Genetic Analysis for Mapping of Physiological Traits Related to Seed Vigour in Rice Through Association Mapping

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Original article

Keywords: Seed vigour, molecular markers, association mapping, physiological traits, indica rice namely rate of imbibition (RI), germination % (GP), germination index (GI), root length (RL), germination rate (GR), seed vigour index-1 (SVI-1), rate of plumule elongation (RPE), shoot length (SL), seedling dry weight (SDW), rate of shoot growth (RSG), SVHI and absolute growth rate (AGR)

Posted Date: March 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-288996/v1>

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Abstract

Background:

Good quality seed is an important factor for a good crop. Vigor is the key performing trait of quality seed. Genomic regions controlling the physiological traits related to seed vigour are not fully reported.

Results: A panel was prepared by including genotypes from all the groups of the fifteen physiological traits representing a population of 250 germplasm lines. Wide variations were observed for the 15 physiological traits in the population. The population was classified into 6 genetic groups. Presence of linkage disequilibrium (LD) was detected in the panel population based on the fixation indices of the subpopulations. Moderate values of gene diversity, polymorphic information content (PIC) and other diversity parameters were estimated from the population by genotyping with 109 SSR markers. The population was classified into subpopulations and sub-clusters showed relation with the genotypes for their physiological traits. Ten physiological traits were detected to be significantly associated with SSR markers analyzed by both the General linear model (GLM) and Mixed linear model (MLM). A total of 19 novel QTLs controlling different physiological traits namely *qGP 6.2* and *qGP 8.2* for germination % (GP); *qGR 9.1* for germination rate (GR); *qGI 1.2*, *qGI 6.1*, *qGI 8.1*, *qGI 11.1* and *qGI 12.1* for germination index (GI); *qRPE1.1* and *qRPE8.1* for rate of plumule elongation (RPE); *qAGR 8.1* for AGR; *qRSG1.2* and *qRSG12.2* for rate of shoot growth (RSG); *qRGR3.1* and *qRGR11.1* for relative growth rate (RGR); *qSVI 1.1* for seed vigour index I (SVI I); *qSVII 1.1*, *qSVII3.1*, and *qSVII12.1* for seed vigour index II (SVI II), and *qRRG8.1* for relative root growth rate were identified. The reported QTL for root length, *qRL 11.1* was validated in this mapping population. Additionally, QTLs, *qRPE8.1* and *qAGR8.1* of traits, RPE and AGR; *qGI 6.1* and *qGP6.2* of GI and GP; *qGI 11.1* and *qRL 11.1* of GI and RL; *qAGR8.1* and *qRRG8.1* of AGR and RRG, and *qRSG3.1* and *qRGR3.1* of traits RSG and RGR were detected for co-localization or co-inheritance.

Conclusion: The traits identified and markers detected in the association analysis will be useful for improvement of seed vigour trait through marker-assisted selection in rice.

Background

The genetic potential of a plant variety is fully manifested by use of quality seed which is considered as the basic and most vital input for crop production. A good quality seed not only ensures high yield but also considered as a mega factor for enhancing crop productivity (Chauhan et al. 2015). Simultaneous increase in population and adverse effects of climate change to rice crop becoming a threat to meet the world food demand which is expected to reach almost double by 2050 (Hunter et al. 2017; Pradhan et al. 2019a). Rice is a life saving grain to feed for more than half of the world population and necessitates increasing the productivity of the crop (Fukagawa and Ziska, 2019). Seed vigour is one of the most important determinant of seed quality, directly influences the crop productivity by delivering the genetic and yield potentials of the seed while ensuring uniformity in seed germination, seedling growth, establishment of seedling in the field and withstanding unfavourable environmental condition (Rajjou et al. 2012; Ventura et al. 2012). In addition, high seed vigour is also equally important for direct seeding as it enhances early crop establishment (Yamauchi and Winn 1996; Mahender et al. 2015) and produces vigorous seedling that can compete with weeds (Dingkuhn et al. 1999; Rao et al. 2007). Improving the seed vigour of rice remains a breeding challenge in crop research (Finch-Savage and Bassel, 2016) as it is not only essential to enhance the yield but also can improve crop resilience against adverse effects of climate change and biotic impediments to crop yields (Daniel, 2017).

Seed vigour via physiological growth parameters is a complex trait controlled by many quantitative trait loci (QTLs). Quantitative inheritance of this trait has made it difficult to identify the contribution of genetic factor by conventional genetic analysis. To investigate the inheritance of such complex trait, the QTL analysis has successfully proven as a powerful tool in the last decades. The detected QTLs for rice seed vigour were associated with many physiological traits viz., root length, shoot length, dry weight of seedling, germination rate, radicle length, root activity, coleoptile length, mesocotyl length, germination potential, germination index and time for 50 % germination (Regan et al. 1992; Redona and Mackill, 1996; Cui et al. 2002; Miura et al. 2002; Zhang et al. 2005; Fujino et al. 2008; Wang et al. 2010; Dang et al. 2014; Xie et al. 2014; Liu et al. 2014; Zhang et al. 2017; To et al. 2019; Zhao et al. 2019).

QTLs governing radicle length and mesocotyl length were mapped which contributed significantly for phenotypic variance (PV) of these traits (Xie et al. 2014; Lee et al. 2012). Control of root length were located on chromosomes 1, 2, 3, 5, 6, 7, 8, 10 and 12, exhibiting 3.9 to 27.1 % PV (Redona and Mackill, 1996; Cui et al. 2002; Zhang et al. 2005; Dang et al. 2014; Zhang et al. 2017). QTLs controlling shoot length were identified using various mapping populations which exhibited PV of 1.1 to 58.8 % (Redona and Mackill, 1996; Huang et al. 2004; Zhang et al. 2005; Abe et al. 2012; Dang et al. 2014; Zhang et al. 2017). QTLs for root dry weight were located on chromosome 1, 5 and 10 and ranged the PV from 2.9 to 24.1% (Cui et al. 2002). Shoot dry weight (SDW) was reported to be controlled by QTLs and located on chromosome 1, 3, 5, 6, 9 and 12 (Cui et al. 2002 and Dang et al. 2014). In addition, QTLs for total seedling dry weight were identified in the regions of chromosome 1, 2, 3, 4, 5, 6, 8, 9, 10 and 12 from different mapping populations contributing PV of 1.2-17.4% (Cui et al. 2002; Zhang et al. 2005; Zhang et al. 2017). QTLs mapping for germination rate were reported on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 10, 11 and 12 by earlier researchers in rice (Cui et al. 2002; Zhang et al. 2005; Wang et al. 2010; Wang et al. 2010; Liu et al. 2014).

Genetic analyses for seed vigour in rice were based on bi-parental segregating population mostly while very few analysis were through association mapping. Linkage disequilibrium (LD) based association mapping is a very successful approach of gene mapping. Using this mapping approach, QTL identification is possible with a higher resolution and multiple complex loci simultaneously by exploiting the available natural variation (Anandan et al. 2016; Pradhan et al. 2016; Pandit et al. 2017). Genome mapping with SSR markers are widely employed to assess genetic diversity and genetic structure in rice and several other crops as they are hyper variable, co-dominant, robust, multi-allelic in nature, chromosome specific and greatly facilitated linkage map construction (Barik et al. 2016; Das et al. 2018; Barik et al. 2020). The genetic diversity and structure of the population need to be studied in association mapping for detecting marker-trait association which could be useful for trait enhancement in molecular breeding programs. For detecting a perfect marker-phenotype association, the population should not show spurious association or unequal relatedness within the population (Mohapatra et al. 2017; Pandit et al. 2017; Pradhan et al. 2020). Therefore, population structure (Q) with relative kinship (K) analyses is used to check and correct the panel population composition for linkage

disequilibrium (LD) mapping analyses (Yu et al. 2006; Pandit et al. 2017). Thus, association estimate based on both the models of General linear model (GLM) and Mixed linear model (MLM) is considered appropriate for mapping complex traits that have shown to perform better than other model analysis. However, limited information is available on genetic analysis of physiological parameters related to seed vigour in rice utilizing SSR markers in a variable natural population.

Therefore, in the present investigation, association mapping of physiological parameters with 109 SSR markers was performed in a representative population shortlisted through phenotyping of 15 physiological traits namely electrical conductivity (EC), RI, GP, GI, SDW, RL, SL, SVI I, SVI II, rate of root growth (RRG), RSG, RPE, relative growth rate relative growth rate (RGR), AGR and MGR from 250 germplasm lines. The study will reveal the population genetic structure, diversity and candidate genes/QTLs involved in the 15 physiological traits associated with seed vigour in rice.

Material And Methods

Seed material

The freshly harvested seeds of 250 diverse germplasm lines collected from five states *viz.*, Assam, MP, Kerala, Odisha and Manipur of India were used for association mapping of physiological growth parameters. The germplasm lines of Odisha state were from the Jeypore tract, the secondary center of origin of rice and known for availability of rich diversity of rice. All the germplasm lines were collected from Gene bank, ICAR-NRRI, Cuttack and grown during wet season, 2017-18 (Supplemental Table 1). The harvested seeds were used for estimation of 15 physiological growth parameters after a storage period of three months to overcome the seed dormancy. A panel population was developed and raised during wet seasons of 2018-19 and 2019-20. The panel population (96) is highlighted in the Supplementary Table 1 were used for mapping of physiological traits (Table 1).

Phenotyping of physiological traits

Seed physiological characteristics such as electrical conductivity, rate of imbibition, germination %, germination index, root length, shoot length, seedling dry weight, seed vigour index, seed vigour index, rate of root growth, rate of shoot growth, rate of plumule elongation, relative growth rate, absolute growth rate and germination rate were estimated for the mapping study.

Seed physiological traits were estimated by sowing 50 seeds in three replications following top of paper method by incubating at 30°C. The EC was determined by measuring the conductivity of the seed leachate of 50 seeds soaked in 150 ml distilled water for 24 hrs at 30°C and expressed as $\mu\text{mhos cm}^{-1}$. Rate of imbibition of the germplasm lines was determined as increase in volume of seed after soaking the seed in water for 24 hours. The percentage of germinated seeds at 10th day was referred to as the final germination percentage. GI was calculated by adopting the method of Maguire, 1962. RL and SL were measured at 10th day of germination and expressed in cm. The increase in plumule length per day were considered as rate of plumule elongation and expressed in cm day^{-1} . The increase in root and shoot length per day recorded at 7th day and 10th day of germination were considered as RRG and RSG and expressed in cm day^{-1} . AGR was calculated as per procedure of Reford (1967) and RGR was determined following the procedure of Fisher (1921). MGR was computed by adopting the procedure of Zuo et al. 2018. The seedlings used for recording rate of growth were subsequently oven dried at 70 °C for 48 hours after removing the cotyledon and seedling dry weight was expressed in gram per seedling. Seed vigour indices (SVI I and II) were calculated using the formula suggested by Abdul-Baki and Anderson (1973). For estimating all the physiological traits except EC, RI and SG (50 seeds in each three replication) observations were recorded on five seedlings selected from each replication and averaged to get the value of each replication.

Analysis of variance (ANOVA) for individual character including the estimation of mean, range, and coefficient of variation (CV %) were estimated by using Cropstat software 7.0. Pearson's correlation coefficients were analyzed to find out the relationship among the various physiological traits, based on the mean values of the 96 genotypes and presented in correlation matrix heatmap. The mean estimates of the 15 physiological parameters were classified into 4 groups as very high, high, medium and low value containing germplasm lines for this study.

Genomic DNA isolation, PCR analysis and selection of SSR markers

Genomic DNA of the germplasm lines was extracted from 15 days-old plant by adopting CTAB method (Murray and Thompson, 1980). The 109 SSR (simple sequence repeat) markers were taken from the data base available in the public domain (Supplementary Table 2). The isolated DNA was quantified by resolving the DNA fragments in gel electrophoresis. PCR analysis was done using the markers selected based on position covering all the chromosomes to illustrate the diversity and to identify the polymorphic loci among the 96 rice germplasm lines (Table 1). Conditions of PCR reaction was set to initial denaturation step (2 min, 95 °C), followed by 35 cycles of denaturation (30 s, 95 °C) and annealing/extension (30 min, 55 °C), extension (2 min, 72°C), final extension (5 min, 72°C) and store at 4°C (infinity). The PCR products were electrophoresed using 2.5% agarose gel containing 0.80g ml⁻¹ ethidium bromide. To determine the size of amplicons, 50 bp DNA ladder was used. The gel was run at 2.5V cm⁻¹ for 4 hrs and photographed using a Gel Documentation System (SynGene).

Molecular data analysis

Data scoring was carried out from the presence or absence of amplified products obtained on the basis genotype-primer combination. A binary data matrix was used as discrete variables for the entry of our result data. Software, 'Power Marker Ver3.25' was used to analyze the parameters namely polymorphic information content (PIC), observed heterozygosity (H), number of alleles (N), major allele frequency (A) and gene diversity (GD) for each SSR locus (Liu and Muse 2005). A Bayesian model based clustering approach STRUCTURE 2.3.6 software was used to analysis genetic data and obtain population structure (Pritchard et al. 2000). To derive the ideal number of groups (K), STRUCTURE software was run with K varying from 1 to 10, with 10 iterations for each K value. A high throughput parameter set of burn-in period of the 150,000 followed by 150,000 Markov Chain Monte Carlo (MCMC) replications was adapted during the

running period. Highest value of ΔK was pick up from Evanno table used to detect the subpopulation groups from the panel of populations in the next step. The maximal value of $L(K)$ was identified using the exact number of sub-populations. The model choice criterion to detect the most probable value of K was ΔK , an *ad-hoc* quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by STRUCTURE (Evanno et al. 2005). Structure Harvester was used for estimation of the ΔK -value as function of K showing a clear peak as the optimal K -value (Earl and Vonholdt, 2012). The principal coordinate analysis of all the genotypes and unweighted neighbor joining unrooted tree for Nei coefficient dissimilarity index (Nei, 1972) with bootstrap value of 1,000 were obtained by using DARwin5 software (Perrier and Jacquemoud-Collet, 2006). The presence of molecular variance across the whole population, within a population and between the sub-population structures (F_{IT} , F_{IS} , F_{ST}) was calculated by the deviation from Hardy-Weinberg expectation and estimated through Analysis of molecular variance (AMOVA) using GenAlEx 6.5 software. All the detailed protocols of the above mentioned softwares were described in earlier publications (Pradhan et al. 2016; Pandit et al. 2017; Pandit et al. 2020; Pradhan et al. 2020).

Software, "TASSEL 5.0" was used to analyze the marker-trait association for mapping study of the seed vigor traits in rice. General linear model and Mixed linear model in TASSEL 5.0 were used to perform the genetic association between the phenotypic traits and molecular markers (Bradbury et al. 2007). By considering the significant *p*-value and r^2 value convincing associated markers were identified. The associations of markers were further confirmed by the Q-Q plot generated by the software. Linkage disequilibrium plot was obtained using LD measured r^2 , between pair of markers is plotted against the distance between the pair. Also, the accuracy of the marker-trait association by estimating the FDR adjusted p-values (q-values) using R software as described in the earlier publications (Pandit et al. 2017; Pradhan et al. 2020).

Results

Phenotyping of the population for physiological traits in rice

The mean values of 250 genotypes for 15 physiological traits *viz.*, EC, RI, G,GI, RL, SL, SDW, SVI-I, SVI-II, RRG, RSG, RPLe, RGR, AGR, and MGR related to seed vigour were estimated during wet seasons of 2019 and 2020 (Supplementary Table 1). Significant differences were noticed among the germplasm lines for these 15 traits. The frequency distribution of the 250 germplasm lines were broadly classified into 4 groups each for the 15 physiological parameters (Fig. 1A-C). The distribution of germplasm lines into various groups were categorized into groups or subpopulations (Fig. 1). A representative panel population containing 96 genotypes was developed from the original population by shortlisting germplasm lines from all the phenotypic groups of each parameter (Table 1; Fig. 2). The mean values of the 15 physiological traits estimated from the studied panel population also showed significant variation among the genotypes for each trait (Table 1). High to very high estimates of seed vigour index I was observed in the germplasm lines MNPAC9005, MNP-AC9006, MNP-AC9021, MNP-AC9038, MNP-AC9043, JBSAC20282, JBSAC20328, JBSAC20362, JBSAC20371, JBSAC20389, JBSAC20770, JBSAC20920, Kantakapura, Kantakaamala, Kapanthi, Champaeisiali, Gondiachampeisiali, Kanar, Adira-2PallakadR3, Pk-21, and AC-10187. High seed vigour index-II were estimated from the lines MNP-AC9030, MNP-AC9038, MNP-AC9043, MNP-AC9044A, MNP-AC9058, MNP-AC9063, MNP-AC9065, MNPAC9090, MNPAC9093, JBSAC20282, JBSAC20328, JBS-AC20362, JBS-AC20371 and JBS-AC20389 (Table 1). The germplasm lines MNP-AC9021, JBS-AC20317, JBS-AC20328, JBS-AC20362, JBS-AC20371, JBS-AC20389, Adira-2PallakadR3, Pk-21, Shayam, Basumati-B, Sugandha-2 and Chatuimuchi showed high germination index. High values for seedling dry weight were recorded from the germplasm lines MNP-AC9028, MNP-AC9030, MNP-AC9035, MNP-AC9043, MNP-AC9058, MNP-AC9063, MNP-AC9065, MNP-AC9090, and MNP-AC9093. Very high RSG estimates was noticed in MNP-AC9030, MNP-AC9035, MNP-AC9038, Kapanthi, Pk-21 while MNP-AC9044A, JBS-AC20423, JBS-AC20920, Champaeisiali, Magra, Balisaralaktimachi-k, AC-6023, AC-6183, AC-10162, AC-10187 exhibited high rate of root growth. Very high relative growth rate was recorded from the genotypes *viz.*, MNP-AC9030, MNP-AC9038, MNP-AC9043, MNP-AC9044A, Kundadhan, Latamahu, Laxmibilash, Kanakchampa, Magura-s, PK-18Ezhoml-2, PK-24Jyothi, PK-25Jaya, PK-33D1, AC-5993, AC-6027, AC-6172, AC-7134 and AC-7269s. Germplasm lines Kantakapura, Champaeisiali and Latamahu showed very high estimates for rate of plumule elongation. Very high shoot length was recorded from MNP-AC9030, MNP-AC9035, MNP-AC9038, MNP-AC9043, MNP-AC9044A, JBS-AC20282 and JBS-AC20770. Very high electrical conductivity was detected from the genotypes, MNP-AC9030, JBS-AC20282, JBS-AC20845, JBS-AC20907 and AC-7008. Germplasm lines namely MNP-AC9038, MNP-AC9043, JBS-AC20282 and BS-AC20845 were categorized for very high Rate of imbibitions. In addition, very high seedling root length was recorded from the germplasm lines MNP-AC9005, MNP-AC9076A, kapanthi and Champaeisiali. Also, very high estimates of rate of plumule elongation were observed in the germplasm lines namely Kantakapura, Champaeisiali and Latamahu. MNP-AC9030, MNP-AC9035, MNP-AC9038, MNP-AC9043, MNP-AC9044A, JBS-AC20282 and JBS-AC20770 had high seedling shoot length in the panel population. The germplasm lines possessing high values of multiple physiological traits for ≥ 10 were in MNP-AC9005, MNP-AC9006, MNP-AC9038, MNP-AC9043, MNP-AC9044A, JBS-AC20282 and JBS-AC20328 (Table 1).

Relatedness among germplasm lines for physiological traits through genotype-by-trait biplot analysis

The scatter diagram was plotted taking the first two principal components to generate genotype-by-trait biplot graph for the 15 physiological traits estimated from the 96 genotypes present in the panel (Fig. 3). The first and second principal components showed 99.651 and 0.2204 of the total variability with eigen value of 102641 and 227.016, respectively (Supplementary Fig. 1). SVI-I contributed maximum diversity among the 15 physiological parameters followed by GI and SVI-II for the panel population based on the principal component analysis (Fig. 3). The scattering pattern of genotypes in the 4 quadrants indicated that genotypes containing high estimates of parameters are placed in opposite direction of the quadrant 1 and II. Higher estimates of physiological parameters containing genotypes have been encircled in the figure (Fig. 3). The top right (1st quadrant) and bottom right (2nd quadrant) accommodated majority of the genotypes containing high estimates of physiological parameters. The 3rd (bottom left) quadrant kept most of the moderate value containing physiological parameters while the 4th quadrant (top left) accommodated majority of poor in values of the physiological parameters (Fig. 3).

Nature of association among seed vigour related traits

The association among 15 physiological traits revealed a strong positive correlation ($r \geq 0.7$) of SVI I with GI and GP; SVI II with SDW; GP with GI, and AGR with RRG and RSG (Fig. 4). Moderate positive correlation (r : 0.5-0.7) of SVI I with RL and SL; SVI II with GI,SVI I and GR; RI with EC; SL with RI and RL; RRG with RL;

RSG with SL; AGR with RL, SL; MGR with GI, SDW, SVI II were observed. Weak positive correlation ($r < 0.5$) was noticed for SVI I with RI, RRG, RSG, AGR and MGR; SVI II with RI, GP, RL and SL; RRG with SL, RSG and AGR; RRG with SL; RSG with GI, RL, SDW and RRG; RPE with RL and SL; AGR with RI, SDW; MGR with Germ, RL, SL, RSG. However, weak negative correlation was estimated for RGR with GI, GP and SVI I (Fig. 4).

Genetic diversity parameters analysis

The constituted panel containing 96 genotypes from the original population which exhibited wide variation for the physiological parameters was genotyped using 109 molecular markers. The gene diversity, loci used for genetic diversity and other diversity related parameters are presented in Table 2. A total of four hundred four markers alleles were obtained with average value of 3.07 alleles per locus. The range of alleles per locus varied from 2 to 7 per marker showing the highest number of alleles by RM220, RM448 and RM493 in the studied panel for the physiological parameters. The average value of the major allele frequency of the parameters linked to the polymorphic markers was observed to be 0.578 which varied from 0.292 (RM488 and 493) and 0.958 (RM22034) (Table 2). The range for PIC value was estimated to be from 0.141 (RM315 and 6054) to 0.771 (RM493) with mean value of 0.477. The observed average heterozygosity (H_o) in the population was 0.117 which varied from 0.00 to 0.958. The gene diversity (H_e) in the panel ranged from 0.061 (RM556) to 0.799 (RM493) showing a mean value of 0.533.

Population Genetic Structure Analysis

The genotypes in the panel exhibiting variation for the studied physiological parameters were evaluated for genetic structure adopting probable sub-populations (K) and selecting higher delta K -value estimated by STRUCTURE 2.3.6 software. The delta K value is related to the rate of change in the log probability of data between successive K values. It categorized the genotypes into two sub-populations (Fig. 3A; Fig.3B) with a high ΔK peak value of 264.2 at $K = 2$ among the assumed K (Fig. 5). The proportions of genotypes in the inferred clusters were 0.875 and 0.125 in subpopulation 1 and subpopulation 2, respectively. However, the two subpopulations did not show correspondence well with the studied physiological parameters. Hence, next peak at the ΔK peak was considered and the population was categorized into 6 subpopulations. The proportions of genotypes in the inferred clusters were 0.179, 0.211, 0.258, 0.081, 0.181 and 0.091 for the sub-population 1, 2, 3, 4, 5 and 6, respectively. The fixation index (F_{st}) values were 0.278, 0.254, 0.201, 0.332, 0.206 and 0.507 for the sub-population 1, 2, 3, 4, 5 and 6, respectively. The expected average distances or heterozygosity were 0.342, 0.348, 0.366, 0.390, 0.373 and 0.331 in the sub-population 1, 2, 3, 4, 5 and 6, respectively. The genotypes with $\geq 80\%$ ancestry value were categorized for that subpopulation (Table 3; Fig. 5).

The physiological parameters showed a relatively fair correspondence at $K=6$ with the structure subpopulations present in the panel population. Majority of the moderate to high seed vigour showing germplasm lines present in the subpopulations SP2 and SP6 while poor vigour containing lines were in subpopulation SP4 and SP5. The panel also showed a low alpha value ($\alpha = 0.0591$) by the structure analysis at $K=6$. Positively skewed leptokurtic distributions were observed for mean alpha-value, F_{st1} , F_{st4} and F_{st5} while mesokurtic distributions detected for F_{st1} , F_{st2} and F_{st6} for the panel population showing a distinct variation in the distribution among the F_{st} values (Supplementary Fig. 2).

Molecular variance (AMOVA) and LD decay plot analysis

The closely related plants in a population are clustered into isolated groups and form various subpopulations. Genetic variations between and within the sub-populations at $K=6$ were detected through analysis of molecular variance (AMOVA) (Table 4). The genetic variations obtained between and within at $K=6$ was computed to be 12% among the populations, 67% among individuals and 21% variation within individuals of the panel population. Deviation from Hardy-Weinberg's prediction was calculated from Wright's F statistics estimates. Different parameters like uniformity of individual within the subpopulation (F_{IS}) and individual within the total population (F_{IT}) were estimated for differentiation of population. The F_{IT} and F_{IS} values of total population and within population based on 109 loci were 0.791 and 0.763, whereas F_{ST} was 0.118 between the two subpopulations. F_{st} is estimated to measure the population differentiation or the subpopulations within the total population. The F_{st} values of each sub-population and their distribution pattern showed a clear differentiation between the six sub-populations from each other (Supplemental Fig. 2).

The nonrandom association of alleles at different loci is successfully utilized for marker-trait association study. The LD decay rate is important factor for getting marker-trait association. The decay rate will facilitate the discovery of reliable markers associated with the physiological parameters and will facilitate the discovery of new genes or allelic variants controlling these traits. Syntenic2 was used to plot the LD decay of the population against the physical distance in million base pair (Fig. 6). Tightly linked markers have the highest r^2 and average r^2 rapidly decreases as linkage distance increases. There was a sharp decline in LD decay for the linked markers at 1-2 mega base pair and thereafter a very slow and gradual decay was noticed. Overall, it is clear that LD decay occur for the physiological parameters.

Genetic relatedness among genotypes by principal coordinates and cluster analyses

The two dimensions diagram for principal coordinate analysis (PCoA) is drawn based on 109 markers which grouped the genotypes as per the genetic relatedness among them (Fig. 7). The component 1 accounted for 11.04% inertia and component 2 for 6.71% of total inertia. The panel genotypes were placed in various spots of the 4 quadrants which formed three major groups (Fig. 7). A total of 30, 46, 11 and 9 number of germplasm lines were distributed in the 1st, 2nd, 3rd and 4th quadrant, respectively. The genotypes belonging to the 6 different sub-populations are grouped in different quadrants. The 1st quadrant genotypes are divided into 3 groups whereas the 2nd quadrant genotypes are divided into two groups of which one group is closer to axis1 and another is to axis 2. This 2nd group genotypes closer to axis2 are admix type depicted in black colour (Fig. 7).

The majority of the germplasm lines containing high to very high mean values of physiological traits were placed in the 1st (top right) and 2nd (bottom right) quadrants of the PCoA. The PCoA distributed the genotypes in the four quadrants forming 7 clusters including the admix type subpopulation. The subpopulations clustered by PCoA are encircled in the figure and showed correspondence with population structure (Fig. 7). Germplasm lines having high to

very high mean values of physiological traits placed in the quadrant II were ARS-AC-6221, MP-Joha, KE-Adira-2-Pallakad-R3, KE-PK-18-Ezhoml-2, KE-PK-14-Vachaw and KE-PK-24-Jyothi. Genotypes namely OD-Landi, OD-Balisaralaktimachi, OD-Kaniar, OD-Kanakchampa, ARS-AC-6023, ARS-AC-6172, MNP-AC-9030, KE-PK-19-Cheruvirippu, JBS-AC-20614, MNP-AC-9005, JBS-AC-20371 and JBS-AC-20423 were observed in the quadrant I. Quadrant III consisted of genotypes mostly from SP4 subpopulation. Majority of the germplasm lines in quadrant IV were from SP6 subpopulation. Majority of the admix genotypes were found in quadrant I and II.

Six sub groups were observed in the dendrogram based on the mean values of studied physiological parameters (Fig. 8A). A total of 15, 4, 11, 21, 19 and 26 genotypes were distributed in the cluster I to VI, respectively. Cluster VI was biggest cluster which accommodated 26 germplasm lines while cluster II was smallest with only 4 genotypes. The germplasm lines present in sub-population 2 of genetic structure were observed in group 6 of the dendrogram. Similarly, four genotypes of structure subpopulation 1 were in the group 4 of the dendrogram. Admix genotypes obtained from the structure were found in all the groups of this phenotype cluster except group 4.

The cluster analysis discriminated the germplasm lines on the basis of genotyping of 109 SSR markers and placed the genotypes into different clusters which corresponded with the studied physiological parameters. The unweighted-neighbour joining tree differentiated the genotypes into 6 different clusters (Fig. 8). Clusters SP₁ was differentiated from SP₃ by the presence of high estimates of root length and rate of plumule in it whereas Seedling dry weight & Seed vigour index II were rich in SP₃. SP₂ and SP₆ had accommodated majority of germplasm lines containing high values for the studied parameters except Seedling dry weight in SP₂ and germination % in SP₆. Sp4 was discriminated from others based on absence of germplasm lines containing high estimates for germination rate and seed vigour index II while root length and seedling dry weight were absent in SP₅.

Association of marker alleles with physiological parameters in rice

Association of molecular markers with 15 physiological parameters was computed using Mixed Linear Model (MLM/ K+Q model) and Generalized Linear Model (GLM) by TASSEL 5 software. The marker-trait comparisons were subjected to filtration at less than 1% error i.e. 99% confidence ($p < 0.01$). Twelve parameters showed significant associations with markers using both the models at $p < 0.01$. A total of 112 and 93 significant marker-trait associations were detected by GLM and MLM, respectively at $p < 0.01$. The marker R^2 values computed by GLM approach was from 0.565 to 22.7 while the range was 0.7009 to 0.175 by Mixed Linear Model (Supplementary Table 3). Significant marker-trait associations were detected for G1 with 5 markers; SVI-II, RSG and MGR with 3 markers; GP, RGR and RP with 2 markers, and RI, RL, SVI, AGR and RRG with 1 marker by both GML and MLM models at $p < 0.01$. Considering trimming at $r^2 > 0.10$ and $p < 0.01$, 6 markers exhibited associations with 4 physiological parameters namely GI with RM225 and RM502; GP with RM225 and RM502; SVI with RM5638 and RP with RM220 (Table 5; Supplemental table 4). The Q-Q plot also confirmed the association of these markers with the associated physiological traits in rice (Fig...).

Four markers showed significant association with GI detected by GLM and MLM models at $p < 0.01$. The genomic regions controlling the trait, GI was detected on chromosome 1, 8, 11 and 12 associated by markers RM5638, RMRM502, RM229 and RM20A, respectively. Among the four markers, RM502 showed highest marker R^2 value of 0.227 analyzed by GLM and 0.175 by MLM. Three markers namely RM5638, RM14723 and RM7003 located at 204, 86 and 132 cM positions on chromosome 1, 3 and 12, respectively were associated with the parameter, SVII. RSG was detected to be associated with RM 6547, RM3701 and RM7003 present on chromosome 1, 11 and 12, respectively. MGR was found to be controlled by the QTLs present on the chromosome 1, 8 and 9 which showed associations with markers RM220, RM502 and RM201, respectively. QTLs for Germination % showed significant associations with RM225 on chromosome 6 and RM502 on chromosome 8. The parameter, RPE was detected to be located on chromosome 1 showing association with RM220 and RM403. Relative growth rate exhibited association with RM468 and RM3701. Marker RM256 showed significant association with RRG and AGR. Significant associations of markers RM248, RM229 and RM5638 with RI, RL and SVI, respectively were detected by both the models. Marker RM256 was strongly associated with parameters, RRG and AGR. In addition, RI, RL and SVI showed significant associations with RM248, RM229 and RM5638, respectively (Table 5). The Q-Q plot also confirmed the associations of these markers with the estimated physiological parameters in rice (Fig. 9).

Common markers were observed to be associated with different physiological parameters in rice. Few markers showed significant associations with two physiological parameters namely RM220 with RP and AGR; RM225 with GI and GP; RM229 with GI and RL; RM256 with AGR and RRG; RM3701 with RSG and RGR, and RM7003 with SVII and RSG by both the models at $< 1\%$ error. Also, marker RM602 showed significant associations with 3 traits namely GI, GP and MGR. In addition, RM5638 also associated with 3 parameters namely GI, SVI and SVII by both the models at $p < 0.01$ (Table 5).

Discussion

The germplasm lines present in the panel population were significantly different from each other with respect to the 15 studied physiological traits (Table 1). Higher magnitude of correlation coefficients was estimated in many studied physiological parameters. This revealed the improvement of seed vigour through the associated traits based on the values of the coefficients of the correlated parameters and availability of higher genetic variations in the population (Table 4). Earlier reports of high variations and genetic advance for many traits were published by many researchers (Bose et al. 2007; Zhao et al. 2013; Anandan et al. 2016; Pradhan et al. 2016; Pandit et al. 2017; Huang et al. 2015; Pradhan et al. 2019b). The existence of diversity based on 109 markers and phenotypic variations of the 15 physiological traits showed clear cut differentiations in the studied population (Table 2). A moderate to high PIC value and more alleles in the population revealed about better informative markers and for use in the breeding program. The germplasms used in this study were collections from the states known for existence of rich rice genetic diversity (Sahoo et al. 2020). Germplasm lines from Jayapur tract of Odisha, the secondary centre of origin were also included in this study. The germplasm lines having high values of multiple physiological traits for ≥ 10 were MNP-AC9005, MNP-AC9006, MNP-AC9038, MNP-AC9043, MNP-AC9044A, JBS-AC20282 and JBS-AC20328. These germplasm lines will be potential donors for seed vigour trait in breeding programs (Table 1). Hence, it is expected that breeding programme with inclusion of parental lines from this population may be effective for seed vigour and its related traits. Many publications on existence of genetic diversity are reported in rice (Bose et al. 2005; Shukla et al. 2006; Chen et al. 2011; Zhang et al. 2011; Singh et

al. 2013; Pandit et al. 2016; Pradhan et al. 2020). Six structure groups and presence of different *Fst* values supported the existence of various LD group in the population. A low alpha value and existence of many admix type germplasm lines in the population revealed that the trait seed vigour evolved from single source and subsequently formed different admix genotypes with different ancestry value during evolutionary process. A good correspondence of genetic structure and physiological parameters was previously published by earlier workers (Anandan et al. 2016; Sahoo et al. 2020). Additionally, publications on phenotype of various traits and structure correlation have been published by many workers (Pradhan et al. 2016; Anandan et al. 2016; Pandit et al. 2017; Zhang et al. 2012; Huang et al. 2011; Zhao et al. 2013; Huang et al. 2015; Pradhan et al. 2019b; Pradhan et al. 2020; Sahoo et al. 2020).

Twelve physiological parameters influencing seed vigour were found to be associated with 10 SSR markers analyzed by both GLM and MLM approaches (Table 5). The markers detected to be associated by both the models at $p < 0.01$ and low 'p' value are considered to be very robust and useful for seed vigour improvement program. Hence, markers namely RM5638, RMRM502, RM229 and RM20A for GI; RM14723, RM7003 and RM5638 for SVII; RM 6547, RM3701 and RM7003 for RSG; RM220, RM502 and RM201 for MGR; RM225 and RM502 for germination %; RM220 and RM403 on chromosome 1 for RPE; RM468 and RM3701 for RGR; RM256 for RRG and AGR; RM248, RM229 and RM5638 for RI, RL and SVI, respectively are useful in molecular breeding for improvement of seed vigour in rice (Table 5). Q-Q plot also confirmed the associations of these markers with various seed vigour influencing traits in rice (Fig. 9).

Many QTLs controlling germination % were reported by earlier researchers in rice (Zhang et al. 2005; Wang et al. 2010; Li et al. 2013; Liu et al. 2014; Jiang et al. 2017; Wang et al. 2017; Jin et al. 2018; He et al. 2019). As per earlier mapping results, germination % is controlled by QTL, *qLTG-3-1* (Fujino et al. 2008). Another QTL, *qGP-9* for the trait was localized to 92.8 kb between two STS markers interval of P6 and P8 (Li et al. 2017). *OsIPMS1* controls seed vigor in rice which is correlated with starch hydrolysis, glycolytic activity and energy levels in germinating seeds (He et al. 2019). As per report of Wang et al. 2010, the QTLs for GP were located on chromosome 6 at 123-132 cM and chromosome 8 at 23.4-34.6cM positions. In our investigation, the RM225 and RM502 were significantly associated with germination % were located on chromosome 6 at 92 cM and chromosome 8 at 177cM, respectively detected by both the models of GLM and MLM. Therefore, these locations are different from the genes reported by Fujino et al. 2008, Jiang et al. 2017, Liu et al. 2014, Li et al. 2017, Wang et al. 2017, Jin et al. 2018 and He et al. 2019. Hence, the two QTLs detected for the trait in this mapping study were novel QTLs and designated as *qGP6.2* and *qGP8.2*. Rate of plumule elongation was observed to be associated significantly with markers RM220 and RM403 on chromosome 1 and 8, at 242 cM and 183 cM positions, respectively. These two QTLs designated as *qRPE1.1* and *qRPE8.1* were considered as novel QTLs as no gene(S)/QTLs were reported at these locations from other studies in rice. Germination rate in rice seed is controlled by the QTLs present on chromosome 3, 5, 6 and 7 (Cui et al. 2002). The QTL present on the chromosome 1, *qGR1* reported for germination rate, is located at 137.5-156.8cM placed away from the detected QTL (Wang et al. 2010). For germination rate, we detected the QTL at 240 cM position on chromosome 1. The detected novel QTL is designated as *qGR1.2* is located on chromosome 1 at 240 cM position. Two QTLs for GR were located on chromosome 8 at 146 cM and 153cM positions reported (Jin et al. 2018). The QTL detected for GR in the present investigation on chromosome 8 was at 177 cM position. Hence, the novel QTL controlling germination rate is designated as *qGR8.3*. The QTL for GR present on chromosome 9 was not reported in earlier studies and hence the QTL is novel which is designated as *qGR9.1*. The QTL for germination index were located on chromosome 1, 3, 7, 8 and 10 (Liu et al. 2014). The detected QTLs for the trait on chromosome 6, 11 and 12 were not reported in earlier studies. Hence, the three novel QTLs are designated as *qGI6.1*, *qGI11.1* and *qGI12.1*. The QTL detected on chromosome 1 were reported earlier by Wang et al. 2010 and Liu et al. 2014. As per Wang et al. 2010, the position of *qGI1.1* was at 88.8-111.5 cM and Liu et al. 2014 located at 52.1-54.2 cM. In the present investigation, we detected the QTL on chromosome 1 at 284cM position. The detected novel QTL is designated as *qGI1.2* is located on chromosome 1. In addition, another QTL reported on chromosome 8 for this parameter was located at 92.2-116.7 cM position (Liu et al. 2014). We detected a QTL on chromosome 8 at 177 cM position. Hence, the detected QTL is a novel one and designated as *qGI8.1*. The QTLs for seedling root length on chromosome 1, 2, 3, 5, 6, 7, 8, 9, 10 and 12 reported by different workers are different from the present investigation detected QTL for root length which is located on chromosome 11 (Cui et al. 2002; Zhang et al. 2005; Zhang et al. 2017; Jin et al. 2018; To et al. 2019; Zhao et al. 2019; Yang et al. 2020). The QTL controlling this trait located on chromosome 12 was within G193-RG574 and linked to RM3455 and RM1065 (Zhang et al. 2005; To et al. 2019; Zhao et al. 2019). The root length-marker linkage reported by Madhuri et al. 2016 is exactly at the same location on the chromosome 11. Hence, this QTL which controlled the seedling root length is validated in the panel population and will be useful in breeding program or improvement of the trait.

Genetic analysis for seedling growth parameters were reported by many workers (Huang et al. 2004; Zhang et al. 2005; Wang et al. 2010; Cordero-Lara et al. 2016; To et al. 2019; Zhao et al. 2019). Relative shoot length showed association with the markers RM3701, RM7003 and RM6547 analyzed by both the models. Mapping results of Han et al. 2002 showed the location of the QTL on chromosome 1 within 117-133.4 cM. We mapped the QTL on the same chromosome but at 145 cM position. Results obtained from Cao et al. 2002, Abe et al. 2012, Dang et al. 2014 and Anandan et al. 2016, also reported the QTL for the trait on different chromosomes. Hence, the QTL located at 145 cM position is a novel QTL and designated as *qRSG1.2*. Another QTL on chromosome 12 at 101-107 cM position was reported by Han et al. 2007 but we detected a QTL for the trait at 132 cM. The novel QTL detected by us is designated as *qRSG12.2*. Relative growth rate was mapped by Kato et al. 2007 on chromosome 4 and 7 within marker interval of RM8213-RM335 and RM8249-RM5120, respectively. The detected QTL in this investigation were located on chromosome 3 and 11. The detected QTLs are novel QTLs and designated as *qRGR3.1* and *qRGR11.1*. Three QTLs located on chromosome 2, 3 and 12 for absolute growth rate by Bharamappanavara et al. 2020. We detected the QTL on chromosome 8 which is not reported in earlier studies. This novel QTL is designated as *qAGR8.1*. 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 Diwan et al. 2013, *qVI* on chromosome 5 was linked with the trait. Anandan et al. 2016 found association of seed vigour index with marker, RM341 on chromosome 2. In our investigation, markers RM5638 located on chromosome 1 showed significant associations with SVI and SVII by both the models. No reports are available for these two traits at this location. These novel QTLs are designated as *qSVI1.1* and *qSVII1.1*. The other two markers, RM14723 on chromosome 3 and RM7003 on chromosome 12 associated with SVII are not reported in earlier studies and are novel QTLs. These two QTLs are designated as *qSVII3.1* and *qSVII12.1*. The QTL for relative root growth, *qRRL-7* was mapped by Masuda et al. 2018 on chromosome 7. In our study, we detected the QTL on chromosome 8 at 97 cM position. No reports are available for RRG at this location and hence this novel QTL is designated as *qRRG8.1*.

Conclusion

Improvement of seed vigour trait is possible in high yielding varieties by employing the strong markers detected for the physiological parameters through marker-assisted breeding program. Fifteen physiological traits showed wide variations present in a panel of 96 genotypes which represented 250 germplasm lines. Presence of linkage disequilibrium (LD) was detected in the panel population based on the fixation indices of the subpopulations. Moderate values of gene diversity, polymorphic information content (PIC) and other diversity parameters were estimated from the population by genotyping with 109 SSR markers. The population was classified into subpopulations and sub-clusters showed relation with the genotypes for their physiological traits. A total of 19 novel QTLs controlling different physiological parameters namely *qGP 6.2* and *qGP 8.2* for germination %; *qGR 9.1* for germination rate; *qGI 1.2*, *qGI 6.1*, *qGI 8.1*, *qGI 11.1* and *qGI 12.1* for germination index; *qRPE1.1* and *qRPE8.1* for rate of plumule elongation (RPE); *qAGR 8.1* for AGR; *qRSG1.2* and *qRSG12.2* for RSG; *qRGR3.1* and *qRGR11.1* for RGR; *qSVI 1.1* for SVI I; *qSVII 1.1*, *qSVII3.1*, and *qSVII12.1* for SVI II, and *qRRG8.1* were identified. The reported QTL for root length, *qRL11.1* was validated in this mapping population. Additionally, QTLs, *qRPE8.1* and *qAGR8.1* of traits, RPE and AGR; *qGI 6.1* and *qGP6.2* of GI and GP; *qGI 11.1* and *qRL 11.1* of GI and RL; *qAGR8.1* and *qRRG8.1* of AGR and RRG, and *qRSG3.1* and *qRGR3.1* of traits RSG and RGR were detected for co-localization or co-inheritance. The traits identified and markers detected in the association analysis will be useful for improvement of seed vigour trait through marker-assisted selection in rice.

Declarations

Ethics approval and consent to participate: The authors declare that this study complies with the current laws of the country in which the experiments were performed.

Consent for publication: Not applicable

Availability of data and material: The data generated or analyzed in this study are included in this article.

Competing interest: The authors declare no conflicts of interest

Funding: No externally aided funding was utilized for this research work

Authors contribution: SKP and PS conceived the study, DKN, SRB, AB, AS, SP, AM, DS and EP conducted the genotyping work, PS, RB, JM and SKP performed the phenotyping work, SRB and EP analyzed the data, SKP, PS wrote the manuscript, all the authors read and approved the final version.

Acknowledgement

The authors acknowledge the support of Director, ICAR-National Rice Research Institute, Cuttack for providing all the necessary facilities including the funding for conducting the experiment.

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Tables

Table 1 Mean values of 15 physiological traits estimated from the panel population containing 96 rice germplasm lines

Sl.No.	VarieAccession No./Name of the germplasm line	E.C.	RI	GI	G	RL	SL	SDW	SVH	SVHI	RRG	RSG	RPE	RGR
1	MNP-AC9005	62.35	0.0079	4.333	48.33	9.47	8.45	0.0120	849.97	0.563	0.803	1.109	0.29	0.077
2	MNP-AC9006	64.95	0.0084	3.917	45.00	7.36	8.612	0.0130	717.36	0.583	0.736	1.227	0.16	0.120
3	MNP-AC9021	32.45	0.0063	5.025	58.33	7.04	7.812	0.0080	865.33	0.477	0.240	1.099	0.23	0.146
4	MNP-AC9028	70.65	0.0077	2.808	30.00	6.85	8.44	0.0210	459.00	0.637	0.923	1.373	0.06	0.114
5	MNP-AC9030	78.85	0.0075	2.892	35.00	5.53	9.29	0.0255	514.27	0.898	0.527	1.767	0.10	0.239
6	MNP-AC9035	48.65	0.0067	2.200	25.00	5.77	9.93	0.0205	397.80	0.515	0.917	1.800	0.14	0.113
7	MNP-AC9038	44.6	0.0086	4.433	53.33	7.87	9.01	0.0190	891.87	1.020	0.903	1.623	0.20	0.276
8	MNP-AC9043	27.95	0.0092	4.708	50.00	6.78	9.28	0.0225	799.87	1.123	0.280	1.363	0.13	0.221
9	MNP-AC9044A	40.95	0.0063	3.875	40.00	8.18	9.07	0.0185	690.00	0.740	1.247	1.103	0.09	0.272
10	MNP-AC9050	34.2	0.0067	1.800	34.00	4.62	3.1	0.0170	261.08	0.564	0.217	0.257	0.25	0.151
11	MNP-AC9053A	38.7	0.0052	0.783	16.00	3.7	2.88	0.0050	105.28	0.080	0.140	0.857	0.27	0.013
12	MNP-AC9058	45.9	0.0062	2.092	38.00	5.29	3.49	0.0250	332.96	0.962	0.237	1.170	0.39	0.115
13	MNP-AC9063	71.4	0.0050	1.833	34.00	6.2	3.45	0.0250	326.12	0.856	0.367	1.190	0.11	0.126
14	MNP-AC9065	52.7	0.0066	2.725	46.00	6.52	4.32	0.0240	498.24	1.094	0.270	0.883	0.38	0.121
15	MNP-AC9076A	52.4	0.0075	0.617	12.00	9.07	5.41	0.0150	173.76	0.180	0.543	0.133	0.58	0.195
16	MNP-AC9090	42.4	0.0064	2.167	38.00	8.18	3.67	0.0275	451.36	1.050	0.477	0.797	0.42	0.147
17	MNP-AC9093	34	0.0062	2.617	44.00	6.19	3.95	0.0295	446.16	1.298	0.457	0.573	0.37	0.150
18	JBS-AC20246	58.8	0.0072	3.208	48.00	5.58	8.26	0.0095	660.96	0.452	0.403	0.893	0.58	0.123
19	JBS-AC20282	78.6	0.0097	4.508	70.00	7.07	9.41	0.0113	1149.28	0.789	0.883	1.150	0.42	0.094
20	JBS-AC20317	50.8	0.0059	5.017	68.00	3.78	4.59	0.0110	569.16	0.442	0.347	0.150	0.33	0.146
21	JBS-AC20328	73.3	0.0073	6.217	86.00	6.21	8.39	0.0135	1255.68	1.161	0.540	1.120	0.34	0.100
22	JBS-AC20362	56.95	0.0063	6.175	84.00	5.61	7.78	0.0105	1129.04	0.886	0.377	0.810	0.55	0.100
23	JBS-AC20371	50.75	0.0075	7.417	94.00	5.74	8.7	0.0140	1356.84	1.318	0.347	1.193	0.19	0.100
24	JBS-AC20389	61.05	0.0062	5.708	80.00	5.57	6.94	0.0115	999.32	0.908	0.513	0.707	0.27	0.109
25	JBS-AC20423	50	0.0071	1.725	32.00	7.67	7.2	0.0100	475.64	0.204	1.090	0.590	0.205	0.142
26	JBS-AC20614	37.75	0.0060	2.475	40.00	4.56	4.73	0.0068	371.20	0.245	0.320	0.193	0.41	0.148
27	JBS-AC20664	52.9	0.0058	0.983	16.00	3.68	3.94	0.0045	121.84	0.078	0.220	0.290	0.43	0.080
28	JBS-AC20686	44.15	0.0041	1.050	16.00	4.91	5.78	0.0030	169.16	0.048	0.147	0.203	0.2	0.159
29	JBS-AC20627	41.45	0.0061	2.667	42.00	4.61	7.1	0.0050	492.52	0.212	0.227	0.700	0.37	0.013
30	JBS-AC20770	44.8	0.0065	3.592	56.00	5.91	9.13	0.0060	842.24	0.336	0.287	1.207	0.25	0.049
31	JBS-AC20845	82.25	0.0094	2.467	42.00	6.78	8.22	0.0107	629.84	0.453	0.510	1.030	0.14	0.101
32	JBS-AC20907	78.45	0.0067	1.733	30.00	4.71	5.67	0.0055	318.60	0.168	0.267	0.523	0.41	0.066
33	JBS-AC20920	55.6	0.0061	3.375	60.00	4.44	8.581	0.0085	771.67	0.504	1.133	0.940	0.26	0.039
34	JBS-AC20347	67.7	0.0076	1.633	28.00	4.04	7.09	0.0045	310.60	0.138	0.563	0.693	0.38	0.130
35	Kantakapura	52.85	0.0063	4.033	70.00	5.23	7.43	0.0075	885.20	0.525	0.447	0.980	0.72	0.121
36	Kantakaamala	52.75	0.0049	3.575	68.00	4.3	6.27	0.0062	713.20	0.419	0.640	0.370	0.67	0.082
37	kapanthi	54.7	0.0054	4.317	76.00	10.02	7.74	0.0090	1349.76	0.684	0.677	1.513	0.54	0.066
38	Karpurkanti	29.35	0.0037	3.717	70.00	4.25	5.21	0.0043	661.12	0.151	0.403	0.707	0.55	0.068
39	Kathidhan	56.65	0.0077	1.983	38.00	7.03	6.74	0.0087	523.20	0.327	0.420	0.447	0.63	0.089
40	Kundadhan	61.25	0.0068	0.867	20.00	4.93	5.72	0.0040	213.92	0.076	0.157	0.777	0.38	0.246
41	Champaesiali	50.8	0.0066	3.308	58.00	9.98	7.24	0.0082	1011.72	0.479	1.113	0.947	0.8	0.104
42	Latamahu	67.5	0.0071	1.367	28.00	4.66	6.77	0.0065	318.48	0.182	0.830	0.323	0.81	0.221

43	Latachaunri	61.4	0.0064	3.458	64.00	5.25	4.74	0.0048	633.20	0.303	0.417	0.237	0.5	0.197
44	Gondiachampeisiali	39	0.0070	3.717	66.00	6.75	8.6	0.0098	1010.48	0.647	0.513	0.910	0.68	0.084
45	Chinamal	47.4	0.0051	2.500	46.00	7.42	6.33	0.0088	637.40	0.411	0.583	0.533	0.41	0.199
46	Magra	38.75	0.0053	1.617	30.00	7.68	5.38	0.0045	390.36	0.138	1.213	0.860	0.09	0.180
47	Landi	38.2	0.0060	2.700	46.00	7.04	7.01	0.0040	672.60	0.184	0.747	0.843	0.65	0.030
48	Lalgundi	63.45	0.0055	2.042	36.00	3.68	5.63	0.0060	334.88	0.054	0.243	0.600	0.68	0.143
49	Balisaralaktimachi-k	53.35	0.0070	2.325	40.00	7.58	7.23	0.0060	579.96	0.244	1.193	0.977	0.51	0.141
50	Laxmibilash	22.7	0.0039	1.108	22.00	5.41	6.24	0.0045	257.00	0.096	0.273	0.377	0.6	0.259
51	Kaniar	38.7	0.0067	3.800	66.00	5.46	8.42	0.0093	918.28	0.619	0.407	0.843	0.56	0.051
52	Kanakchampa	35.5	0.0062	1.092	24.00	5.6	5.97	0.0085	277.68	0.204	0.733	0.457	0.33	0.308
53	Magura-s	35.1	0.0054	1.575	32.00	5.46	7.17	0.0040	400.40	0.136	0.647	1.350	0.45	0.246
54	Adira-1Pallakad	47.4	0.0061	1.617	28.00	6.56	7.15	0.0073	380.12	0.216	0.230	0.887	0.4	0.069
55	Adira-2PallakadR3	56.65	0.0049	5.083	84.00	7.9	7.42	0.0068	1273.20	0.592	0.753	0.890	0.43	0.065
56	Adira-3Pallakad	40	0.0046	2.892	50.00	6.35	5.69	0.0058	602.68	0.293	0.533	0.840	0.21	0.109
57	PK6	44.7	0.0058	2.400	46.00	5.28	3.37	0.0048	400.24	0.178	0.463	0.620	0.15	0.157
58	PK14-Vachaw	44.2	0.0060	4.192	74.00	4.05	2.98	0.0053	523.72	0.316	0.463	0.413	0.19	0.038
59	Pk-16Kozhivalan	40.9	0.0055	3.767	66.00	4.47	4.2	0.0053	571.12	0.355	0.093	0.527	0.31	0.184
60	PK-17Marathondi	50	0.0078	1.408	26.00	4.01	6.17	0.0043	264.20	0.112	0.360	1.067	0.37	0.057
61	PK-18Ezhoml-2	51.6	0.0071	4.192	74.00	3.51	3.5	0.0028	526.84	0.215	0.243	0.677	0.05	0.257
62	PK-19Cheruvirippu	51.5	0.0070	3.242	56.00	3.63	3.71	0.0060	411.04	0.149	0.300	0.503	0.275	0.084
63	Pk-21	48.25	0.0056	5.842	100.00	5.53	5.98	0.0040	1151.00	0.400	0.787	1.613	0.27	0.059
64	PK-22Gandhakasala	36	0.0035	3.342	58.00	3.96	2.91	0.0028	397.76	0.152	0.720	0.670	0.14	0.091
65	PK-23Sreyas	53.25	0.0051	1.792	32.00	3.92	3.42	0.0038	235.36	0.127	0.667	0.707	0.19	0.064
66	PK-24Jyothi	43.55	0.0047	1.500	28.00	3.51	3.26	0.0040	190.40	0.112	0.423	0.737	0.19	0.251
67	PK-25Jaya	41.4	0.0064	3.625	64.00	3.89	3.01	0.0047	442.00	0.300	0.527	0.507	0.24	0.273
68	PK-28Mahamaga	42.7	0.0071	2.350	42.00	2.92	3.07	0.0015	250.08	0.066	0.443	0.617	0.19	0.100
69	PK-33D1	42.3	0.0054	1.725	34.00	2.72	2.67	0.0038	185.36	0.130	0.357	0.483	0.2	0.241
70	Jhagrikartik	24.4	0.0048	1.233	20.00	6.54	3.37	0.0028	202.12	0.057	0.970	0.337	0.53	0.096
71	Dadghani	39.35	0.0051	3.400	50.00	3.29	3.86	0.0028	357.40	0.137	0.110	0.340	0.15	0.096
72	Shayam	36.75	0.0046	5.667	78.00	4.86	3.84	0.0016	676.52	0.127	0.810	0.473	0.24	0.070
73	Basumati-B	26.05	0.0021	5.792	80.00	2.32	3.7	0.0026	481.60	0.100	0.290	0.663	0.29	0.066
74	Bharati	51.4	0.0062	0.842	14.00	4.07	5.32	0.0050	131.64	0.070	0.127	0.747	0.19	0.183
75	Joha	40.05	0.0051	1.050	20.00	2.47	2.7	0.0050	103.60	0.100	0.220	0.280	0.2	0.183
76	Jhingesal	51.2	0.0042	2.142	34.00	5.77	5.12	0.0050	370.44	0.169	0.610	0.937	0.37	0.182
77	Sugandha-2	21.2	0.0028	6.000	84.00	3.75	2.69	0.0025	540.80	0.208	0.457	0.433	0.24	0.029
78	Gochi	53.8	0.0048	1.667	26.00	2.71	3.9	0.0045	171.96	0.120	0.250	0.447	0.09	0.159
79	Uttarbangalocal-9	61.4	0.0056	0.617	10.00	3.38	2.31	0.0045	56.92	0.048	0.200	0.107	0.14	0.159
80	Chatuimuchi	27.7	0.0046	5.900	84.00	3.89	3.36	0.0039	608.80	0.329	0.663	0.527	0.22	0.142
81	Palinadhan-1	32.8	0.0033	0.967	16.00	3.29	3.14	0.0024	102.88	0.038	0.267	0.660	0.28	0.075
82	AC-5993	57.5	0.0072	0.733	16.00	3.13	2.38	0.0155	88.16	0.248	0.260	0.317	0.35	0.347
83	AC-6007	38.45	0.0058	1.517	30.00	5.85	4.88	0.0070	323.28	0.093	0.973	0.880	0.21	0.034
84	AC-6023	48.4	0.0077	0.733	16.00	5.5	3.99	0.0100	151.84	0.160	1.070	0.947	0.38	0.126
85	AC-6027	31.55	0.0054	0.833	18.00	3.94	3.93	0.0075	141.92	0.134	0.553	1.017	0.17	0.241

86	AC-6170	48.7	0.0053	4.300	84.00	3.42	2.92	0.0022	532.16	0.179	0.447	0.577	0.2	0.100
87	AC-6172	66.7	0.0065	4.367	80.00	4.31	4.25	0.0061	684.80	0.490	0.600	0.440	0.21	0.261
88	AC-6183	62.75	0.0048	1.942	36.00	8.85	3.69	0.0055	451.44	0.198	1.617	0.520	0.31	0.186
89	AC-6221	48.75	0.0050	3.742	70.00	3.9	2.99	0.0055	483.56	0.310	0.617	0.667	0.16	0.029
90	AC-7008	82.95	0.0071	0.733	16.00	4.18	3.93	0.0055	129.76	0.088	0.660	0.700	0.11	0.191
91	AC-7134	35.6	0.0058	0.917	20.00	3.87	3.27	0.0075	142.72	0.140	0.687	0.690	0.29	0.233
92	AC-7269	32.45	0.0037	0.833	18.00	3.32	2.96	0.0110	113.00	0.194	0.570	0.640	0.66	0.244
93	AC-7282	49.3	0.0072	0.917	20.00	4.21	4.11	0.0035	166.32	0.074	0.927	0.990	0.35	0.075
94	AC-10162	67.5	0.0070	1.283	28.00	5.12	4.03	0.0033	256.24	0.071	1.067	0.890	0.35	0.073
95	AC-10187	51.85	0.0059	4.292	80.00	8.15	5.1	0.0051	1060.2	0.412	1.917	0.667	0.22	0.157
96	AC-10608	39.4	0.0048	1.958	38.00	4.4	4.03	0.0046	320.51	0.175	0.440	0.850	0.12	0.121
LSD _{5%}		10.14	11.23	10.25	11.15	9.61	10.14	11.92	11.14	11.8	12.25	11.18	10.27	12.16
CV%		11.15	0.0039	1.21	10.14	1.25	1.34	0.0058	1575	0.153	0.305	0.141	0.091	0.095

E.C: E.C (µmhos/cm/10seed); RI: Rate of imbibition; GI: Germination index; GP: Germination %; RL: Root length (cm); SL: Shoot length (cm); SDW: Seedling dry weight (g); SVI-I: Seed Vigour index I; SVI-II: Seed Vigour index II; RRG: Rate of root growth; RSG: Rate of shoot growth; RPE: Rate of plumule elongation; RGR: Relative growth rate.

Table 2 Estimation of genetic diversity parameters based on 109 SSR marker loci in a panel containing 96 rice germplasm lines

Sl. No	Marker	No. of Alleles	Major allele frequency	Gene diversity	Heterozygosity	PIC	f
1	RM5310	4	0.781	0.370	0.042	0.345	0.888
2	RM582	4	0.760	0.400	0.042	0.374	0.897
3	RM13335	4	0.536	0.525	0.010	0.417	0.980
4	RM6275	4	0.740	0.428	0.063	0.400	0.855
5	RM50	4	0.375	0.692	0.031	0.634	0.955
6	RM85	4	0.401	0.690	0.156	0.633	0.776
7	RM222	4	0.672	0.505	0.010	0.465	0.980
8	RM247	5	0.531	0.599	0.083	0.530	0.862
9	RM328	3	0.708	0.448	0.000	0.397	1.000
10	RM337	5	0.448	0.641	0.135	0.573	0.791
11	RM340	5	0.672	0.501	0.115	0.457	0.773
12	RM470	5	0.464	0.692	0.813	0.648	-0.168
13	RM472	3	0.516	0.509	0.115	0.390	0.777
14	RM506	3	0.646	0.494	0.167	0.419	0.665
15	RM1812	3	0.479	0.604	0.000	0.521	1.000
16	RM3701	3	0.656	0.496	0.542	0.432	-0.086
17	RM6947	3	0.875	0.225	0.000	0.210	1.000
18	RM14978	3	0.458	0.573	0.000	0.479	1.000
19	RM18776	3	0.828	0.293	0.031	0.265	0.895
20	RM22034	3	0.958	0.081	0.000	0.079	1.000
21	RM24161	4	0.625	0.530	0.146	0.467	0.727
22	RM223	5	0.651	0.534	0.073	0.498	0.865
23	RM440	5	0.417	0.655	0.292	0.589	0.558
24	RM201	3	0.568	0.559	0.031	0.480	0.945
25	RM216	4	0.547	0.593	0.156	0.527	0.739
26	RM258	3	0.417	0.654	0.000	0.580	1.000
27	RM286	4	0.448	0.655	0.115	0.589	0.827
28	RM3735	4	0.313	0.732	0.990	0.682	-0.347
29	RM1347	3	0.542	0.531	0.000	0.428	1.000
30	RM7571	3	0.880	0.218	0.010	0.207	0.953
31	RM14723	4	0.552	0.597	0.250	0.533	0.585
32	RM103	3	0.500	0.520	0.958	0.405	-0.842
33	RM315	2	0.917	0.153	0.000	0.141	1.000
34	RM225	3	0.552	0.504	0.229	0.387	0.549
35	RM486	3	0.771	0.362	0.063	0.310	0.829
36	RM256	3	0.849	0.262	0.073	0.237	0.724
37	RM1113	3	0.630	0.487	0.073	0.395	0.852
38	RM3423	3	0.500	0.588	0.000	0.504	1.000
39	RM6100	3	0.417	0.656	0.042	0.582	0.937
40	RM590	3	0.677	0.483	0.083	0.428	0.829
41	RM5739	3	0.667	0.500	0.021	0.449	0.959
42	RM405	3	0.833	0.291	0.000	0.270	1.000
43	RM547	5	0.474	0.568	0.177	0.474	0.691

44	RM7364	5	0.771	0.391	0.000	0.373	1.000
45	RM205	3	0.729	0.431	0.021	0.392	0.952
46	RM167	4	0.875	0.228	0.115	0.219	0.502
47	RM229	4	0.354	0.720	0.146	0.667	0.799
48	RM20A	3	0.563	0.582	0.021	0.514	0.965
49	RM235	5	0.401	0.711	0.083	0.661	0.884
50	RM7003	4	0.760	0.388	0.104	0.348	0.734
51	RM4536	4	0.453	0.630	0.073	0.558	0.885
52	RM25181	5	0.474	0.681	0.208	0.633	0.697
53	RM469	3	0.578	0.558	0.052	0.484	0.908
54	RM6547	3	0.844	0.276	0.021	0.258	0.925
55	RM152	4	0.479	0.642	0.021	0.577	0.968
56	RM148	2	0.667	0.444	0.104	0.346	0.768
57	RM421	3	0.438	0.640	0.000	0.564	1.000
58	RM2634	3	0.417	0.653	0.021	0.579	0.968
59	RM248	4	0.333	0.737	0.073	0.688	0.902
60	RM7179	5	0.406	0.710	0.427	0.660	0.403
61	RM215	2	0.615	0.474	0.021	0.362	0.956
62	RM324	3	0.667	0.498	0.198	0.445	0.606
63	RM317	2	0.677	0.437	0.000	0.342	1.000
64	RM174	3	0.625	0.521	0.083	0.451	0.842
65	RM556	2	0.969	0.061	0.042	0.059	0.317
66	RM257	4	0.438	0.630	0.063	0.555	0.902
67	RM502	3	0.854	0.256	0.000	0.236	1.000
68	RM331	4	0.365	0.710	0.073	0.657	0.898
69	RM403	4	0.495	0.638	0.104	0.576	0.838
70	RM309	3	0.693	0.456	0.031	0.395	0.932
71	RM6641	3	0.583	0.573	0.000	0.510	1.000
72	RM3	3	0.385	0.662	0.010	0.588	0.984
73	RM594	3	0.557	0.561	0.010	0.480	0.982
74	RM3392	3	0.521	0.600	0.125	0.525	0.794
75	RM1278	3	0.771	0.378	0.083	0.344	0.781
76	RM168	3	0.625	0.490	0.188	0.397	0.621
77	RM3375	3	0.573	0.568	0.042	0.497	0.927
78	RM282	3	0.677	0.488	0.000	0.438	1.000
79	RM26632	4	0.375	0.701	0.198	0.646	0.720
80	RM1314	3	0.578	0.554	0.031	0.477	0.944
81	RM4112	3	0.568	0.583	0.198	0.518	0.664
82	RM20377	4	0.745	0.402	0.083	0.354	0.795
83	RM210	5	0.333	0.741	0.635	0.694	0.148
84	RM218	4	0.604	0.564	0.042	0.511	0.927
85	RM494	5	0.406	0.713	0.031	0.666	0.957
86	RM336	4	0.406	0.652	0.031	0.586	0.953
87	RM3475	4	0.479	0.604	0.052	0.524	0.915

88	RM480	4	0.630	0.539	0.031	0.487	0.943
89	RM566	4	0.438	0.648	0.021	0.580	0.968
90	RM11701	3	0.646	0.465	0.000	0.366	1.000
91	RM220	6	0.313	0.759	0.229	0.720	0.701
92	RM488	6	0.292	0.752	0.240	0.710	0.684
93	RM6374	6	0.307	0.770	0.094	0.733	0.879
94	RM233	5	0.365	0.713	0.292	0.660	0.594
95	RM112	3	0.854	0.254	0.000	0.231	1.000
96	RM13600	4	0.484	0.658	0.125	0.602	0.812
97	RM495	3	0.625	0.534	0.042	0.472	0.923
98	RM493	7	0.292	0.799	0.698	0.771	0.132
99	RM444	5	0.339	0.761	0.198	0.722	0.742
100	RM468	3	0.724	0.436	0.031	0.394	0.929
101	RM6054	3	0.917	0.156	0.021	0.150	0.868
102	RM509	3	0.813	0.314	0.000	0.281	1.000
103	RM5638	5	0.693	0.485	0.167	0.450	0.659
104	RM8044	6	0.349	0.764	0.281	0.728	0.635
105	RM8271	5	0.411	0.704	0.167	0.654	0.766
106	RM171	4	0.469	0.653	0.073	0.592	0.889
107	RM16686	3	0.396	0.655	0.000	0.581	1.000
108	RM434	4	0.479	0.650	0.031	0.587	0.952
109	RM6091	4	0.823	0.311	0.000	0.294	1.000
	Mean	3.706	0.578	0.533	0.117	0.477	0.783

Table 3 The population structure group based on the inferred ancestry value of the germplasm lines present in the panel population for 15 physiological parameters

Sl. No.	Accession No./ Vernacular name of germplasm line	Inferred ancestry value at K=6							physiological parameters with very high/high estimates in the genotype
		Q1	Q2	Q3	Q4	Q5	Q6	Group	
1	ARS-AC-5993	0.678	0.006	0.308	0.003	0.003	0.002	Admix	EC, RI, GI, RL, SL, SVII, RRG, RSG, AGR, MGR
2	ARS-AC-6221	0.989	0.002	0.003	0.002	0.002	0.001	SP1	EC, RI, GI, RL, SL, SVH, RRG, RSG, RGR, AGR,
3	ARS-AC-6183	0.869	0.004	0.118	0.002	0.004	0.002	SP1	RI, GI, RL,SL, SVH, RSG, RGR, AGR, MGR
4	ARS-AC-6170	0.539	0.003	0.443	0.004	0.008	0.001	Admix	EC, RI, SL,SDW, RRG, RSG, RGR, AGR, MGR
5	ARS-AC-6023	0.01	0.005	0.978	0.001	0.005	0.001	SP3	EC, RI, SL, SDW, SVH-II, RSG, RGR, AGR, MGR
6	ARS-AC-6172	0.038	0.05	0.902	0.002	0.006	0.002	SP3	RI,SL,SDW, RRG, RSG,RGR, AGR,MGR
7	ARS-AC-6027	0.004	0.003	0.087	0.002	0.003	0.902	SP6	RI,GI,RL,SL,SDW,SVH,SVH-II, RRG, RSG, RGR, AGR,MGR
8	ARS-AC-6007	0.024	0.004	0.957	0.008	0.005	0.002	SP3	RI,GI,SL,SDW,SVH,SVH-II, RSG, RGR, AGR,MGR
9	MNP-AC-9006	0.066	0.009	0.729	0.023	0.166	0.007	Admix	RI,GI,RL,SL,SDW,SVH-II, RRG, RSG, RGR, AGR,MGR
10	MNP-AC-9021	0.005	0.021	0.961	0.004	0.009	0.002	SP3	RI,SDW, RGR
11	MNP-AC-9028	0.003	0.005	0.943	0.005	0.041	0.003	SP3	Low for majority parameter
12	MNP-AC-9030	0.042	0.008	0.929	0.002	0.018	0.001	SP3	RI,SDW,SVH-II, RSG, RGR, AGR,MGR
13	MNP-AC-9035	0.059	0.016	0.671	0.033	0.205	0.014	Admix	EC, SDW, SVH-II, RSG, RGR, AGR,MGR
14	MNP-AC-9038	0.019	0.022	0.799	0.003	0.154	0.002	SP3	RI,SDW,SVH-II,RGR,AGR,MGR
15	MNP-AC-9043	0.108	0.012	0.14	0.007	0.732	0.001	Admix	RI,RL,SDW,RPE,RGR
16	MNP-AC-9044A	0.011	0.003	0.284	0.005	0.694	0.003	Admix	RI,RL,SDW,SVH-II,RGR,AGR,MGR
17	JBS-AC-20920	0.216	0.749	0.017	0.002	0.008	0.008	Admix	RI,SDW,SVH-II,RGR,AGR,MGR
18	JBS-AC-20907	0.652	0.242	0.013	0.003	0.089	0.001	Admix	RI,GI,SL,RPE,RGR,AGR,MGR
19	JBS-AC-20845	0.132	0.843	0.016	0.002	0.006	0.001	SP2	EC,RI,GI, G,RL,SL,SVH,SVH-II,RRG,RSG,AGR,MGR
20	JBS-AC-20770	0.026	0.018	0.692	0.004	0.253	0.007	Admix	GI,RGR,MGR
21	JBS-AC-20627	0.004	0.043	0.004	0.003	0.945	0.001	SP5	EC,RI,GI,G,SL,SVH,SVH-II,RSG,AGR,MGR
22	JBS-AC-20686	0.302	0.034	0.004	0.002	0.656	0.002	Admix	RI, GI, G,SL,SVH,SVH-II,RPE,AGR,MGR
23	JBS-AC-20664	0.009	0.967	0.008	0.003	0.011	0.001	SP2	RI, GI, G,SL,SVH,SVH-II,RSG,AGR,MGR
24	JBS-AC-20614	0.009	0.09	0.004	0.012	0.882	0.002	SP5	EC,RI,GI, G,SVH,SVH-II,RGR,AGR,MGR
25	MP-Jhagrikartik	0.643	0.177	0.01	0.001	0.166	0.002	Admix	RI,RL,SL,RRG,RGR,AGR
26	MP-Dadghani	0.28	0.02	0.687	0.003	0.008	0.002	Admix	Low for majority parameter
27	MP-Shayam	0.011	0.002	0.002	0.004	0.002	0.978	SP6	Low for majority parameter
28	MP-Basumati-B	0.01	0.003	0.006	0.301	0.006	0.673	Admix	RGR
29	MP-Bharati	0.549	0.326	0.004	0.003	0.116	0.002	Admix	RI,SL,
30	MP-Joha	0.819	0.143	0.007	0.009	0.021	0.002	SP1	RI,GI,SL,SVH,II,RSG,AGR
31	KE-Adira-1-Pallakad	0.026	0.005	0.064	0.885	0.015	0.005	SP4	EC,RI,SL,RSG,RGR,AGR
32	KE-Adira-2-Pallakad-R3	0.816	0.048	0.076	0.004	0.054	0.001	SP1	EC,RI
33	KE-Adira-3-Pallakad	0.024	0.016	0.015	0.915	0.023	0.006	SP4	RI,GI,SL,SVH,II,RRG,AGR
34	KE-PK6	0.836	0.002	0.007	0.144	0.006	0.004	SP1	EC,RI,SL,RGR,AGR
35	KE-PK14-Vachaw	0.961	0.009	0.008	0.003	0.007	0.013	SP1	RI,GI,SL,SVH,II,RPE,RGR,AGR
36	KE-PK16-Kozhivalan	0.616	0.003	0.369	0.002	0.009	0.001	Admix	GI, SVH,II,RPE,AGR
37	KE-PK17-Marathondi	0.002	0.006	0.002	0.98	0.002	0.009	SP4	GI,G,RL,SL, SVH,II,RSG,RPE,AGR
38	KE-PK18-Ezhoml-2	0.968	0.014	0.007	0.004	0.005	0.002	SP1	GI,G,RPE,AGR
39	KE-PK24-Jyothi	0.912	0.015	0.026	0.022	0.023	0.001	SP1	RI,RL,RPE
40	OD-Kantakopura	0.065	0.382	0.006	0.064	0.481	0.002	Admix	EC,RI,RGR
41	OD-Kantakaamala	0.011	0.137	0.016	0.813	0.022	0.002	SP4	RI,GI,RL, SL, SVH,II,RRG,RPE,RGR,AGR

42	OD-Kapanthi	0.002	0.001	0.001	0.993	0.002	0.002	SP4	EC,RI,RRG, RPE,RGR,AGR
43	OD-Karpurkanti	0.001	0.025	0.001	0.033	0.002	0.937	SP6	EC,RI, GI,RPE,RGR
44	OD-Kathidhan	0.369	0.541	0.028	0.004	0.046	0.012	Admix	RI,GI,SL, SVH, RPE, AGR
45	OD-Kundadhan	0.011	0.973	0.006	0.004	0.005	0.001	SP2	RL,RGR, AGR
46	OD-Champaeisiali	0.017	0.942	0.008	0.011	0.021	0.002	SP2	RL, RRG, RGR, AGR
47	OD-Latamahu	0.038	0.928	0.01	0.012	0.009	0.002	SP2	RL,SL,RRG,RPE,AGR
48	OD-Latachaunri	0.04	0.94	0.005	0.006	0.008	0.002	SP2	EC,RPE,RGR
49	ARS-AC-10608	0.984	0.005	0.004	0.002	0.005	0.001	SP1	RI,RL,SL,RRG ,RPE,RGR,AGR
50	ARS-AC-10187	0.57	0.006	0.117	0.002	0.304	0.002	Admix	RPE,RGR
51	ARS-AC-10162	0.648	0.006	0.243	0.025	0.03	0.048	Admix	GI,SL, SVH,I,RPE, AGR
52	ARS-AC-7282	0.002	0.001	0.002	0.001	0.002	0.992	SP6	RI,RRG,RGR,AGR
53	ARS-AC-7269	0.01	0.003	0.979	0.002	0.006	0.001	SP3	SL,RSG, RGR,AGR
54	ARS-AC-7134	0.01	0.013	0.739	0.016	0.015	0.208	Admix	RI,SL, AGR
55	ARS-AC-7008	0.184	0.07	0.157	0.002	0.586	0.001	Admix	GI,G,RL,SL,SVH,I,RRG,AGR
56	MNP-AC-9093	0.023	0.009	0.782	0.002	0.18	0.004	Admix	RGR,AGR
57	MNP-AC-9090	0.007	0.009	0.796	0.014	0.153	0.021	Admix	RGR,AGR
58	MNP-AC-9076A	0.008	0.407	0.553	0.003	0.027	0.001	Admix	GI,G,
59	MNP-AC-9065	0.008	0.009	0.401	0.069	0.5	0.012	Admix	GI,RGR
60	MNP-AC-9063	0.115	0.595	0.128	0.017	0.144	0.001	Admix	RI,RSG, AGR
61	MNP-AC-9058	0.012	0.012	0.78	0.002	0.192	0.001	Admix	RI,GI,G, RGR
62	MNP-AC-9053A	0.004	0.005	0.073	0.012	0.879	0.028	SP5	RI,GI
63	MNP-AC-9050	0.017	0.024	0.016	0.027	0.913	0.003	SP5	GI,G, SVH, RRG, RSG, AGR
64	MNP-AC-9005	0.091	0.005	0.087	0.007	0.808	0.002	SP5	GI, RRG, AGR
65	JBS-AC-20389	0.017	0.006	0.6	0.23	0.146	0.002	Admix	AGR
66	JBS-AC-20371	0.065	0.01	0.088	0.002	0.833	0.002	SP5	RGR, AGR,MGR
67	JBS-AC-20423	0.018	0.02	0.079	0.003	0.88	0.001	SP5	RI,GI,RGR, AGR
68	JBS-AC-20362	0.084	0.014	0.018	0.006	0.873	0.006	SP5	RI,AGR
69	JBS-AC-20328	0.007	0.005	0.007	0.002	0.974	0.005	SP5	RGR
70	JBS-AC-20317	0.008	0.01	0.004	0.002	0.972	0.004	SP5	RRG, RPE, AGR
71	JBS-AC-20282	0.014	0.265	0.008	0.198	0.512	0.003	Admix	GI
72	JBS-AC-20246	0.019	0.006	0.005	0.165	0.787	0.018	Admix	GI,G, RRG, AGR,MGR
73	JBS-AC-20347	0.32	0.063	0.568	0.034	0.012	0.002	Admix	GI,G, MGR
74	MP-Palinadhan-1	0.002	0.003	0.058	0.81	0.018	0.107	SP4	RI,RGR
75	MP-Chatuimuchi	0.001	0.001	0.001	0.005	0.001	0.99	SP6	RGR, MGR
76	MP-Uttarbangalocal-9	0.12	0.124	0.528	0.212	0.015	0.001	Admix	RGR, AGR
77	MP-Gochi	0.3	0.011	0.14	0.166	0.382	0.002	Admix	GI,G, MGR
78	MP-Sugandha-2	0.001	0.001	0.002	0.002	0.002	0.991	SP6	RGR
79	MP-Jhingesal	0.117	0.846	0.023	0.003	0.01	0.001	SP2	EC, RGR
80	KE-PK-19-Cheruvirippu	0.009	0.019	0.961	0.004	0.006	0.001	SP3	GI,G, RGR, AGR,MGR
81	KE-PK-28-Mahamaga	0.003	0.156	0.794	0.026	0.02	0.001	SP3	Low for majority parameter
82	KE-PK-25-Jaya	0.007	0.006	0.98	0.003	0.004	0.001	SP3	RI,SDW,RGR
83	KE-PK-33-D1	0.02	0.003	0.766	0.204	0.004	0.003	Admix	RRG, AGR
84	KE-PK-21	0.011	0.187	0.774	0.02	0.007	0.001	Admix	RI, RRG, RGR, AGR
85	KE-PK-22-Gandhakasala	0.002	0.016	0.002	0.002	0.003	0.975	SP6	RSG, RGR, AGR

86	KE-PK-23-Sreyas	0.41	0.048	0.492	0.015	0.032	0.003	Admix	GI, G, AGR,MGR
87	OD-Gondiachampeisiali	0.007	0.949	0.018	0.008	0.016	0.002	SP2	EC,RI,GI, G, RGR, AGR
88	OD-Chinamal	0.008	0.614	0.28	0.003	0.087	0.009	Admix	EC, RL, RRG, RGR, AGR,MGR
89	OD-Magra	0.006	0.69	0.285	0.009	0.006	0.003	Admix	GI, AGR
90	OD-Landi	0.005	0.982	0.006	0.002	0.004	0.001	SP2	EC,RI, RGR, AGR
91	OD-Lalgundi	0.002	0.982	0.004	0.003	0.006	0.004	SP2	RGR, AGR
92	OD-Balisaralaktimachi-K	0.002	0.984	0.003	0.003	0.006	0.002	SP2	RPE, RGR, AGR
93	OD-Laxmibilash	0.011	0.399	0.008	0.015	0.008	0.558	Admix	RI, RRG, AGR
94	OD-Kanar	0.006	0.973	0.011	0.002	0.004	0.004	SP2	EC,RI, RRG, AGR
95	OD-Kanakchampa	0.068	0.879	0.023	0.011	0.014	0.005	SP2	GI, G,RL, SVI-I, RRG, RGR, AGR,MGR
96	OD-Magura-S	0.003	0.957	0.003	0.005	0.007	0.025	SP2	RGR, AGR

Table 4 Analysis of molecular variance (AMOVA) of the sub-populations present in the panel population at K=6 for 15 physiological parameters in 96 rice germplasm lines

Source	df	SS	MS	Est. Var.	%
Among Pops	6	820.641	136.774	3.616	12%
Among Indiv	89	4225.400	47.476	20.553	67%
Within Indiv	96	611.500	6.370	6.370	21%
Total	191	5657.542		30.593	100%
F-Statistics	Value	P(rand >= data)			
Fst	0.118	0.001			
Fis	0.763	0.001			
Fit	0.791	0.001			
Fst max	0.509				
F'st	0.233				
Nm	1.861				

Table 5 Marker alleles association with physiological parameters detected by both the models of GLM and MLM analyses at p<0.01 rice germplasm lines present in the panel.

SL.No	Traits	Marker	Position (cM)	GLM				MLM			
				Marker_F	Marker_p	Marker_R ²	q-value	Marker_F	Marker_p	Marker_R ²	q-value
1	RI	RM248	155	8.98583	0.00353	0.07563	0.005814	7.17908	0.00879	0.07053	0.009116
2	GI	RM225	92	13.59483	3.90E-04	0.12244	0.001041	12.50148	6.48E-04	0.12881	0.004536
3	GI	RM229	125	7.37835	0.00793	0.07074	0.008369	7.28108	0.00834	0.07502	0.009116
4	GI	RM20A	127	8.03947	0.00566	0.07655	0.007773	7.22205	0.00859	0.07442	0.009116
5	GI	RM502	177	29.01418	5.81E-07	0.22718	1.63E-05	16.95205	8.56E-05	0.17467	0.001198
6	GI	RM5638	284	14.91459	2.13E-04	0.13263	0.001041	8.7923	0.00388	0.09059	0.00776
7	GP	RM225	92	13.74601	3.63E-04	0.12756	0.001041	10.84335	0.00142	0.11297	0.005507
8	GP	RM502	177	18.43784	4.46E-05	0.16363	0.000416	10.41549	0.00175	0.10851	0.005507
9	RL	RM229	125	13.55245	3.97E-04	0.11864	0.001041	9.59904	0.00261	0.09878	0.006644
10	SVI-1	RM5638	284	15.51412	1.62E-04	0.14194	0.001041	10.39564	0.00177	0.11096	0.005507
11	SVI-2	RM14723	86	7.52465	0.00736	0.06014	0.008369	8.94579	0.0036	0.09572	0.00776
12	SVI-2	RM7003	132	7.34405	0.00807	0.05881	0.008369	9.70644	0.00247	0.10386	0.006644
13	SVI-2	RM5638	284	7.98095	0.00583	0.06349	0.007773	8.84629	0.00378	0.09466	0.00776
14	RRG	RM256	97	13.93515	3.33E-04	0.12877	0.001041	8.11243	0.00546	0.08683	0.009116
15	RSG	RM3701	45	14.75164	2.29E-04	0.14083	0.001041	7.96593	0.00588	0.08679	0.009116
16	RSG	RM7003	132	7.26461	0.00841	0.07475	0.00841	12.99803	5.14E-04	0.14162	0.009116
17	RSG	RM6547	145	7.56795	0.0072	0.07763	0.008369	7.27751	0.00835	0.07929	0.004536
18	RPE	RM403	183	11.73481	9.30E-04	0.08487	0.00186	7.46605	0.00758	0.06992	0.009116
19	RPE	RM220	242	20.617	1.75E-05	0.13702	0.000245	17.75344	6.01E-05	0.16625	0.001198
20	RGR	RM3701	55	7.84584	0.00625	0.07272	0.007955	7.59213	0.00711	0.07971	0.009116
21	RGR	RM468	275	8.73351	0.004	0.08021	0.006222	7.49192	0.00748	0.07866	0.009116
22	AGR	RM256	97	9.3763	0.00291	0.09252	0.005093	6.94416	0.00992	0.07508	0.00992
23	MGR	RM201	68	12.23415	7.35E-04	0.09561	0.001583	7.9956	0.00579	0.08063	0.009116
24	MGR	RM502	177	8.07998	0.00555	0.06585	0.007773	10.92039	0.00137	0.11013	0.005507
25	MGR	RM220	240	12.40579	6.78E-04	0.09679	0.001582	10.73664	0.0015	0.10828	0.005507

Figures

Figure 1

Frequency distribution of germplasm lines for each of the physiological parameters for (A) EC, RI, GI, GP and RL (B) SL, SDW, SVI-I, SVI-II and RRG, and (C) RSG, RPE, RGR, AGR and GR present in 250 germplasm lines.

Figure 2

Frequency distribution of germplasm lines in the panel population for (A) Spider graph based on seed vigour index-I and (B) Based on 15 physiological parameters. VH: very high; H: high; M: medium; L: low. Classification of each trait into distinct group (VH, H, M, and L) is represented by proportion of colour.

Figure 3

Genotype-by-trait bi-plot diagram showing 96 germplasm lines in two PCs for 15 physiological traits. E.C: E.C ($\mu\text{mhos/cm/10seed}$); RI: Rate of imbibition; GI: Germination index; G: Germination %; RL: Root length (cm); SL: Shoot length (cm); SDW: Seedling dry weight (g); SVI-I: Seed vigour index I; SVI-II: Seed vigour index II; RRG: Rate of root growth; RSG: Rate of shoot growth; RPE: Rate of plumule elongation; RGR: Relative growth rate. The dot numbers in the diagram depict the serial number of the germplasm line listed in Table 1.

Figure 4

Heat map showing Pearson's correlation coefficients for physiological traits. Significant correlations are colored either in red (negative) or blue (positive). Shades of blue indicate increasing positive correlation coefficient; shades of red indicate increasing negative correlation coefficient. EC: electrical conductivity; RI: rate of imbibition; G: germination %; RL: root length; SL: shoot length; 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; RPE: rate of plumule elongation; RGR: rate of growth rate; AGR: absolute growth rate and GR: mean germination rate.

Figure 5

A) Graph of ΔK value, to the rate of change in the log probability of data between successive K values; B) Population structure of the 96-panel population placed based on membership probability fractions of individual genotypes at K = 2, c) Population structure of the panel population at K = 6. The genotypes with the probability of $\geq 80\%$ membership proportions were assigned as subgroups while others grouped as admixture group. The numbers in the diagram depict the serial number of the germplasm lines listed in Table 1.

Figure 6

The physical distance (base pairs, bp) between pairs of loci on chromosomes against linkage disequilibrium (LD) decay (r^2) curve plotted in rice. The decay started in million bp estimated by taking 95th percentile of the distribution of r^2 for all unlinked loci.

Figure 7

Principal coordinate analysis (PCoA) of 96 genotypes in the panel population for 15 physiological traits using 109 molecular markers. The dot numbers in the figure represent the serial number of the genotypes enlisted in Table 1. The numbers are coloured on the basis of sub-populations obtained from structure analysis (SP1-Orange; SP2-Pink; SP3-Brown; SP4-Green; SP5-Red; SP6-Light Blue; admix type-Black).

Figure 8
Unrooted tree using unweighted-neighbour joining method depicting clustering pattern of 96 germplasm lines genotyped by 109 SSR markers coloured on the basis of (A) Fifteen physiological parameters, (B) sub-populations obtained from structure analysis at K=6 (SP1-Pink; SP2-Green; SP3-Red; SP4-Brown; SP5-Light Blue; SP6-Violet; admix type-Black).

Figure 9

Distribution of marker-trait association and quantile–quantile (Q-Q) plot generated from Generalized Linear Model analysis for 15 physiological parameters at (A) $p < 0.05$ and (B) at $p < 0.01$.

Supplementary Files

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- [SupplementaryFig.2.tif](#)
- [SupplementaryTable1.xlsx](#)
- [SupplementaryTable2.docx](#)
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