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RESEARCH ARTICLE

Identification and analysis of low light tolerant rice genotypes in field conditions and their SSR-based diversity in various abiotic stress tolerant lines

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Abstract. The yield potentiality of kharif rice is not completely used even under well-irrigated agro-ecosystem, mainly due to low irradiance by overcast cloud throughout the growing season in eastern India. We observed more than 50% yield reduction compared to the performance of 100 high-yield genotypes for consecutive three years both under open and 30–35% reduced light intensity, mainly by 34%, 25% and 12% reduction of panicle number, grains per panicle and test weight. As per the analysis of variance, genotypic variance explained 39% of the total yield-variation under shade with 58% heritability. Overall, the maintenance of equal panicle per plant in both open and shade has the highest association with shade tolerance. Purnendu, Sashi and Pantdhan19 showed less than 28% yield-reduction by maintenance or even by increasing grain numbers under shade and test weight. On the other hand, maintenance of an equal number of panicle under both situations was the key to the tolerance of Bhasamanik, Sasarang, Rudra and Swarnaprabha. As compared to open, we noticed the improvement of chlorophyll a and b under shade but saw a poor correlation with the shade tolerance index. Comparing the net photosynthesis rate (P_n) in eight genotypes, we found the best tolerant line ranked last with least P_n at low light intensity (<400 μmol m^{-2} s^{-1}). We also identified diverse parental combinations between newly identified shade tolerant and abiotic stress tolerant high-yielding rice lines following diversity analysis using 54 simple-sequence repeats. Thus, the selected tolerant lines from a large set of genotypes with different adjustment ability to keep up high yield under low light intensity can be used for physiological, molecular analysis as well as pyramiding of traits.

Keywords. shade-tolerance; panicle number; rice; diversity.

Introduction

In India and South Asia, rice is cultivated mainly in rainy season (kharif rice) when it receives significantly less irradiation (below 500 $\mu m/m^{-1}/s^{-1}$) due to the overcast cloud throughout the growing season. Irradiance reduces further according to location and solar movement within a day. Low irradiance reduces the photosynthesis vis-a-vis dry weight, as rice exhibits optimum net photosynthesis at the range of 800–1000 $\mu m/m^{-1}/s^{-1}$ (Murchie *et al.* 2002; Kasajima *et al.* 2011). Depending on the stage of rice, low light intensity can reduce 34–55% grain-yield (Janardhan *et al.* 1980; Nayak and Murty 1980; Voleti and Singh 1996; Dutta *et al.* 2018) by limiting tiller number, dry weight (Venkateswarlu 1996; Singh 2005), spikelet number (Adhya *et al.* 2008), test weight (Voleti and Singh 1996; Jiao and Li 2001). It also hampers the quality (Wang *et al.* 2013, Liu *et al.* 2014) of rice. Genotypes with higher net photosynthesis rate with less reduction of test weight are identified behind the shade tolerance mechanism (Liu *et al.* 2014). Although, several works indicated genes and pathways related to photoprotection vis-a-vis photoinhibition might have a role in improving yield under higher light intensity (Jiao and Li 2001; Kasajima *et al.* 2011), there is no such report on the

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mechanism behind acclimatization for insufficient light intensity except one (Dutta *et al.* 2018). It is also suggested that autophagy recycling of chloroplast and root plastid has some role in nutrient remobilization for growing organs or grain filling in rice during energy limitation (Izumi *et al.* 2015). Shading, either at vegetative or grain filling stage enhances the leaf area as well as chlorophyll a and b content, eventually, enhanced synthesis of chlorophyll a and b (under shade) associated with higher net photosynthesis rate help in augmenting yield (Liu *et al.* 2014; Janardhan and Murty 1980). On the other hand, several reports indicated that mechanism of high yielding ability under shade varies according to varieties (Janardhan and Murty 1980; Nayak and Murty 1980). In summary, tolerant varieties adjust the low irradiance by (i) improving net photosynthesis rate with stronger antioxidant activities and higher amount of chlorophyll a and b; (ii) better translocation efficiency from source organ to sink organs vis-a-vis prevents impaired sink size. As variety specific tolerance mechanism is reported earlier screening of a large set of genotypes in field will be useful in identification of tolerance genotypes with diverse mechanism of low light tolerance which will finally help in pyramiding of target traits. Another aim of this study was to identify diverse tolerant lines relative to existing high yielding varieties and other abiotic stress tolerant lines so that parental combination can be selected easily for the introgression of the traits.

Therefore, 300 genotypes were screened initially for one year and finally 100 high yielding lines were assessed for consecutive three years in the field, both under incidental light (open) and low light for the selection of stable shade tolerant lines. Studied genotypes are mainly landraces or landrace-derived varieties of the east and northeast states of India where irradiation in kharif season is below the average of the rest of the country. We estimated percentage of variation controlled by genotypes as well as correlation between shade tolerance index with yield attributing and other associated index. To find the diverse crossing combinations, simple-sequence repeat (SSR)-based diversity analysis was performed between a set of abiotic stress tolerance lines with newly identified low light tolerant lines.

Materials and methods

Plant materials

Three-hundred rice genotypes, predominantly landraces or its derived lines, collected from eastern and northeast India were grown in university experimental field (latitude 22.87° and longitude 88.20) for the first year (2015). Based on the high yielding ability under open field, 100 genotypes were selected and grown further for consecutive three kharif seasons (2016, 2017 and 2018). Transplanting was made on 15 July of each year at 20 x 15 cm with single seedling per hill. Sixty kg/ha N, 30 kg/ha P and 40 kg/ha K were applied before transplanting.

Screening for shade tolerant genotypes

Three rows (5-m long) of each 300 rice genotypes (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/), were grown both under white shade net (30–35% light-cut) and open conditions following 'augmented design'. We segregated experimental plot into 12 blocks each with 25 genotypes keeping IR64, Swarnaprabha and Sasarang as the check varieties in each block. IR64 is a popular cultivar whereas Swarnaprabha is reported as low light tolerance line and Sasarang is a popular cultivar of Meghalaya. Mean values of each genotype under each block was adjusted after calculating the adjusted value in 12 blocks from three check varieties. We classified all the genotypes based on their height and days to 50% flowering and transplanting (not nursery type direct seedling) was done accordingly, both under open and shade conditions. By this way we had planned to reduce mutual shading by tall variety next to dwarf. Shade was provided at the height of 3 m, keeping 1.5 m open from the ground so that sufficient air can flow (figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). In both situations (open and shade-net) photosynthetically active radiation (PAR), temperature and relative humidity was measured everyday at 9 h, 13 h and 16 h during the period, transplanting to maturity, by portable automatic weather station (table 2 in electronic supplementary material). No significant difference in relative humidity and temperature was observed between open and shade during growing period. Based on the adjusted mean of 300 lines in first year, we grew 100 genotypes (table 3 in electronic supplementary material) for consecutive three years with three rows for each genotypes planted in three replicated randomized block design. A replication plot consisted of three rows with 4-m long and three such plots were considered for each genotype in the randomized fashion. No extra spacing was allowed between two replications of two genotypes, i.e. 25 cm normal spacing between both rows and genotypes and 20 cm between two hills within a row. Five randomly selected plants from each row were used for taking observations, like, number of panicles per plant (PN), dry weights (50 days after transplanting) (DW), days to 50% flowering (DFF) and grain yield per plant (YP). In each year, the observations were recorded from five plants per genotype from each rows (15 plants/plot). Yield-related traits were recorded at maturity. For yield data, mature seeds were collected, oven-dried for three days at 45°C and then weighed with average values expressed on per plant basis. DFF were recorded when 50% of the plants in a row started flowering. Thousand randomly selected filled grains in three replications were used for 1000 grain weight by using simple weighing balance.

Chlorophyll and P_n estimation

During heading, flag leaves in three replications were collected from 10 am to 11 am, and used to estimate chlorophyll a and b. The chlorophyll content was calculated following the equations proposed by Barnes *et al.* (1992). Rice leaf tissues (100 mg) were placed in a graduated tube containing 25 mL of 80% buffered acetone (80 mL of acetone made up to 100 mL with 20 mL of 2.5 mM sodium phosphate buffer, pH 7.8) in refrigerator at 4°C and the chlorophyll was extracted without grinding and centrifugation. The spectrophotometer was calibrated to zero absorbance both at 663 nm and 645 nm using a blank of 80% acetone.

Flag leaves were considered for the estimation of net photosynthesis rate (P_n) at the panicle initiation stage (within two days of emergence) using portable photosynthesis analyser Li6800 of LiCor, USA, using various intensity of light ranging from 50 to 900 μm photon m^{-2} s^{-1}. Observations were taken from five places of each leaf of five randomly selected plants.

Diversity analysis

Genomic DNA was extracted from the selected 50 rice genotypes (table 5 in electronic supplementary material) which were selected based on their high yielding and stress tolerance abilities. Genomic DNA were utilized for PCR reactions to amplify with 54 SSR primers of rice (*Oryza sativa*) following methods as described earlier (Chattopadhyay *et al.* 2008) to assess polymorphism and diversity analysis. In brief, 25 μL PCR reaction mixture contained 20 ng of genomic DNA, 1 U of *Taq* DNA polymerase (Promega), 0.25 mM dNTPs and 1.5 mM $MgCl_2$ and 10 pmol of the forward and reverse primers. PCR reactions were performed with a Thermal Cycler (Applied Biosystem) and amplified products were separated in 2.0–2.5% agarose gel. Amplified bands were scored as present (1) and absent (0) in the binary data matrix as well as size of the allele. The resultant data matrix was used to calculate genetic similarity based on Nei's genetic distance. Dendrogram displaying relationships among 50 genotypes was constructed using the unweighted pair group method with arithmetic mean (UPGMA) and neighbour-joining (NJoin) random module of the NTSYS-pc. The polymorphism information content (PIC), number of alleles, unique alleles, low frequency allele, high frequency allele of each SSRs were calculated following POWER marker software.

Data analysis

Mean value of consecutive three years' data as well as proportion of performance under low light and open expressed as index were considered for PCA, Pearson correlation and two-tailed *t*-test analysis using XLSTAT 2019. Two-way ANOVA was made using Graph Pad Prism 6. Stable genotypes were identified based on stability analysis (Eberhart and Russel Model 1966) and as per model, stable genotypes were those where mean square deviation from regression line (S^2_{di}) not significantly (at 5% level) different from zero.

Results

Photosynthetic active radiation (PAR) was recorded daily at 9th h, 13th h and 16th h in both conditions. Plants grown under shade-net received almost 35–40% less solar irradiance than that of open conditions, thus crop did not suffer high light stress (table 1 in electronic supplementary material). Also no significant deviation was recorded for temperature and relative humidity in both conditions. Mean yield and panicle number of each genotype was adjusted considering the block adjusted value for both the parameters in each of the 12 blocks after analysis of three check varieties (table 2 in electronic supplementary material). Hundred high yielding genotypes were selected for growing consecutive three years for analysis of yield attributing and other parameters. Index is calculated as the ratio of performance under shade by open. The mean value of per-plant yield (PY) under low light intensity was 8.19 g compared to the open 16.81 g with more than 50% reduction whereas 38%, 34% and 22% reduction of panicle number (PN), grain number (GN) and test weight (TW) were observed in the same condition (table 3 in electronic supplementary material). Under shade, 39% and 19.7% of the total yield-variation is controlled by genotypes and years respectively where as 71% and 2% under open grown plants (table 1), eventually, heritability (broad sense) of the yield under shade is low (58% vs 83%) relative to open.

Minimum, maximum, mean for open and shade grown plants along with correlation (r) between open and shade are given in figure 1. GN, TW and DW showed significant correlation between open and shade and no correlation was observed for PY, PN and Chla/b. Purnendu showed best tolerance ability (STI, 0.79) but Pantdhan19 yielded highest, 15.8 g. Purnendu, Sashi and Pantdhan19 showed less yield

Table 1. Heritability and ANOVA for the estimation of percentage of total yield-variation controlled by genotype and year.

	Open		Shade	
Source of variation	Per cent of total variation	*P* value	Per cent of total variation	*P* value
Genotypes	70.95	< 0.0001	39.08	< 0.0001
Years	2.161	0.0005	19.72	< 0.0001
Heritability	81%		58%	

reduction by maintaining or even by increasing grain numbers under shade and test weight (table 2). On the other hand, maintenance of equivalent number of panicle under both conditions was the key for the tolerance of Bhasamanik, Sasarang, Rudra and Swarnaprabha. Both open and shade grown plants showed wide range of variation both for chlorophyll a and b with mean increase of 12% Chla/b under shade. This study observed 34% reduced mean dry weight ranging from 32 to 90% where Sasarang, Rudra and Purnendu limited less than 20% reduction under shade.

Correlation and PCA analysis

Index (I), as calculated from the ratio of shade by open for all six parameters were used in correlation and PCA analysis. Minimum, maximum, mean and SD are given in table 3. Less PN reduction under shade showed high positive correlation ($r = 0.602$) with yield index, although PN is negatively (-0.56) correlated with yield under open condition.

PCA identifies the mutual relationship between characters and help us to decide the important characters explaining maximum contribution towards variation and related genotypes for those contributing character. Three eigen-vectors with near one eigen value (table 4 in electronic supplementary material) explain almost 70% of the total variation. First component explains 35% variability and STI and PNI, have the major contribution with their positive association. The second component explains around 19% variability, mainly contributed by GNI, TWI and DWI. GNI and SWI are negatively associated with DWI and PNI. Chla/b is the major variable of third eigen vector but not associated with any other character. Tolerant genotypes like Purnendu, Sashi, Mahananda, Pantdhan19 are equidistance position with the variables GNI, TWI, DW and PNI, implied that these genotypes showed yield reduction mainly restricting reduction of all four parameters, GN, PN, TW and DW uniformly. Maintenance of high tiller number is the main reason behind the tolerance of Bhasamanik, Rudra, Laldhan, Swarnaprabha as observed from their location near the variable, PNI where as Santhi and Swarna kept their biomass high. Presence of Sarathi, Udaygiri, Red Tribeni in opposite quadrate of tolerant lines can be considered as susceptible (figure 2 in electronic supplementary material).

The study identified stable genotypes considering three year's PY and PN of 20 contrasting genotypes following Eberhart and Russel model (1966). Mean, regression coefficient (b_i) and mean square deviation (S^2_{di}) are given in table 5 in electronic supplementary material. Nonsignificant (at 5% level, $F_{1,18}$ df) deviation from the unit regression coefficient value, zero deviation from regression line (S^2_{di}) helps us in identifying stable genotype. Chamarmani, Laldhan, Sasarang are found to be unstable in yield over three years and Pyari is unstable for panicle per plant, remaining genotypes were considered stable over three years. Mahananda, Kataribhog and Chamarmani with high negative b_i value but zero deviation from regression line (S^2_{di}) indicated their preference for low light environment only. Purnendu and Sashi showed almost unit regression coefficient value, zero deviation from regression line.

Comparison of net photosynthesis rate at the low light intensity

We compared the P_n at wide range of PAR starting 50 μm photon m^{-2} s^{-1} to 900 μm photon m^{-2} s^{-1} for six genotypes having a wide range of low light tolerance index (ratio of yield under shade by open) ranging from 0.39 to 0.79 as shown in bracket of each genotype. Rudra (0.72), Swarnaprabha (0.71) Bhasamanik (0.73), showed high P_n at 400 μm photon m^{-2} s^{-1} but Satabdi being a susceptible genotype, ranked first among the studied genotypes (figure 2). On the other hand, best tolerant genotype, Purnendu (0.79) ranked last with another susceptible variety, IR64.

Diversity with other high yielding and abiotic stress tolerance genotypes

Other than newly identified low light tolerant genotypes, we included submergence, salinity, drought, phosphate deficiency tolerance high-yielding for diversity analysis by 54 SSRs, those were selected from all twelve chromosomes.

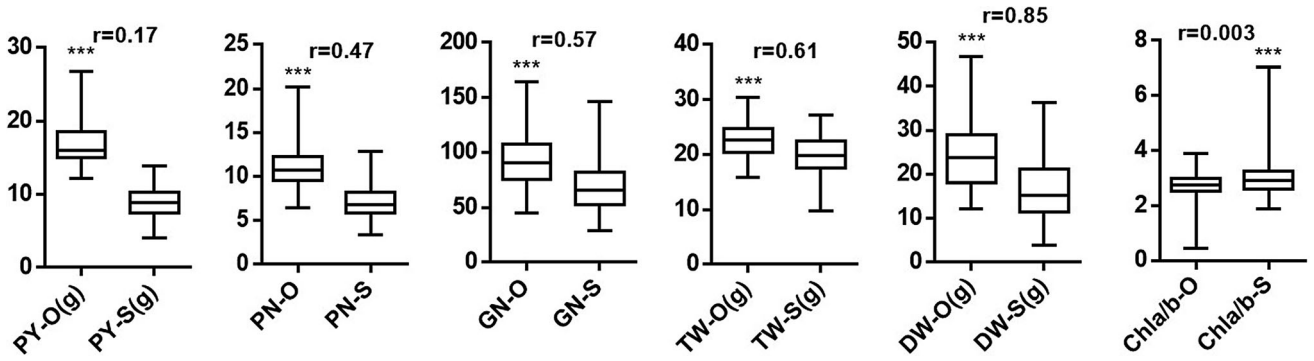

Figure 1. Comparison of minimum, maximum and mean value yield and its associated parameters between open and shade grown 100 genotypes; r value between open and shade grown plants are also given; ***difference is significant at $P< 0.0001$.

Table 2. Mean data and tolerance index value (ratio of performance under shade by open) of selected genotypes.

Genotype	YP-O (g)	YP-S (g)	STI	PN-O	PN-S	PNI	GN-O	GN-S	GNI	TW-O (g)	TW-S (g)	TWI	Chla-O (mg/g)	Chla-S (mg/g)	Chlb-O (mg/g)	Chlb-S (mg/g)	Chl a/b O	Ca/b-S	DW-O mean	DW-S (g)	DWI
Purnendu	17.59	13.93	0.79	8.69	6.57	0.76	126.22	146.00	1.16	22.59	22.40	0.99	2.12	2.48	0.76	0.98	2.80	2.54	37.68	30.36	0.81
Mahananda	13.80	10.41	0.75	8.72	6.70	0.77	97.71	79.14	0.81	19.72	18.79	0.95	1.60	2.17	0.61	0.31	2.63	7.03	35.97	21.31	0.59
Bhasamanik	14.67	10.77	0.73	9.33	9.20	0.99	100.24	61.39	0.61	21.04	20.45	0.97	1.96	1.54	0.74	0.70	2.64	2.21	28.81	21.54	0.75
Pantdhan-19	21.71	15.80	0.73	12.33	7.92	0.64	111.70	103.10	0.92	28.06	27.17	0.97	1.68	4.00	0.59	1.63	2.84	2.45	25.51	18.34	0.72
Sasarang	13.46	9.63	0.72	7.74	7.56	0.98	91.17	63.25	0.69	17.96	17.04	0.95	1.52	2.01	0.41	0.57	3.68	3.53	27.61	24.84	0.90
Swarnaprabha	17.67	12.62	0.71	8.54	7.58	0.89	164.61	123.02	0.75	23.74	21.94	0.92	2.23	3.05	0.58	0.85	3.87	3.57	27.91	21.32	0.76
Sashi	14.28	10.15	0.71	8.08	6.21	0.77	88.36	92.55	1.05	20.38	22.46	1.10	2.36	2.35	0.89	0.81	2.64	2.90	34.62	24.13	0.70
Rudra	15.14	10.40	0.69	9.33	9.48	1.02	100.19	53.13	0.53	21.59	20.60	0.95	2.25	2.25	0.70	0.78	3.21	2.89	15.42	12.76	0.83
Red Tribani	15.31	5.26	0.34	11.38	5.59	0.49	78.31	61.48	0.79	19.61	19.72	1.01	2.67	3.86	1.70	1.18	1.57	3.26	20.04	12.57	0.63
Sarathi	15.71	5.28	0.34	12.52	4.90	0.39	99.75	58.77	0.59	22.80	15.20	0.67	1.39	2.04	0.58	0.77	2.39	2.64	15.26	6.00	0.39

O, open; S, shade.

Summary analysis of SSR derived alleles of the 50 rice accessions using 54 SSR markers is given in table 4. Characteristics of the chosen 50 genotypes are given in table 6 in electronic supplementary material. All 54 polymorphic SSRs identified total of 158 alleles where the number of alleles ranged from 2 to 4 with an average of 2.93 per SSR with 10 unique alleles. Unique alleles were produced by RM125, RM118, RM142, RM484, RM161, RM332, RM452, RM316, RM507 and RM307. Eight genotypes, DRR39, Kasalath, Barhabalidhan, Rasi, Purnendu, Panthdhan19, Vandana and Mallicksail, amplified single unique allele where as Bardogidhan produces two unique alleles. Mean PIC value ranged from 0.038 to 0.557 with an average of 0.276. Black rice landrace of Manipur, Chakao Nungshi is most diverse genotypes as compared to others as included in this study (figure 3). Other than Chakao Nungshi, the dendogram showed five clusters. Clusters are not based on their adoptive ecology. A few diverse genotypic pairs are IR64 (high yielding) and Sashi; Kasalath and Purnendu; DRR39 and Pantdhan 19, Sashi and N22, etc.

Discussion

We analysed the performance of 100 genotypes in completely randomized block design for three years. Here, in our experiment, as plot sizes (0.75 m x 4 m) are small (3 rows of 4 m with 25 cm spacing), the requirement of the total area is not too big and the chance of fertility gradient is limited if not nil. As genotypes were randomized and replicated within a small area of land; thus heterogeneity in the experimental field was addressed more accurately than that of augmented design. In the field, we recorded 35% reduction of natural irradiance just after transplanting till harvesting. Thus all plants suffered low light stress throughout the life cycle as compared to open grown plants. High yielding ability under open with less than 20–30% yield reduction under shade is considered tolerance against low light intensity which will be useful in rice breeding programme. Thus, all the plants suffered low light stress throughout the life cycle as compared to open grown plants. We considered tolerance when a high yielding line showed less than 20–30% yield reduction under low light which will be useful in the rice breeding programme directly. We observed 50% mean yield reduction when plants are grown under the low light intensity during transplanting to maturity; earlier workers also observed 34%, 15% and 55% shade-imposed reduction during transplanting to booting, heading to grain filling (12 days) and heading to maturity (Liu *et al.* 2009; Janardhan *et al.* 1980; Janardhan and Murty 1980). Therefore, tolerant genotypes identified in this study will be suitable for the improvement of shade tolerance more broadly than a stage-specific way. As heritability of yield under low light intensity is not so high, selection of tolerant line should be done carefully based on the performance at multi-locations in a number of years. Purnendu and Sashi

Table 3. Min, max, mean, standard deviation (SD) of tolerance index and their correlation coefficient.

Variable	*n*	Min.	Max.	Mean	SD	STI	PNI	GNI	TWI	Ca/b-S
STI	100	0.261	0.792	0.502	0.122	**1**				
PNI	100	0.391	1.080	0.660	0.175	**0.602**				
GNI	100	0.328	1.157	0.749	0.185	**0.215**	0.038			
TWI	100	0.547	1.102	0.876	0.118	**0.338**	**0.191**	**0.256**		
Chl a/b-S	100	1.900	7.035	3.008	0.688	**0.158**	0.140	0.053	0.127	
DWI	100	0.319	0.986	0.658	0.134	**0.266**	**0.392**	0.079	0.015	0.065

Bold values are significant at 5% level.

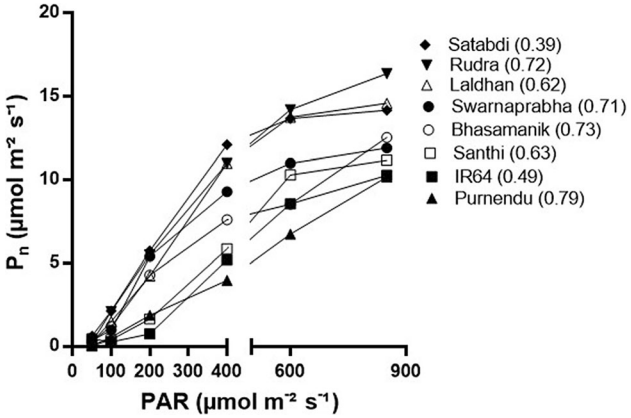

Figure 2. Net photosynthesis rate (P_n) at the low irradiance ranging from 50–800 μm photon m^{-2} s^{-1} in the flag-leaf of selected eight genotypes; ratio of yield under shade by open is given beside the name of each genotype in parenthesis.

with unit regression coefficient value and zero deviation from regression line indicated less responsive among the years where Pantdhan19 and Neeroja are identified as the most responsive over the years as observed by high positive b value with zero deviation from regression line.

Genotype dependent shade tolerance was manifested by either improving or maintaining equal amount of panicle number or grain number or test weight. Previous reports also showed that rice yield of low-light grown plants exhibit differential effects by varietal dependent way (Voleti and Singh 1996; Liu *et al*. 2012). We considered a large number of high yielding genotypes in this experiment and low light was imposed throughout the growth cycle, thereby identified genotypes can be used for deciphering the pathways for genotypic plasticity under low light by analysing at the physiological and molecular levels. Similarly, crossing among contrasting tolerant genotypes maybe useful in pyramiding lowlight responsive traits, like Purnendu and Rudra where duration of both genotypes is same but Purnendu showed less yield-reduction mainly by improving grains/panicle and TW but Rudra maintains almost equal panicle number. Both genotypes maintained the equivalent amount of dry weight at the preflowering stage. Despite the low net photosynthesis rate under low light intensity, better sink size in Purnendu may be due to low light responsive yield-enhancing genes and higher partitioning efficiency. As per the principal component analysis, less grain number reduction under shade is positively associated with high seed weight index, thus genotypes with good translocation efficiency has some role in low light tolerance ability in agreement with the earlier observation that poor yield under low light intensity at the grain filling stage is mainly due to poor translocation efficiency (Nayak and Murty 1980). Dry weight index positively correlated with yield but not so strong, means, other than translocation efficiency, variety specific acclimatization has a significant role in maintaining good yield, rather than a universal phenomenon. Kobata *et al.* (2000) observed that shading during the early grain filling period did not affect potential grain dry matter increase in rice. Both Chl a and b improved under shade and as a result ratio of Chl a/b remain almost same. However, their association to yield improvement is not so strong. Enhanced Chl b vis-a-vis reduced Chl a/b ratio involved in higher rate of photosynthesis in tobacco both under excess and limited light intensity (Biswal *et al.* 2012) but not significantly positively correlated with CO_2 fixation.

We also observed that the rate of net photosynthesis in unit leaf-area is not directly correlated with low light tolerance abilities. Satabdi, being the susceptible one ranked first in photosynthesis rate at low light intensity, below 400 μm photon m^{-2} s^{-1} contrarily, best tolerant genotype, Purnendu ranked last. Ability to high net photosynthesis under low light for the tolerant genotypes, Bhasamanik, Rudra and Swarnaprabha can be used in rice breeding programme. Purnendu did not exhibit higher rate of photosynthesis in spite of its stable and best performance under shade, thus, it can be utilized in crossing with Rudra, Swarnaprabha, Bhasamanik, etc. for the further improvement and mapping of the low light responsive yield in rice. This again confirms that higher net photosynthesis rate under low light is not the only factor behind better yield under shade and strengthen the principles, genotypic dependent tolerance ability. Genotypes with below eight panicles under open condition showed more than 20% yield advantage under low light, probably, due to reduction of mutual shading to the lower leaves. Low PN helps doubling P_n in low-tillering, NPT rice than that of IR72 (Murchie *et al.* 2002) at low light intensity. Diversity, analysed in this study

Table 4. Name, chromosomal location and map position of 54 SSRs in rice.

	Chromosome no.	Map position (cM)	Marker	N_A	N_{PA}	N_{UA}	F_{LA}	F_{HA}	PIC
1	1	29.7	RM1	3	3	0	0.23	0.43	0.57
2	1	51	RM490	3	3	0	0.03	0.06	0.34
3	1	115.2	RM237	2	2	0	0.06	0.94	0.11
4	2	34.7	RM555	3	3	0	0.10	0.76	0.36
5	2	58.4	RM452	3	3	0	0.04	0.94	0.35
6	2	92.5	RM475	3	3	0	0.08	0.76	0.35
7	3	15.7	RM231	3	3	0	0.04	0.70	0.37
8	3	64	RM7	2	2	0	0.06	0.94	0.11
9	3	79.1	RM251	3	3	0	0.12	0.72	0.40
10	3	216.4	RM514	3	3	0	0.04	0.64	0.40
11	4	0	RM307	3	3	1	0.02	0.90	0.17
12	4	68.5	RM142	4	4	0	0.02	0.86	0.24
13	4	94.4	RM273	2	2	0	0.18	0.82	0.25
14	4	118.3	RM317	2	2	1	0.02	0.84	0.25
15	4	150.1	RM124	2	2	0	0.35	0.59	0.44
16	5	0	RM507	3	3	1	0.02	0.90	0.17
17	5	20.8	RM510	2	2	0	0.12	0.88	0.19
18	5	26.7	RM413	3	3	0	0.04	0.88	0.21
19	5	96.9	RM161	4	4	1	0.02	0.76	0.35
20	5	99.3	RM454	2	2	1	0.02	0.88	0.20
21	5	101	RM3476	2	2	0	0.10	0.90	0.16
22	5	108.3	RM162	2	2	0	0.08	0.74	0.37
23	5	141.8	RM334	2	2	0	0.30	0.70	0.33
24	5	188.8	RM178	2	2	0	0.18	0.82	0.25
25	6	0	RM133	3	3	0	0.13	0.43	0.52
26	6	7.4	RM190	3	3	0	0.04	0.84	0.26
27	6	74.3	RM3	2	2	0	0.24	0.76	0.30
28	7	24.8	RM125	2	2	1	0.02	0.98	0.04
29	7	96.9	RM118	3	3	0	0.02	0.92	0.14
30	7	115.3	RM420	3	3	0	0.02	0.90	0.17
31	8	9.4	RM152	2	2	0	0.14	0.86	0.21
32	8	52.2	RM25	2	2	0	0.18	0.82	0.25
33	8	80.5	RM223	2	2	0	0.29	0.71	0.32
34	8	103.7	RM149	3	3	0	0.04	0.78	0.32
35	8	116	RM433	2	2	0	0.10	0.90	0.16
36	9	1.8	RM316	3	3	1	0.02	0.94	0.11
37	9	11.7	RM219	3	3	0	0.20	0.46	0.56
38	9	32.1	RM105	2	2	0	0.12	0.88	0.11
39	9	99.4	RM215	2	2	0	0.04	0.96	0.07
40	9	114.7	RM205	3	3	0	0.08	0.84	0.26
41	10	0	RM474	3	3	0	0.05	0.84	0.26
42	10	59.4	RM271	3	3	0	0.06	0.68	0.40
43	10	97.3	RM484	3	3	1	0.02	0.85	0.24
44	11	27.9	RM332	3	3	1	0.02	0.90	0.17
45	11	40.6	RM552	4	4	1	0.02	0.56	0.49
46	11	55.1	RM536	3	3	0	0.18	0.59	0.50
47	11	102.9	RM206	3	3	0	0.04	0.52	0.43
48	11	123.2	RM144	2	2	0	0.14	0.80	0.31
49	12	20.9	RM19	3	3	0	0.06	0.78	0.33
50	12	43.2	RM512	3	3	0	0.04	0.76	0.34
51	12	51.5	RM1337	2	2	0	0.10	0.90	0.16
52	12	57.2	RM277	3	3	0	0.04	0.90	0.18
53	12	91.3	RM270	2	2	0	0.04	0.96	0.08
54	12	109.1	RM17	3	3	0	0.12	0.52	0.50

N_A, number of alleles; N_{PA}, number of polymorphic allele; N_{UA}, number of unique allele; F_{LA}, low frequency allele; F_{HA}, high frequency allele; PIC, polymorphic content of each SSRs used in this study.

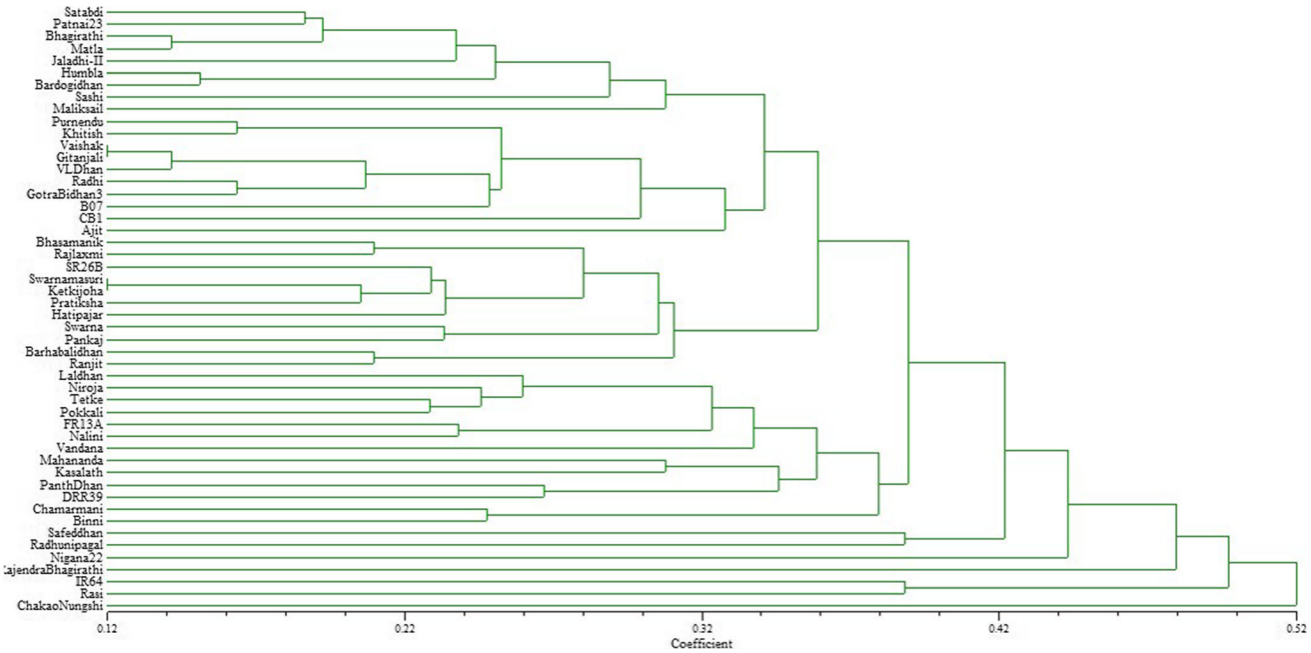

Figure 3. Dendogram of 50 genotypes showing relationship between newly identified tolerance lines with other high yielding and abiotic stress tolerant genotypes using similarity coefficient deduced from polymorphic alleles of 54 SSRs.

will be utilized in selecting diverse parental pairs in rice breeding programme for the pyramiding of low light tolerance ability in the background of other abiotic and biotic stress tolerance abilities. 54 SSRs amplified average 2.93 alleles which was similar to earlier studies by Singh *et al.* (2016) 2.75 and Vanniarajani *et al.* (2012) 2.50. Frequency of unique alleles was around 6% which was amplified by nine genotypes. Unique alleles can be used for the identification of low light tolerance genotypes like Purnendu, Bardogidhan, etc. As diversity analysis was executed selecting 50 genotypes which are diverse in terms of their ecological adoptability and source of abiotic stress tolerance, so selection of diverse parental pairs will be useful in rice breeding programme. Thus, stable shade-tolerant rice lines, Purnendu, Sashi, Pantdhan19 from a large set of genotypes with different adjustment ability to keep up to 72% or more yield of open grown plants can be used further for the yield improvement in kharif season as well as analysis at the physiological and molecular levels. Genotypic variance explained 39% of the total yield-variation under shade with 58% heritability and high photosynthesis rate per unit leaf-area is not the sole cause of low light tolerance. Diversity analysis identified diverse parental pairs, which will be used in pyramiding of low light tolerance ability in the background of other abiotic stress tolerances and choice of suitable SSRs for the heterozygosity confirmation.

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References

Adhya T. K., Singh O. N. and Ghosh A. 2008 Rice in Eastern India: causes for low productivity and available options. *J Rice Res.* **2,** 1–5.

Barnes J. D., Balaguer L., Manrique E., Elvira S. and Davison A. W. 1992 A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ Exp. Bot*. **32,** 85–100.

Biswal A. K., Pattanayak G. K., Pandey S. S., Leelavathi S., Reddy V. S. and Tripathy B. C. 2012 Light intensity-dependent modulation of chlorophyll b biosynthesis and photosynthesis by overexpression of chlorophyllide a oxygenase in tobacco. *Plant Physiol.* **159,** 433–449.

Chattopadhyay T., Biswas T., Chatterjee M., Mandal N. and Bhattacharyya S. 2008 Biochemical and SSR marker based characterization of some Bengal landraces of rice suffixed with 'sail' in their name. *Ind. J. Genet. Plant Breed.* **68,** 15–20.

Dutta S. S., Tyagi W., Pale G., Pohlong J., Aochen C., Pandey A., Pattanayak A. and Rai M. 2018 Marker–trait association for low-light intensity tolerance in rice genotypes from Eastern India. *Mol. Genet. Genomics* **293**, 1493–1506.

Eberhart S. A. and Russel W. L. 1966 Stability parameters for comparing varieties. *Crop Sci.* **6,** 36–40.

Janardhan K. V., Murty K. S. and Dash N. B. 1980 Effects of low light during ripening period on grain yield and translocation of assimilates in rice varieties. *Ind. J. Plant Physiol.* **23,** 163–168.

Janardhan K. V. and Murty K. S. 1980 Effect of low light during vegetative stage on photosynthesis and growth attributes in rice. *Ind. J. Plant Physiol.* **23**, 156–162.

Jiao D. and Li X. 2001 Cultivar differences in photosynthetic tolerance to photooxidation and shading in Rice (*Oryza sativa* L.). *Photosynthetica* **39,** 167–175.

Izumi M., Hidema J., Wada S., Kondo E., Kurusu T., Kuchitsu K. *et al*. 2015 Establishment of monitoring methods for autophagy in rice reveals autophagic recycling of chloroplasts and root plastids during energy limitation. *Plant Physiol.* **167,** 1307–1320.

Kasajima I., Ebana K., Yamamoto T., Takaharac K., Yanob M., Kawai-Yamadaa M. *et al*. 2011 Molecular distinction in genetic regulation of nonphotochemical quenching in rice. *Proc. Natl. Acad. Sci. USA* **108,** 13835–13840.

Kobata T., Sugawara M. and Takatu S. 2000 Shading during the early grain filling period does not affect potential grain dry matter increase in rice. *Agron. J.* **92**, 411–417.

Liu L., Wang F., Deng Y., Huang D.Y., Liu W. J, Ren W. Y. and Yang W. Y. 2012 Osmotic regulation substance contents and activities of protective enzymes in leaves of different hybrid rice combinations as affected by shading. *Chin. J. Rice. Sci.* **26**, 569–575.

Liu Q., Wu X., Chen B., Ma J. and Gao J. 2014 Effect of low light on agronomic and physiological characteristics of rice including grain yield and quality. *Rice Sci.* **21**, 243−251.

Liu Q. H., Zhou X. B., Yang L. Q., Li T. and Zhang J. J. 2009 Effects of early growth stage shading on rice flag leaf physiological characters and grain growth at grain-filling stage. *Chin. J. Appl. Ecol.* **20**, 2135–2141.

Murchie E. H., Hubbart S., Chen Y., Peng S. and Horton P. 2002 Acclimation of rice photosynthesis to irradiance under field conditions. *Plant Physiol.* **130**, 1999–2010.

Nayak S. K. and Murty K. S. 1980 Effects of varying light intensities on yield and growth parameters in rice. *Ind. J. Plant Physiol.* **23**, 309–316.

Singh S. 2005 Effect of low-light stress at various growth phases on yield and yield components of two rice cultivars. *Int. Rice Res. Notes* **30**, 36–37.

Singh N., Choudhury D. R., Tiwari G., Singh A. K., Kumar S., Srinivasan K. *et al*. 2016 Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC Genet.* **17,** 127.

Vanniarajani C., Vinod K. K. and Pereira A. 2012 Molecular evaluation of genetic diversity and association studies in rice *Oryza sativa* L. *J. Genet.* **91**, 9–19.

Venkateswarlu B. 1996 Influence of low light intensity on growth and productivity of rice (*Oryza sativa* L.). *Plant Soil* **47**, 713–719.

Voleti S. R. and Singh V. P. 1996 Influence of low light irradiance on grain filling in rice (*Oryza sativa* L.) cultivars. *J. Agron. Crop Sci.* **176**, 1–4.

Wang L., Deng F., Ren W. J. and Yang W. Y. 2013 Effects of shading on starch pasting characteristics of indica hybrid rice (*Oryza sativa* L.). *PLoS One* **8**, e68220.

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