index II (SVI-II), rate of root growth (RRG), rate of shoot growth (RSG), absolute growth rate (AGR), relative growth rate (RGR) and mean germination time (MGT) were analyzed in order to study the marker-trait association. Fifty seeds were sown in three replications on the top of paper method and maintained at 30 °C for analyzing the physiological attributes. The RI of the genotypic accessions were determined as increase in volume of seed after soaking the seed in water for 24 h. The percentage of germinated seeds at 10th day was considered as the germination % (GP). GI was estimated following the procedure of Maguire, 1962. The increase in root and shoot length per day recorded at 7th day and 10th day of germination

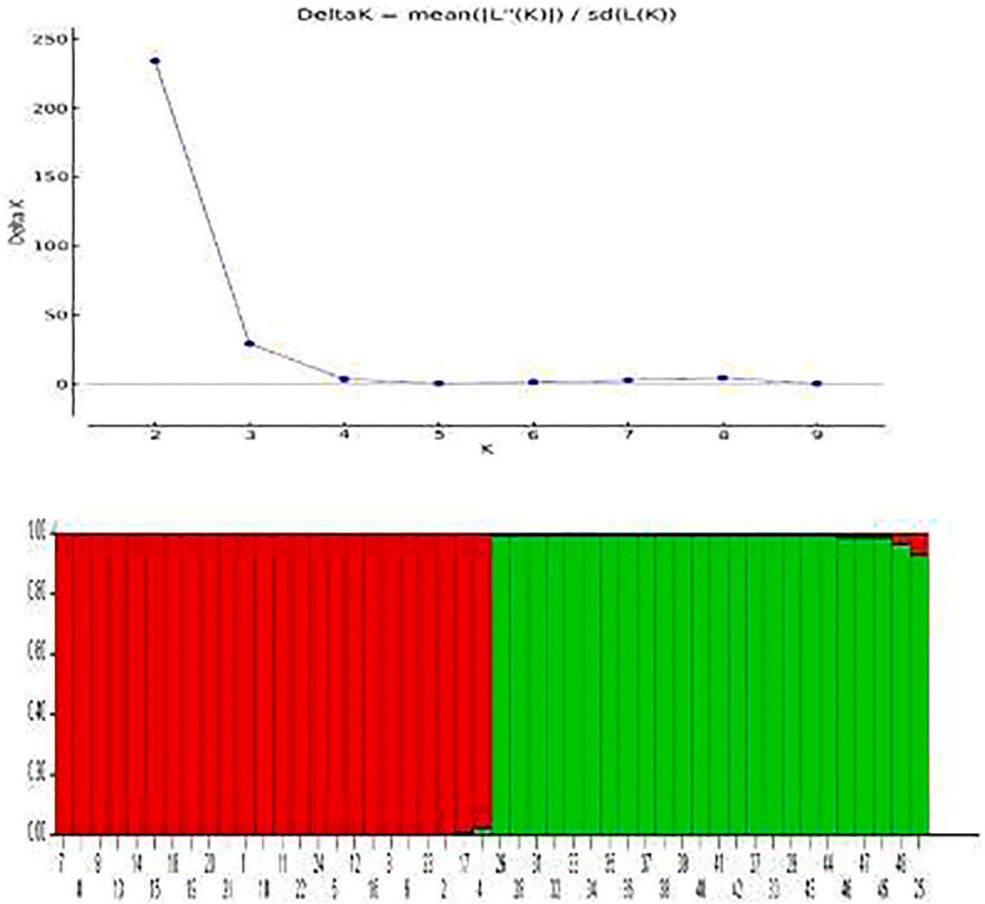

Fig. 4. (A) Graph of ΔK value for estimation peak value between successive K values for measuring the highest ΔK and (B) population structure obtained based on inferred ancestry value on the basis 50 molecular marker.

were considered as RRG and RSG and expressed as cm/day. AGR was calculated as per procedure of Redford (1967) and RGR was determined following the procedure of Fisher (1921). MGT of genotypes were computed by adopting the procedure of Zuo et al., 2018. The seedlings used for recording rate of growth were subjected to drying in hot air oven at 70 °C for 48 h after discarding the cotyledon. The dry weight of seedling was recorded in gram seedling^{-1}. Procedure of Abdul-Baki and Anderson (1973) was used to estimate seed vigour indices (SVI- I; SVI-II). For estimating all the physiological traits except RI and SG, observations were recorded from five seedlings of each replication (total three replications) and averaged to get the value of each replication.

Analysis of variance (ANOVA) for each trait was performed by Cropstat software version 7.0. Phenotypic (PCV), genotypic (GCV) and environmental coefficient of variation (ECV) were calculated following formulae of Singh and Choudhury (1985). Procedure of Allard (1960) was used to calculate broad sense heritability (BSH) and genetic advance (GA) of all the traits. Correlation heatmap matrix based on pearson correlation coefficient represents association of all the physiological traits on the basis of mean values of the germplasm lines.

2.3. Isolation of genomic DNA, PCR and SSR markers selection

Genomic DNA was extracted from 25 days old seedlings by adopting CTAB method (Murray and Thompson 1980). DNA fragments were resolved in gel electrophoresis for quantification of the isolated DNA. Fifty SSR markers were selected from the public database for the study (Supplementary Table 2). Selection of these markers was based on the positions across the 12 chromosomes for estimation of diversity, to identify the polymorphic loci and market-trait associations using the panel accommodating the 48 rice genotypic accessions (Table 1). PCR reaction was set for running of initial denaturation step (2 min, 95 °C), denaturation for 35 cycles (30 s, 95 °C) and annealing/extension (30 min, 55 °C), extension (2 min, 72 °C), final extension (5 min, 72 °C) followed by storage at 4 °C (infinity). Electrophoresis of PCR products was done using agarose gel (2.5%) containing 0.80 g ml^{-1} ethidium bromide and was run at 2.5V cm^{-1} for 4 h. DNA ladder of 50 bp was run to determine the size of amplicons and a Gel Documentation System (SynGene) was used to photograph the amplified products. The PCR reactions, electrophoresis and gel documentation were performed following previous publications (Pradhan et al., 2015; Barik et al., 2016; Pradhan et al., 2019b; Mohapatra et al., 2021).

2.4. Molecular data analysis

The scoring of the total amplicons was based on the presence or absence of the amplified products for each genotype-primer combination. Power marker version 3.25 was used to estimate the diversity parameters (Liu and Muse 2005). Jaccard's coefficients were used to generate similarity matrix from binary data and data analysis was performed. The cladogram was drawn by using unweighted pair group method arithmatic average (UPGMA) algorithm employing the Free Tree software (Page, 1996; Hampl et al., 2001; Pavalicek et al., 1999). Population structure and cluster analysis were performed using STRUCTURE 2.3.6 and Darwin 5 softwares following the procedure described in the earlier publications (Pandit et al., 2020; Pradhan et al., 2020). The software was run at K value of 1 to 10, allowing 10 runs per each K value for estimation of the optimal number of groups (K). ΔK, an adhoc statistic was used to estimate the actual value of K (Evanno et al.,

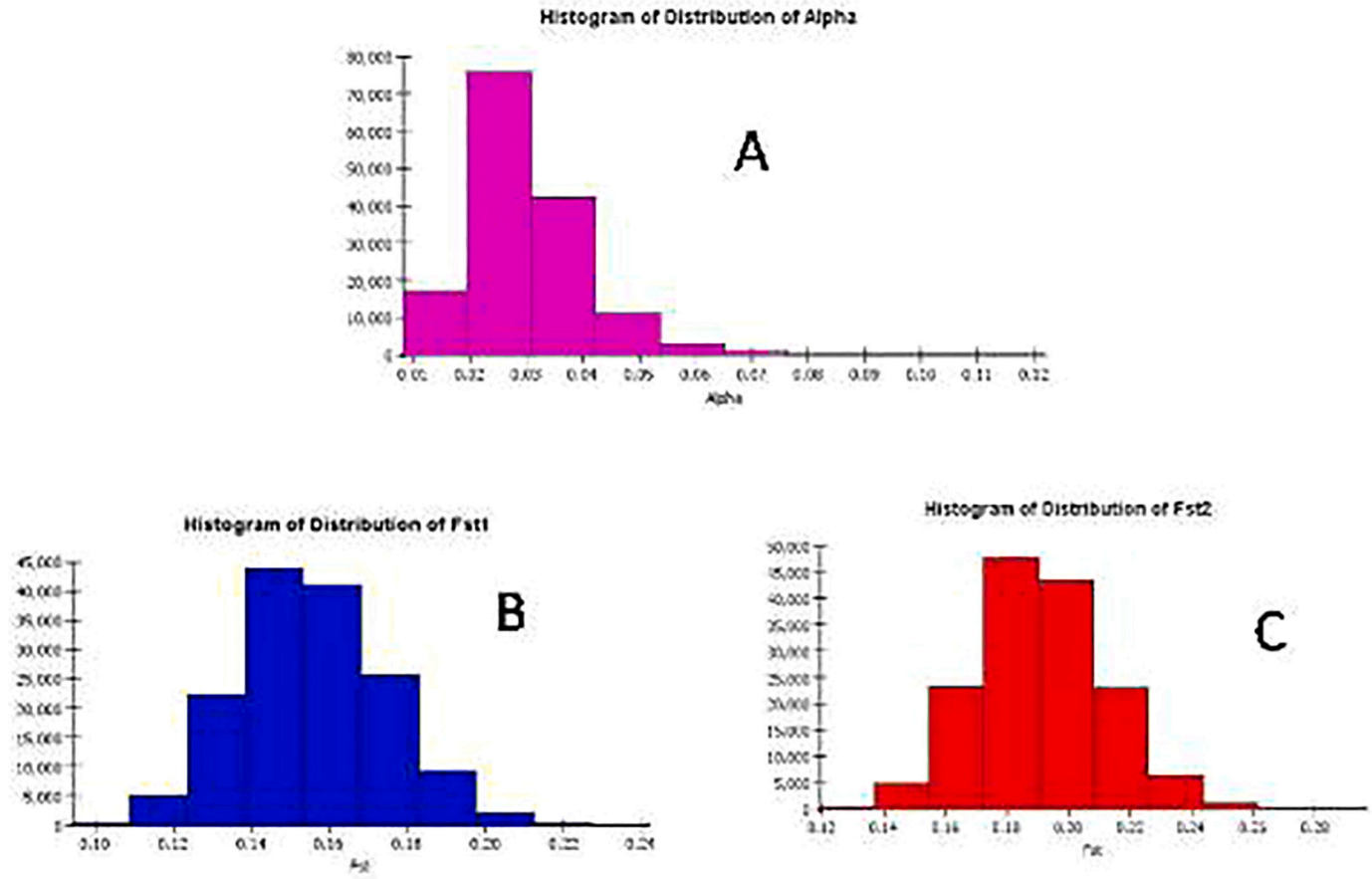

Fig. 5. Distribution of (A) alpha value (B) Fst1 values in the sub-population1 (C) Fst2 values in the subpopulation 2 at K = 2 for the population 48 genotypes relating to seed vigour traits in rice.

2005). Parameters were set for the burn-in periods and was set at 150000 Markov Chain Monte Carlo (MCMC).

2.5. Marker-trait association analysis

TASSEL5 software was used for marker-trait association analysis. Two models namely General linear model (GLM) and Mixed linear model (MLM) approach were followed for the genetic association between phenotypic traits and the used SSR makers (Bradbury et al., 2007). Markers' R^2 and *p*-values were considered for identifying significantly association of markers with the traits.

3. Results

3.1. Phenotyping for the physiological traits

Eleven physiological traits were recorded from a population of 120 genotypic accessions (Supplemental Table 1). A small panel was prepared by selecting genotypes from all the phenotypic groups of the studied 11 traits. There was 48 out of 120 shortlisted genotypic accessions accommodated from this study (Table 1). Analysis of variation and mean values estimated for the 11 physiological traits showed clear difference for the studied traits among the genotypes present in the panel population (Table 1; Table 2). High values of GCV (20.24–76.11%) and PCV (22.48–78.11%) were observed for the traits GI, SG, SDW, SVI-I, SVI-II, RRG and RGR. The physiological parameters namely AGR (PCV: 31.54%; GCV: 31.12%) and RI showed moderate (PCV: 22.48%; GCV: 20.24%) while MGT and RSG had low estimates of PCV (6.52%; 18.92% respectively) and GCV (5.01%; 18.16%). All the traits had high heritability (81.03–98.68%) except MGT (59.16%). Low genetic advance (0.14–2.84) was observed for all the physiological traits except SVI-I (673.92) and SG (50.64).

3.2. Genotype-trait biplot analysis

To know the relationships between genotypes and physiological parameters, and among the parameters, the genotype-trait biplot diagram was constructed. The estimated mean physiological parameters for the 11 traits were taken as the variables. The biplot placed the genotypes into the 4 quadrants. Majority of the genotypes are placed in the 3rd quadrant (bottom left). Most of the genotypes present in the 3rd quadrant showed poor to moderate in the physiological parameters (Fig. 1). The 2nd quadrant accommodated genotypes of which majority were good for the physiological parameters. The desirable genotypes are encircled in the in the diagram. The landraces present inside the circle are useful as donor parents for the improvement physiological parameters in rice.

3.3. Nature of association among the physiological traits

The correlation coefficients estimated for the 11 physiological traits showed strong positive correlation ($r \geq 0.7$) of SVI- I with GI and SG. SVI- II was strongly correlated with SDW (Fig. 2). In addition, strong positive association of SG with GI (0.972); AGR with RRG (0.794) and RSG (0.772) were also observed. MGT showed strong negative association with SVI-II (−0.703). Moderate positive correlation value (r: 0.5–0.7) of SVI II with GI (0.561), SG (0.528), SVI I (0.630) and negative correlation of MGT with GI (−0.588) and SDW (−0.569) were estimated using the panel population. A weak positive correlation ($r < 0.5$) for SVI I and SVI II with RI (0.381; 0.356 respectively) and RSG (0.473; 0.350

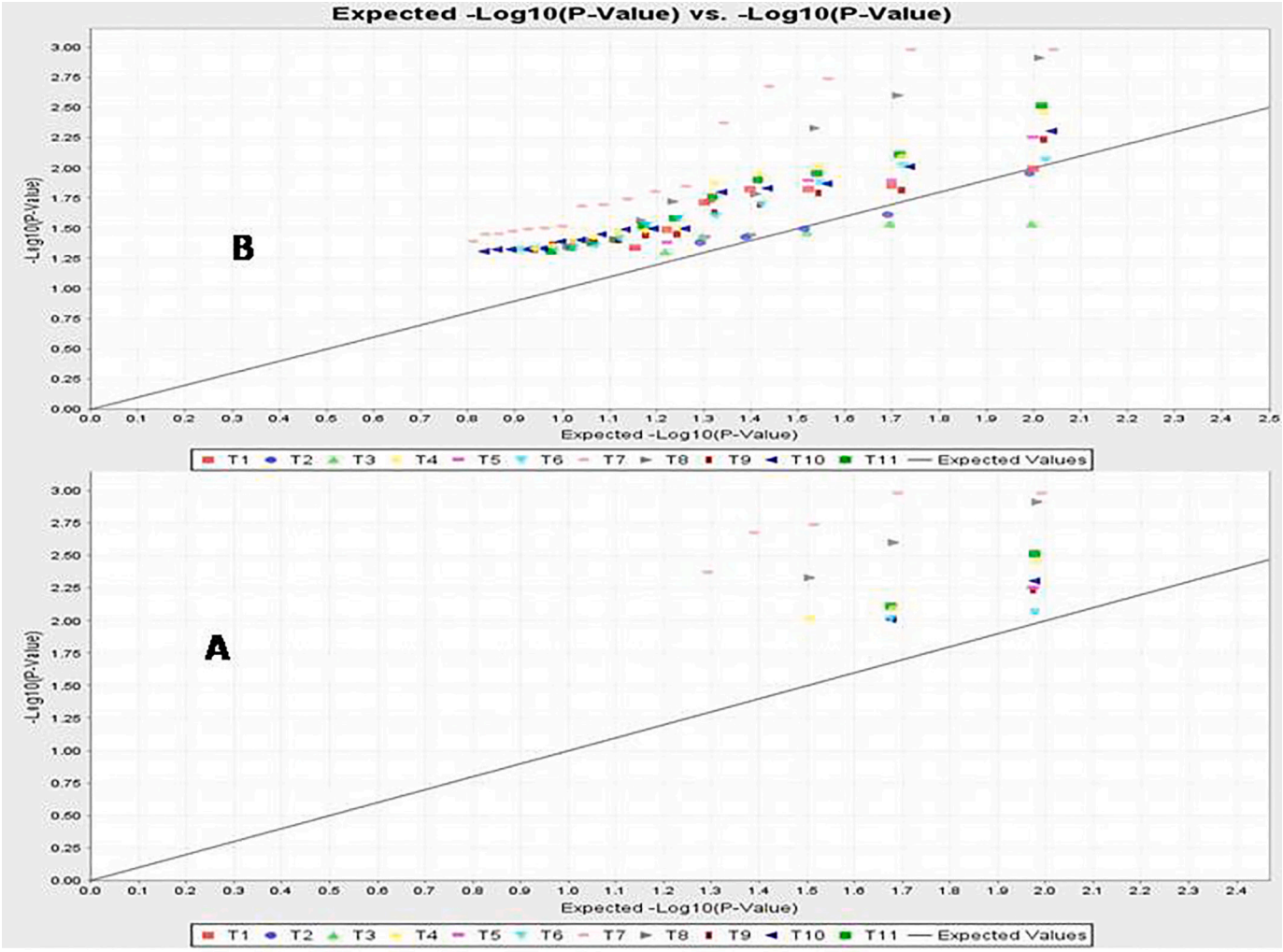

Fig. 6. Quantile–Quantile (Q-Q) plot and distribution of marker-trait association from MLM analysis for physical-physiological traits related to seed vigour. A: at $p < 0.05$, B: at $p < 0.01$.

Table 3
Nature of association of 11 physiological traits estimated from 48 rice genotypes.

	RI	GI	SG	SDW	SVI I	SVI II	RRG	RSG	RGR	AGR
GI	0.146									
SG	0.132	0.972**								
SDW	0.274	0.074	0.049							
SVI I	0.381**	0.871**	0.878**	0.151						
SVI II	0.356*	0.561**	0.528**	0.812**	0.630**					
RRG	0.190	0.051	0.131	−0.247	0.244	−0.162				
RSG	0.230	0.277	0.308*	0.129	0.473**	0.350*	0.226			
RGR	−0.128	−0.373**	−0.355*	0.027	−0.340*	−0.178	0.047	−0.345*		
AGR	0.268	0.207	0.278	−0.081	0.454**	0.113	0.794**	0.772**	−0.185	
MGT	−0.141	−0.588**	−0.491**	−0.569**	−0.454**	−0.703**	0.363*	−0.065	0.417**	0.196

RI:Rate of imbibitions, GI:Germinationindex,SG:Seed germination, SDW:Seedling dry weight,SVI-I:Seed vigour index I, SVI-II:Seed vigour index II, RRG:Rate of root growth, RSG:Rate of shoot growth, RGR:Relative growth rate, AGR:Absolute growth rate,MGT:Mean germination time. * $P = 0.05$ level **$P = 0.01$level.

respectively); RSG with SG; AGR with SVI I; RRG and RGE with MGT. Weak negative correlation of GI, SG, SVI I, RSG with RGR (−0.373;-0.355;-0.340;0.345 respectively); SG and SVI I with MGT(−0.491; −0.454 respectively) were also observed (Fig. 2).

3.4. Molecular diversity parameters estimation from the panel population

The mini core germplasm consisting of 48 genotypic accessions was used for genetic diversity study by employing 50 simple sequence repeat markers is presented in Supplementary Table 2. A total of 310 marker alleles were estimated from the panel population. The total number of alleles per marker varied from 2 (RM14978) to 12 (RM 547) and the mean value was 6.08. High major specific allele frequency was shown by the genotype, AC7282 as detected by marker RM6547. The mean frequency for specific allele in the population was 0.436. The gene diversity value in the population varied from 0.2494 assessed by marker RM582

Table 4
Genetic diversity parameters associated with the germplasm lines used for marker-trait association study.

SL No.	Marker	Major. Allele. Frquency	Genotype No	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Heterozygosity	PIC	F
1	RM315	0.667	3	48	48	3	1.000	0.478	0.000	0.409	1.000
2	RM486	0.427	7	48	48	5	1.000	0.709	0.146	0.662	0.798
3	RM225	0.383	9	48	47	5	0.979	0.727	0.213	0.680	0.713
4	RM256	0.543	7	48	47	5	0.979	0.564	0.043	0.480	0.926
5	RM1113	0.337	6	48	46	5	0.958	0.730	0.065	0.683	0.913
6	RM3423	0.421	5	48	44	5	0.917	0.699	0.023	0.646	0.968
7	RM6100	0.233	9	48	30	7	0.625	0.823	0.067	0.799	0.922
8	RM14960	0.695	6	48	41	5	0.854	0.483	0.024	0.449	0.951
9	RM590	0.719	6	48	32	4	0.667	0.455	0.125	0.425	0.733
10	RM5793	0.283	7	48	46	7	0.958	0.803	0.000	0.776	1.000
11	RM405	0.333	8	48	27	7	0.563	0.785	0.074	0.754	0.909
12	RM547	0.217	15	48	46	12	0.958	0.872	0.196	0.859	0.780
13	RM 7364	0.434	9	48	38	8	0.792	0.708	0.079	0.664	0.891
14	RM205	0.412	6	48	34	6	0.708	0.718	0.000	0.675	1.000
15	RM167	0.239	10	48	46	9	0.958	0.842	0.087	0.823	0.899
16	RM229	0.478	9	48	23	7	0.479	0.707	0.174	0.675	0.763
17	RM558	0.286	6	48	35	6	0.729	0.789	0.000	0.757	1.000
18	RM20A	0.439	8	48	33	7	0.688	0.751	0.030	0.727	0.961
19	RM235	0.214	12	48	42	7	0.875	0.833	0.119	0.811	0.860
20	RM7003	0.531	5	48	32	4	0.667	0.582	0.031	0.503	0.948
21	RM5436	0.514	6	48	37	5	0.771	0.633	0.108	0.575	0.833
22	RM25181	0.261	15	48	46	10	0.958	0.838	0.217	0.818	0.746
23	RM469	0.611	5	48	36	3	0.750	0.548	0.083	0.486	0.852
24	RM6547	0.723	4	48	47	4	0.979	0.432	0.021	0.384	0.952
25	RM406	0.585	4	48	41	3	0.854	0.552	0.024	0.478	0.957
26	RM152	0.250	7	48	20	7	0.417	0.810	0.000	0.784	1.000
27	RM148	0.300	8	48	40	6	0.833	0.773	0.075	0.736	0.905
28	RM421	0.517	3	48	29	3	0.604	0.595	0.000	0.516	1.000
29	RM2634	0.467	4	48	30	4	0.625	0.667	0.000	0.610	1.000
30	RM248	0.600	3	48	20	3	0.417	0.560	0.000	0.499	1.000
31	RM13335	0.575	6	48	47	6	0.979	0.605	0.000	0.561	1.000
32	RM1347	0.462	4	48	26	4	0.542	0.585	0.039	0.497	0.937
33	RM14978	0.546	2	48	11	2	0.229	0.496	0.000	0.373	1.000
34	RM18776	0.304	7	48	46	6	0.958	0.739	0.022	0.692	0.971
35	RM50	0.281	8	48	32	7	0.667	0.817	0.031	0.792	0.963
36	RM3735	0.438	8	48	48	6	1.000	0.674	0.167	0.618	0.757
37	RM582	0.862	6	48	47	6	0.979	0.249	0.064	0.238	0.749
38	RM328	0.394	7	48	33	7	0.688	0.735	0.000	0.694	1.000
39	RM337	0.178	12	48	45	9	0.938	0.861	0.133	0.845	0.848
40	RM340	0.565	9	48	46	8	0.958	0.637	0.109	0.608	0.833
41	RM470	0.286	11	48	42	10	0.875	0.810	0.048	0.785	0.943
42	RM506	0.703	5	48	37	4	0.771	0.464	0.162	0.422	0.659
43	RM1812	0.433	6	48	30	6	0.625	0.687	0.000	0.636	1.000
44	RM286	0.189	14	48	37	10	0.771	0.876	0.108	0.863	0.880
45	RM258	0.447	3	48	47	3	0.979	0.589	0.000	0.501	1.000
46	RM201	0.386	5	48	44	5	0.917	0.719	0.000	0.670	1.000
47	RM216	0.441	9	48	42	9	0.875	0.717	0.095	0.678	0.870
48	RM440	0.330	14	48	47	11	0.979	0.832	0.192	0.816	0.774
49	RM7571	0.464	5	48	42	4	0.875	0.678	0.071	0.625	0.897
50	RM223	0.413	10	48	46	9	0.958	0.753	0.044	0.723	0.943
	Mean	0.436	7	48	39	6	0.803	0.680	0.066	0.636	0.905

while high value of 0.8755 detected by marker RM286. The average gene diversity value in the population was found to be 0.6796.The allele heterozygosity varied from 0.00 (RM315, RM5793, RM205, RM558, RM152, RM421, RM2634, RM248, RM3335, RM4978, RM328, RM1812, RM258, RM201) to 0.2174 (RM25181) with a mean of 0.0662 in the representative population. The polymorphic information content showed mean value of 0.6796 which varied from 0.2375 (RM582) to 0.8628 (RM286) in the panel population.

3.5. Cluster analysis

The euclidean distance based clustering classified the 48 genotypes broadly into two groups (Fig. 3). Thirty-eight genotypic accessions were found in the cluster I while remaining 10 were found in the cluster II. The classification showed correspondence with SVI for inclusion of genotypes in the groups. The genotypes present in the cluster II were having high seed vigour index I while genotypic accessions in cluster I had very low or low and low-medium seed vigour index I. The cluster I is further divided into two subgroups *i.e*, sub cluster-I (a) which accommodated 11 genotypes and sub cluster- I (b) placed 27 genotypes. Two sub-sub-clusters were seen from both the sub-cluster I (a) and I (b). SVI- I discriminated the genotypes which accommodated 6 in sub-sub cluster I (a)-I showing medium value while and 5 were present in the sub-sub cluster I(a)-II having low to medium value for the index. The sub-cluster I (b) is again sub divided into two groups *i.e,* sub-sub cluster I(b)-I containing 10 genotypes with low vigour index I and sub-sub cluster I-(b)-II accommodates 17 genotypes.

The cluster II broadly categorized into two sub clusters. Eight

Table 5
The inferred ancestry values and structure sub-population groups in the 48 germplasm lines.

Sl No.	Genotype(AC No./ vernacular name)	Q1	Q2	Sub population	Remark
1	AC-10608	0.998	0.002	SP1	M
2	AC-10187	0.993	0.007	SP1	H
3	AC-10162	0.996	0.004	SP1	M
4	AC-7282	0.974	0.026	SP1	M
5	AC-7269	0.997	0.003	SP1	L
6	AC-7134	0.996	0.004	SP1	L
7	AC-7008	0.999	0.001	SP1	L
8	AC9093	0.999	0.001	SP1	M
9	AC9090	0.999	0.001	SP1	M
10	AC9076A	0.998	0.002	SP1	M
11	AC9065	0.998	0.002	SP1	M
12	AC9063	0.997	0.003	SP1	M
13	AC9058	0.999	0.001	SP1	M
14	AC9053A	0.999	0.001	SP1	L
15	AC9050	0.999	0.001	SP1	M
16	AC9005	0.997	0.003	SP1	H
17	AC20389	0.989	0.011	SP1	H
18	AC20371	0.999	0.001	SP1	H
19	AC20423	0.999	0.001	SP1	M
20	AC20362	0.999	0.001	SP1	H
21	AC20328	0.999	0.001	SP1	H
22	AC20317	0.998	0.002	SP1	M
23	AC20282	0.995	0.005	SP1	H
24	AC20246	0.998	0.002	SP1	M
25	AC20347	0.069	0.931	SP2	M
26	Palina dhan-1	0.001	0.999	SP2	L
27	Chatuimuchi	0.002	0.998	SP2	M
28	Uttarbanga local 9	0.001	0.999	SP2	L
29	Gochi	0.004	0.996	SP2	L
30	Sugandha-2	0.002	0.998	SP2	M
31	Jhingesal	0.001	0.999	SP2	M
32	PK-19Cheruvirippu	0.001	0.999	SP2	M
33	PK-28Mahamaga	0.001	0.999	SP2	M
34	PK- 25 Jaya	0.001	0.999	SP2	M
35	PK-33 D1	0.001	0.999	SP2	L
36	Pk-21	0.001	0.999	SP2	H
37	PK-22	0.001	0.999	SP2	M
38	PK- 23	0.001	0.999	SP2	M
39	Gondiachampeisiali	0.001	0.999	SP2	H
40	Chinamal	0.001	0.999	SP2	M
41	Magra	0.001	0.999	SP2	M
42	Landi	0.001	0.999	SP2	M
43	Lal gundi	0.004	0.996	SP2	M
44	Balisaralaktimachi-K	0.006	0.994	SP2	M
45	Laxmibilash	0.011	0.989	SP2	L
46	Kaniar	0.008	0.992	SP2	H
47	Kanakchampa	0.008	0.992	SP2	L
48	Magura-s	0.035	0.965	SP2	M

H:High seed vigour index M: Medium seed vigour index L:Low seed vigour index

genotypes were observed under the sub cluster 2a and 2 lines in the sub cluster 2b. Again, 2a was grouped into two sub-sub group containing two genotypes in cluster II (a)-I and six genotypes in sub-sub cluster II (a)-II (Fig. 3).

3.6. Genetic structure analysis

The grouping of population based on genetic structure helps in assessing the gene pool present in the population. The gene pool provides initial idea about the genetic makeup of the parental lines present provide idea about the desired traits in a population for improvement programs. The STRUCTURE software categorized the representative population into two different sub-populations based on the genotyping results by 50 SSR markers at a peak ΔK value (Fig. 4). The genotypes with ≥80% ancestry value were categorized for that sub-population (Table 5). The sub-population 1 showed proportions of 0.502 while sub-population 2 had 0.498 genotypes in the inferred clusters. Sub-population 1 showed fixation index (F_{st}) values of 0.1552 while sub-population 2 had 0.1908. The allele-frequency divergences based on the net nucleotide distance were 0.1454 and 0.1454 for sub-population 1 and 2, respectively. The expected heterozygosity (average distance) in sub-population 1 was 0.6275 while it showed 0.6104 in the sub-population 2.

The seed vigour traits in the genotypes of a structure group showed relatively fair relationship among themselves in the sub-populations present in the panel population. Majority of the moderate to high seed vigour containing genotypic accessions were seen in the sub-population 1. While lower to moderate vigour containing genotypes were in the sub-population 2. Structure analysis showed low alpha value (alpha = 0.0297) for the studied population using the peak at K = 2. The alpha-value detected from the panel population exhibited a leptokurtic distribution while a symmetrical distribution was observed for both the F_{st} values and a clear variation in distribution was noticed for the F_{st} values (Fig. 5A-C).

3.7. Marker-trait association of physiological traits influencing seed vigour

Association analysis for marker-trait was performed to know the association of molecular markers with seed vigour related traits analyzed by the GLM (Generalized Linear Model) and MLM/ K + Q (Mixed Linear Model) employing the software. The parameters detected from the marker-trait study were trimmed at <5% and < 1% error. Analysis by GLM approach showed association of 165 markers with the entire studied traits except absolute growth rate (Supplementary Table 3). However, analyzing with MLM model, all the traits except rate of shoot growth were found associated with the 105 markers at above threshold level (Supplementary Table 4). Marker-trait associations showing high R^2 value (>0.1) and low *p*-value (<0.01) were considered for further trimming of parameters to identify effective associations.

Marker-trait association by GLM revealed three SSR markers viz., RM486, RM20A, and RM152 associated with rate of imbibition while by MLM no marker was detected. Germination index showed association with RM225, RM235, RM6547, RM216 and RM 440 by GLM while no markers associated by MLM at this stringent trimming but RM440 showed association at >0.05 R^2 and < 0.01 p-value. Seed germination percentage showed significant association with three SSR markers of which RM440 had highest phenotypic variability (21%) by GLM model while no markers were found by the MLM model. For seedling dry weight, two markers RM440 (chromosome 5) and RM223 (chromosome 8) were found to associated by both GLM and MLM model (Table 6; Table 7). Seed vigour index-I showed significant association with RM167 by both the models and present on chromosome 11 exhibiting marker R^2 value of 0.177. Marker RM7364 and RM235 associated with seed vigour index- II by MLM and GLM analysis. The trait, rate of root growth (cm/day) showed association with RM256, RM6547, RM25181, RM328 and RM201 markers analyzed by both the models (Table 6; Table 7). Rate of shoot growth (cm/day) trait showed significant association with marker, RM20A, RM13335, RM216 detected by both the models analyses. RM405 showed strong association with growth parameter, RGR analyzed by both the models. Two markers, RM20A and RM201 showed significant association values for the growth parameter, absolute growth rate analyzed by the both models. SSR markers RM235 and RM286 showed significant association by both the models for the trait, mean germination time (Table 6; Table 7). The Q-Q plot also confirmed the detected marker-association for various seed vigour influencing traits (Fig. 6).

4. Discussion

Improvement of seed vigour in rice is considered as a very important breeding objective by the rice breeders. The genotypic accessions used for the diversity and association study will be effective as the accessions were different from each other for the studied 11 physiological traits

Table 6
Marker-trait associations for seed vigour traits using GLM analysis at high R^2 (>0.1) and low p-value (<0.01) in rice.

Sl. No.	Trait	primer	marker_F	marker_p	marker_R^2	Sl. No.	Trait	primer	marker_F	marker_p	marker_R^2
1.	Rate of imbibitions (ml/h)	RM486	8.12423	0.00681	0.1247	24	Rate of shoot growth (cm/day)	RM20A	13.31075	7.38E-04	0.23118
2.	Rate of imbibitions (ml/h)	RM20A	8.92706	0.00473	0.13482	25	Rate of shoot growth (cm/day)	RM13335	17.72381	1.36E-04	0.2847
3.	Rate of imbibitions (ml/h)	RM152	8.12423	0.00681	0.1247	26	Rate of root growth(cm/day)	RM3735	10.43775	0.00244	0.17692
4.	Germination index(GI)	RM225	7.52406	0.00899	0.10863	27	Rate of root growth(cm/day)	RM328	24.77692	1.21E-05	0.32843
5.	Germination index(GI)	RM235	11.28625	0.0017	0.15122	28	Rate of root growth(cm/day)	RM337	12.60205	9.83E-04	0.20499
6.	Germination index(GI)	RM6547	8.36974	0.00608	0.11876	29	Rate of root growth(cm/day)	RM201	15.67231	2.93E-04	0.24112
7.	Germination index(GI)	RM216	7.34508	0.00977	0.10643	30	Rate of shoot growth (cm/day)	RM582	8.02079	0.00714	0.15434
8.	Germination index(GI)	RM440	21.92069	3.10E-05	0.24406	31	Rate of shoot growth (cm/day)	RM216	10.90228	0.002	0.19814
9.	Final germination (%)	RM225	10.38031	0.0025	0.15702	32	Relative growth rate(g/day)	RM1113	7.64556	0.00849	0.13362
10.	Final germination (%)	RM235	7.59911	0.00868	0.12153	33	Relative growth rate(g/day)	RM405	11.03104	0.00189	0.18025
11.	Final germination (%)	RM440	15.59556	3.02E-04	0.21417	34	Relative growth rate(g/day)	RM558	7.69253	0.00831	0.13431
12.	Seedling dry weight(g)	RM440	13.4085	7.10E-04	0.10083	35	Absolue growth rate (cm/day)	RM256	10.10791	0.00281	0.18543
13.	Seedling dry weight (g)	RM223	15.74376	2.85E-04	0.11352	36	Absolue growth rate (cm/day)	RM20A	8.25873	0.0064	0.15719
14.	Seed Vigour index I	RM225	7.42764	0.0094	0.10369	37	Absolue growth rate (cm/day)	RM13335	9.94811	0.00301	0.18307
15.	Seed Vigour index I	RM167	15.00818	3.78E-04	0.18116	38	Absolue growth rate (cm/day)	RM3735	9.64842	0.00343	0.17861
16.	Seed Vigour index I	RM25181	7.65878	0.00844	0.10641	39	Absolue growth rate (cm/day)	RM201	12.19876	0.00116	0.21499
17.	Seed Vigour index I	RM328	7.65878	0.00844	0.10641	40	Mean germination time (day)	RM3423	9.9051	0.00307	0.11067
18.	seed Vigour index II	RM 7364	8.74614	0.00513	0.103	41	Mean germination time (day)	RM235	18.20261	1.14E-04	0.17487
19.	seed Vigour index II	RM235	9.09605	0.00438	0.10637	42	Mean germination time (day)	RM5436	11.38118	0.00163	0.12358
20.	Rate of root growth (cm/day)	RM256	13.30653	7.39E-04	0.21364	43	Mean germination time (day)	RM328	9.82893	0.00317	0.10998
21.	Rate of root growth (cm/day)	RM167	7.95482	0.00736	0.14168	44	Mean germination time (day)	RM286	17.63086	1.41E-04	0.17103
22	Rate of root growth (cm/day)	RM25181	24.77692	1.21E-05	0.32843	45	Mean germination time (day)	RM440	9.24747	0.0041	0.10467
23	Rate of root growth (cm/day)	RM6547	11.35181	0.00165	0.18906						

(Table 1). High PCV (22.48–78.11%) and GCV (20.24–76.11%) were estimated for majority of the studied traits viz., GI, SG, SDW, SVI- I, SVI-II, RRG, RPG, RGR and therefore the landraces present in the panel are very useful for selection of the trait. In addition, a high heritability values were found for all the traits under study except MGT. Traits namely MGT and RSG showed poor statistical parameters and hence selection is difficult. Similar high PCV, GCV combined with high heritability were reported for seedling vigour trait such as shoot length, root length, coleoptile length and mesocotyl length by Akshaya et al., (2020); Akshitha et al., (2020). Availability of high genetic variations and heritability for the target traits were useful for improvement of the traits in rice reported in many previous studies (Singh et al., 2012; Pradhan et al., 2019a; Mohapatra et al., 2021). Landraces with superior value for multiple physiological traits such as AC. 20,371 (SG, SVI-I and SVI-II) and PK 21 (SG, RSG and AGR) are useful donor parents in the breeding for improvement of seed vigour in rice.

Genetics of seed vigour is very complex and hence its association with 11 physiological traits related to vigour were taken up to understand its association with SSR markers for improvement of the trait. The SSR markers are highly variable, codominant, chromosome specific and multi allelic are extensively used for genetic diversity and genetic structure assessment in rice and also in other crops (Pandit et al., 2016; Pradhan et al., 2016a; Barik et al., 2019; Barik et al., 2020). Knowledge on the association among the traits will help in selecting the desired correlated trait. The positive and strongly associated desired trait with another targeted trait will help in simultaneous improvement of both the traits. Higher magnitude of correlation coefficients were observed for seed vigour with RI, GI, SG and SDW traits will also be helpful in deciding the traits associated with the seed vigour improvement (Table 3). Such association among physiological traits for seed vigour were also observed by Zhang et al., 2005a; Lu et al., 2007; Zhang et al., 2017; Chen et al., 2019. The molecular diversity analysis based on the genotyping of 50 SSR markers showed clear differentiation within the studied population (Table 4). A high number of alleles (310) were detected from the panel population showing mean value of 6.08 alleles per locus. Detection of 0.67 average PIC value indicated a rich population with respect to allele present in the population along with highest PIC value obtained by RM 286. A moderate to high polymorphic information content value from the population revealed about the better informative markers for genotype selection. Information on the availability of high, medium and low genetic diversity for various traits in rice have already been published by previous studies (Bose, 2005; Jin et al., 2010; Zhao et al., 2013; Pradhan et al., 2016b; Mohapatra et al., 2018; Pawar et al., 2017; Das et al., 2018; Pandit et al., 2018; Pradhan

Table 7
Marker-trait associations for seed vigour traits in rice using MLM analysis at high R^2 (>0.1) and low p-value (<0.01) in rice.

Sl. No.	Trait	primer	marker_F	marker_p	marker_R^2	Sl. No.	Trait	primer	marker_F	marker_p	marker_R^2
1	Seedling dry weight(g)	RM486	7.76763	0.00802	0.13749	9	Seedling dry weight(g)	RM486	7.76763	0.00802	0.13749
2	Seedling dry weight(g)	RM440	7.36845	0.00967	0.13043	14	Rate of shoot growth (cm/day)	RM20A	10.37058	0.00251	0.23295
3	Seedling dry weight(g)	RM223	9.6359	0.00345	0.17056	15	Rate of shoot growth (cm/day)	RM13335	12.0683	0.00122	0.27109
4	Seed Vigour index I	RM167	8.55637	0.00559	0.17959	16	Rate of shoot growth (cm/day)	RM216	8.95893	0.00466	0.20124
2	seed Vigour index II	RM 7364	7.38425	0.0096	0.103	17	Relative growth rate(g/day)	RM405	8.45835	0.00584	0.16994
6	seed Vigour index II	RM235	7.62604	0.00857	0.10637	18	Absolute growth rate (cm/day)	RM20A	7.34117	0.00979	0.15259
7	Rate of root growth (cm/day)	RM256	9.18057	0.00422	0.20851	19	Absolute growth rate (cm/day)	RM201	8.8193	0.00496	0.18332
8	Rate of root growth (cm/day)	RM6547	10.79568	0.00209	0.2452	20	Mean germination time (day)	RM235	9.91285	0.00306	0.19631
11	Rate of root growth (cm/day)	RM25181	12.4554	0.00104	0.28289	21	Mean germination time (day)	RM286	7.85395	0.00771	0.15554
12	Rate of root growth (cm/day)	RM328	12.4554	0.00104	0.28289						
13	Rate of root growth (cm/day)	RM201	11.11715	0.00182	0.2525						

et al., 2019c).

Two genetic structure groups were obtained for the seed vigour related traits in the studied population by the distribution of the genotypes in the F_{st} graph. A low alpha value was estimated by the structure software which revealed that the trait for seed vigour initially evolved from single source and subsequently formed different admix genotypes with different ancestry value of seed vigour through evolutionary process. Majority of the moderate to high seed vigour containing genotypes were located in sub-population 1 while the lower seed vigour genotypes grouped in the sub-population 2. Few reports are available on genetic structure analysis based on seed vigour in rice. In earlier publications reported a fair correspondence of genetic structure and seedling vigour in rice by Anandan et al., 2016 and Sahoo et al., 2020. Phenotypic group of various traits and structure correlation were reported by many earlier research workers in rice (Huang et al., 2011; Zhang et al., 2012; Zhao et al., 2013; Mohapatra et al., 2017; Pandit et al., 2017; Das et al., 2018; Pradhan et al., 2020; Pandit et al., 2020; Pawar et al., 2021).

The 11 traits related to seed vigour showed signification association with SSR markers detected by using both the models of GLM and MLM in the analyses (Table 6; Table 7). However, all the traits except germination percentage and germination index were found to be significantly associated with SSR markers by both the models. The markers detected to be significantly associated by using both the models are considered to be robust associations and will be useful in seed vigour improvement programs. The markers namely RM167 present on chromosome 11 for seed vigour index-I; Marker RM7364 on chromosome 9 and RM235 on chromosome 12 associated with seed vigour index- II by MLM and GLM analysis will be highly useful. In addition, the markers detected by both the models viz., RM 440, RM223 for seedling dry weight (g); RM25181, RM 256, RM6547, RM328 and RM201 for rate of root growth (cm/day); RM 20A, RM13335 and RM216 for rate of shoot growth (cm/day); RM 405 for relative growth rate (g/day); RM20A and RM201 for absolute growth rate and markers RM235 and RM286 for mean germination time showed strong association will be useful in molecular breeding for enhancement of seed vigour related traits in rice. The Q-Q plot further confirmed the associations of these markers with various physiological traits (Fig. 6).

Reports on the QTLs governing seed germination have been published in rice (Fujino et al., 2008; Li et al., 2013; Jiang et al., 2017; Li et al., 2017; Wang et al., 2017; Jinet al., 2018; He et al., 2019). In our investigation, the 3 markers, RM225, RM235 and RM440 associated strongly with seed germination detected by GLM analysis are different from the genes reported by Fujino et al., 2008, Li et al., 2017, Wang et al., 2017, Jin et al., 2018 and He et al., 2019. Huang et al., 2004 detected linkage of RM21, RM224, RM336, RM30 and RM225 with growth rate at seedling stage in rice. Zhang et al., 2005 also reported linkage of RM225, RM85, RM230 and RM264 with this trait. Wang et al., 2010 reported linkage of RM7075, RM6091 and RM9 for growth rate in rice. GR in rice is controlled by the QTL, *qGR2* and mapped on chromosome 2 (Cordero-Lara et al., 2016). In our study, we detected strong association of RM25181 (chromosome 10), RM 256, (chromosome 8), RM6547(chromosome 1), RM328 (chromosome 9), RM 201 (chromosome 9) for rate of root growth, RM 20A (chromosome 12), RM13335 (chromosome 2) and RM216 (chromosome 10) for rate of shoot growth, RM 405 (chromosome 5) for relative growth rate, RM20A and RM201 (chromosome 9) for absolute growth rate by both GLM and MLM approach.

Markers RM336, RM19 and RM21 located on chromosome7, 2 and 11, respectively were significantly associated with QTL controlling shoot dry weight trait (Huang et al., 2004). Linkage of RM13, RM334, RM225, RM16 (Zhang et al., 2005a), RM 315, RM 250,RM166 on chromosome 1 and 2 (Cairns et al., 2009), RM3839 on chromosome 4 (Cheng et al., 2013), RM341 and RM224 markers showed strong association with shoot dry weight at seedling stage (Anandan et al., 2016). The QTLs for seedling dry weight at initial seedling stage *qFV-5-1* (Zhou et al., 2007); *qSDW5–1* and *qDW5* (Cui et al., 2002); *qSDW4.2* and *qSDW8.2* (Cheng et al., 2013); *qSDW2* (Han et al., 2007) and *qTDW 12–1* (Kanbar et al., 2006) was reported earlier. Of all these QTLs, only QTL *qSV7c* was fine mapped and the candidate gene was Os07g0600400 found controlling seedling dry weight, plant height and tiller number (Chen et al., 2019). We found strong association of this trait with two markers, RM440 (chromosome 5) and RM223 (chromosome 8) analyzed by both GLM and MLM model. The trait, seed vigour index was reported from linkage analysis of Zhang et al., 2005 in which RM249 on chromosome 5 and RM225 on chromosome 6 controlled the physiological parameter. As per report of on Diwan et al., 2013, *qVI* on chromosome 5 was linked with the trait. Anandan et al., 2016 detected association of seed vigour index with marker, RM341 on chromosome 2. In our investigation, markers RM167, RM7364 and RM235 were detected to be associated with seed vigour index by both the models.

5. Conclusion

The panel containing the landraces showed high variations for the 11

physiological traits. The population showed two distinct genetic groups by the STRUCTURE soft ware. The panel containing the landraces exhibited higher diversity parameters and the population is useful for improvement of the physiological traits. The importance of this investigation is that the strongly associated molecular markers with physiological parameters namely RM167 for seed vigour index-I; RM7364 and RM235 for seed vigour index- II; RM486 for seedling dry weight; RM216 for rate of shoot growth while RM20A and RM201 for absolute growth rate were detected by both GLM and MLM analysis. High yielding varieties may be improved for seed vigour trait via the associated physiological parameters by employing these strong markers in marker-assisted breeding program. The markers detected for the physiological traits need to be validated using more genotypes and markers.

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Authors' contributions

SKP and PS conceived the study, NN, EP, SRB, SS conducted the genotyping work, PS and TBB performed the phynotyping work, EP analyzed the data, SKP, PS: wrote the manuscript, all authors read and approved the final version.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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