



Marker–trait association for low-light intensity tolerance in rice genotypes from Eastern India

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Abstract

Light intensity is a crucial environmental factor that affects photosynthesis and ultimately, grain yield in rice. However, no gene or marker directly associated with improved performance under low-light intensity under field conditions has been identified till date. With an aim of identifying genes and markers associated with improved performance (measured in terms of better yields) under low-light intensity, an integrated field screening, in silico and wet lab validation analysis was performed. Field-based screening of a diverse set of 110 genotypes led to the identification of two physiological and three morphological parameters critical for low-light tolerance in rice. In silico analysis using information available in public databases led to the identification of a set of 90 potential candidate genes which were narrowed to thirteen genic targets for possible marker–trait association. Marker–trait association on the panel of 48 diverse rice genotypes varying in their response to low-light intensity led to the identification of six markers [HvSSR02-44 (biological yield), HvSSR02-52 (spikelet fertility), HvSSR02-54 (grain yield), HvSSR06-56 (spikelet fertility), HvSSR06-69 (spikelet fertility; biological yield), HvSSR09-45 (spikelet fertility)] lying on chromosomes 2, 6 and 9 showing significant association ($R^2 > 0.1$) for traits like grain yield/plant, biological yield and spikelet fertility under low light. Eight rice genes [including member of BBX (B-box) family] lying within 10 kb distance of these identified markers already reported for their role in response to stress or change in plant architecture in rice were also identified. The eight rice genotypes, five traits, eight genes and six markers identified in the current study will help in devising strategies to increase yield under low light intensity and pave way for future application in marker-assisted breeding.

Keywords Low light tolerance · Marker-trait association · Candidate genes · Rice

Introduction

Light is a crucial parameter affecting various metabolic processes including photosynthetic efficiency, as a result of which the type, duration and intensity of light is of vital importance in determining agronomic performance of all crop species. Several studies have been conducted on genetic and physiological basis of light regulation (Jiao et al. 2005), photoreceptors (Crocco et al. 2010; Franklin 2008; Li et al. 2012a; Reed et al. 1993) and plant growth in response to light (Petrillo et al. 2014). However, genes/genic regions involved in imparting tolerance to low-light intensity under field conditions across crop species have not been reported till date. Therefore, it is crucial to understand the molecular mechanism involved in low-light intensity tolerance and use the genes and markers underlying such genes for breeding for better performance in such adverse environmental conditions. One of the ways this can be achieved is by identifying

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candidate genes for the trait under study from the rich genomic resources available for various crops and then use such target genes and markers for marker–trait association across a diverse set of genotypes screened under field conditions. The markers and genes thus identified can be used for marker-assisted selection for the trait in crop improvement programmes.

About 90% of the rice production takes place in the tropical/sub-tropical Asia where 60% of the world population lives (Sridevi and Chellamuthu 2015). The Eastern and North Eastern Hill regions of India occupy 58% of rice growing area but contribute less than 48% to the national rice production. One of the reasons of low productivity is presence of overcast skies during most of the rice growing season. Light is one the most important environmental cues as well as the source of energy for plants (Schmoll et al. 2012). Plants have adopted the ability to sense multiple parameters of light signals, including light quantity (fluence), quality (wavelength), direction and duration (Petrillo et al. 2014). The world's top rice-yielding countries are those that receive the highest solar radiation. New South Wales, Australia, is at high southern latitude, having long summer days and this region usually records the highest rice yields. It has been previously suggested that higher yields in the north-western regions of India are mainly due to higher radiation (380–460 cal/cm²/h) (Venkateswarlu and Visperas 1987). Changes in light conditions impact photosynthetic electron flux and redox state of the components involved in it (Pfalz et al. 2012). In the case of rice, high tiller mortality at vegetative stage, low spikelet number at reproductive stage and dry weight after flowering are the major symptoms in response to low-light intensity (Adhya et al. 2008). Solar radiation intercepted by rice canopy plays a major role in determining biomass and grain yield. Light is a key regulator for seedling development as well. At seedling stage, as much as 18% of the rice genome is regulated by light (Jiao et al. 2005).

The red/far-red-light (R/FR) absorbing photoreceptor phytochrome B (PHYB) is the major sensor of shade light (Reed et al. 1993). It is known that in shaded conditions the ratio of R–FR is low (< 1) leading to inactivation of far-red-absorbing form of PHYB. This causes the phytochrome-interacting factors (PIFs) to accumulate and interact with genes causing hypocotyl elongation (Franklin 2008). This shade avoidance syndrome (SAS) is well worked out in model species, *Arabidopsis*. At least three PIFs, PIF4 (Lorrain et al. 2008), PIF5 (Leivar and Quail 2011) and PIF7 (Li et al. 2012a) along with *AtBBX21* (B-box zinc finger transcription factor) (Crocco et al. 2010) have been implicated in SAS in *Arabidopsis*. The role of PIF7, a basic helix–loop–helix (bHLH) transcription factor (TF), in regulating an auxin-mediated pathway leading to shade-induced growth is now well understood (Li et al. 2012b). This TF

directly links light quality signal to rapid growth response. Increase in photosynthetic pigments, mostly chlorophyll *a* and *b*, in response to low irradiation has been reported in shade tolerant species (Flexas et al. 2012). The increase in chlorophyll was proportionately more in chlorophyll *b* fraction leading to lower chl *a/b* ratio (Liu et al. 2014). Reduction of light intensity beyond 50% of the normal light inhibited chlorophyll synthesis resulting in decreased chlorophyll content.

In the case of rice, it is known that very low light reduces the rate of overall growth and photosynthesis and impaired translocation of carbohydrates to developing grain under subdued light results in high sterility (Sridevi and Chellamuthu 2015).

Microarray analysis on rice seedlings transferred to high light from low light has revealed a downregulation of light-harvesting genes, upregulation of photo protection genes and no change in the expression level of RUBISCO (Murchie et al. 2005). Now it is known that, apart from photoreceptor like phytochromes which absorb light and transduce signals into chromatin, transcriptional and post-transcriptional changes, chloroplast itself acts as a light sensor (Petrillo et al. 2014). Chloroplast-derived retrograde signals regulate transcription, as well as alternative splicing decisions in the nucleus (Petrillo et al. 2014). A yellow-green leaf (*vgl*) mutant of rice is now characterised and it encodes a novel, chloroplast transit peptide protein, *YGL9*, involved in chloroplast development (Wang et al. 2016).

To develop rice genotypes with higher yield and greater stability under low light stress, knowledge about morpho-physiological, molecular and genetic basis of low-light intensity tolerance will be crucial. Identification of candidate genes (CG) and development and utilization of molecular markers underlying such CG could be one of the ways of achieving the target by marker-assisted breeding.

The current study, aimed (a) to identify contrasting rice genotypes by field screening under adverse light conditions and (b) to identify markers associated with key traits for use in breeding for increased yield under low light conditions. We identified eight low-light intensity tolerant rice genotypes and short-listed a set of candidate genes for future association studies for studying light regulation in rice. Markers tightly linked to yield related traits, as well as candidate genes in response to low-light intensity for use in molecular breeding programme have also been identified.

Materials and methods

Plant materials and growth conditions

A set of one hundred and ten genotypes from different parts of Eastern India including tolerant and susceptible

check (supplementary data 1) were used in this study. The study was conducted at plant breeding field of ICAR-NEH (Indian Council of Agricultural Research-North Eastern Hill Research Station, Umiam, Meghalaya located at 25° 41' 18.36"N Latitude and 91° 55' 04.38"E Longitude) during the rice growing season of 2015. Each genotype consisted of 20 plants grown in two sets (normal field conditions and 30% less light intensity) (Fig. 1a). The main rice growing season (Kharif/Sali) extends from June to November, depending on duration of rice variety. Since July and August are heavy rainfall months (Fig. 1c) with excessive cloud cover, the experimental material was sown in nursery on 1st of July, transplanted on 1st of August, and shading treatment was provided from 10th of August to allow maximum contrast between ambient and shaded conditions during the active tillering and reproductive stages of crop growth (September–November) (Fig. 1b). Thirty percent shading effect was achieved by high-density polyethylene (HDPE) 30% white coloured agro shade net (B&V Agroshade company, India). Ten 30-day-old seedlings of each line were transplanted with a uniform spacing of 15 cm between plants and 20 cm

between rows. The ambient temperature ranged from 13.7 to 33 °C and relative humidity was 65–90%. Field management was done according to standard agronomic practices.

Phenotyping for low light intensity tolerance

The light intensity ($\mu\text{mol quantum m}^{-2} \text{s}^{-1}$ units) was measured using a light meter (TES Electronic Corp., China) three times in a day during the entire kharif season of 2015.

All the genotypes were evaluated for 14 agro-morphological and physiological characters. The length of each randomly sampled plant was measured (in cm) at harvest from the base of the lowest culm of the plant to the tip of the farthest leaf. Total number of tillers present in each plant was counted manually at physiological maturity stage. Leaf area was measured in cm^2 using leaf area meter (CDI Bio-Science, Inc., USA). The collected leaf samples were oven-dried (70 °C) for 96 h and weighed in an electronic balance. Specific leaf area was calculated as the ratio of mean leaf area to the mean leaf dry weight. The tillers carrying ear was recorded from each plant of

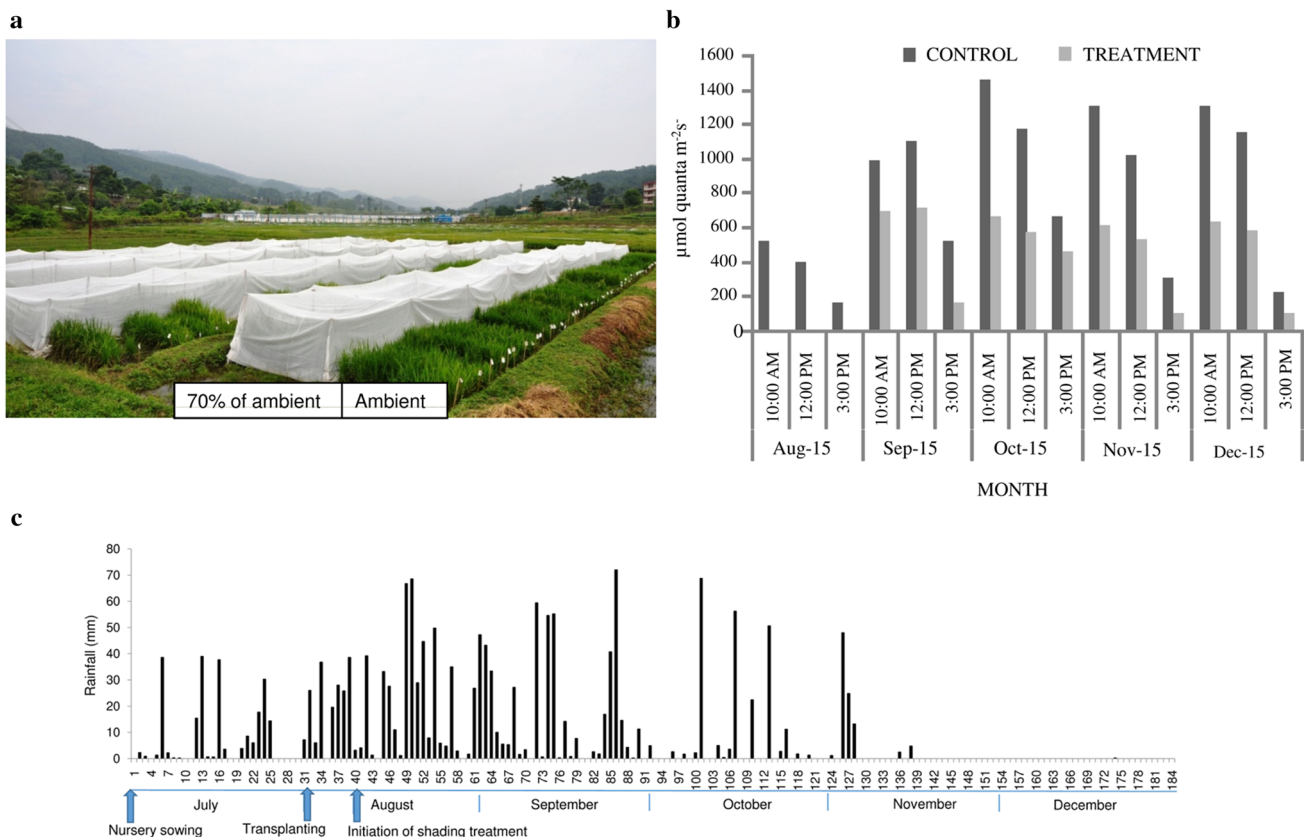


Fig. 1 Screening of genotypes under ambient and low (70% of ambient) conditions (a), variation in light intensity from August to December (b) and daily rainfall pattern at Umiam, Meghalaya during field experimentation period (c). One set of genotypes was grown under low light (covered with white color net) and another set in ambient

light. For each genotype, two rows (ten plants/row) were grown, both in low light and natural light conditions. Variation in light intensity ($\mu\text{mol quantum m}^{-2} \text{s}^{-1}$ units) during rice growing season was measured at 10 a.m., 12 p.m. and 3 p.m. daily

the genotypes. The panicle of each plant of genotype carrying filled grain was recorded. Chlorophyll content of the leaf next to flag leaf at the flowering stage was measured spectrophotometrically (Eppendorf, India) using DMSO as solvent (Hiscox and Israelstam 1979) and chlorophyll content calculated using the following formulae: chlorophyll $a = [0.0127 \times \lambda 663 - 0.00269 \times \lambda 645] \times 100$ (g/L), chlorophyll $b = [0.0229 \times \lambda 645 - 0.00468 \times \lambda 663] \times 100$ (g/L) and total chlorophyll = $[0.0202 \times \lambda 645 + 0.00802 \times \lambda 663] \times 100$ (g/L). Leaf chlorophyll content was also measured using chlorophyll meter (Spectrum Technologies, Inc., USA).

The length of each panicle was measured in cm from panicle neck node to the tip of the panicle. The weight of individual panicles from the three sampled plant of each genotypes was taken using electronic weighing balance. From each panicle of sampled plant, filled grains were separated and counted using a seed counter. From each panicle of sampled plant, unfilled spikelet were separated and counted. Total number of filled and unfilled spikelets of all panicles in a plant was added to get the total no. of spikelets/plant. One hundred grains from each genotype were counted and weighed in an electronic balance. The spikelet fertility percentage was calculated using the following formula, (healthy grain per plant/total spikelets per plant) $\times 100$. After harvesting total plant weight was taken and expressed in grams. Total grains per plant were weighed to determine the grain yield/plant in grams. The harvest index was calculated as: (total grain weight/total grain weight + dry shoot weight) $\times 100$.

In silico analysis for identification of putative candidate genes for low-light intensity response

The selection of candidate genes (CG) for low-light intensity was carried out based on genes underlying traits already reported (Liu et al. 2014) or identified in the current study. The field data generated on screening of 110 rice genotypes under 30% less light as discussed above was used to identify key traits. Oryzabase database (<http://shigen.nig.ac.jp/rice/oryzabase/>) was searched to identify genes based on the traits identified. This large set of genes was further narrowed down by selecting only functionally annotated genes. Using locus search tool (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml) of TIGR rice database, complete information about genes like nucleotide length and sequence were obtained. From this list of identified genes, at least two genes per selected trait were identified for initial validation. A set of 20 potential genes were selected for testing marker–trait association. These genes were mapped on rice chromosomes and SSR markers (Singh et al. 2010) reported in these selected regions identified. Sequences of the selected genes were retrieved from Rice Genome Annotation Project database (<http://rice.plantbiology.msu.edu>).

Using GRAMENE (<http://www.gramene.org>) database information pertaining to reported QTLs lying within 1 Mb region of identified genes was obtained.

Genotyping

A panel of 48 contrasting genotypes consisting of top tolerant and susceptible was made (supplementary data 2). DNA extraction was done using cetyltrimethyl ammonium bromide (CTAB) method of Murray and Thompson (1980). CG primers (700–1000 bp) were designed for each of the selected genes as previously described (Dkhar et al. 2014) using software Primer3. Primers were synthesized by Eurofins Genomics India Pvt. Ltd., India. Primer standardization was done with one tolerant and one susceptible genotype. Subsequent to primer standardization, polymerase chain reaction (PCR) was performed in a thermal cycler (Thermo scientific, India) using a total reaction volume of 10 μ L. PCR reaction mixture contained 30 ng of template DNA, 10 pmol of each forward and reverse primer, 25 mM MgCl₂ (Sigma, India), 2 mM dNTPs (Sigma, India) and 0.5 U of Taq polymerase (Sigma, India). Polymerase chain reaction comprised one cycle of denaturation at 95 °C for 5 min, followed by 33 cycles at 95 °C for 1 min, 55 °C/58 °C/60 °C for 1 min and 72 °C for 2 min, with a final extension of 72 °C for 5 min. The amplified products were resolved on 1–2% agarose (Sigma, India) gel containing 0.1 mg/mL of ethidium bromide along with a DNA size standard 100 bp ladder (Fermentas, India) and visualized under ultraviolet trans-illuminator (AlphaImager Mini, USA). HvSSR and CG markers spanning chromosomal segments where majority of the candidate genes were located were screened for polymorphism on a panel of eight contrasting genotypes and primers showing polymorphism were used for association study.

Statistical analysis

The phenotypic data for different traits under low-light intensity were arranged in Microsoft Excel 2010 for calculation of mean and correlation analysis. Least significant difference values among means were tested at 5% level of significance. Correlation for all the traits was performed using Pearson's correlation coefficient. Principle component analysis was carried out using EXCEL Stat software.

The genotypic data generated on 48 genotypes using polymorphic HvSSR and CG markers were used for studying marker–trait association using t test taking single marker at a time. Additive gene action was assumed for performing t test. For each marker locus, phenotypic means of the two homozygous genotypic groups were compared. Significant t test ($1 - P \geq 0.95$) indicated marker–trait association. Coefficient of determination (R^2) for each marker–trait pair was calculated to know the proportion of phenotypic variation

explained by a marker. Marker-trait regression analysis was also performed using Microsoft Excel 2010 to confirm marker–trait association.

Results

Evaluation of 110 rice genotypes collected from different regions of Eastern India for response to low-light intensity

The highest average light intensity in Kharif, 2015 was received during the month of October (booting stage of majority of genotypes) and the lowest during August (tillering initiation stage of the genotypes) under ambient light condition during the growth period. Under low-light treatment condition (30% shading) (Fig. 1a), the highest and the lowest average light intensity was received during the month of September (active tillering or pre booting stage of the genotypes) and December 2015 (maturity stage of late maturing genotypes), respectively (Fig. 1b). It is also found that during morning 10 a.m. highest light intensity was received on an average. The cloud cover measured was variable, with the highest cloud cover (nearly 7 OKTA) observed during August 2015 (data not shown). Then the cloud cover was found decreasing with the crop growing season.

Variance due to genotypes and genotype \times light interaction was found to be significant for all the traits under study (supplemental data 3) indicating that selection for low-light intensity tolerance within 110 accessions would be effective. Under low-light conditions, grain yield showed highest correlation with plant height (Pearson correlation coefficient 0.461), ear bearing tillers (0.404), number of effective

panicles (0.591), biological yield (0.305) and panicle weight (0.787) (Table 1).

Principle component analysis (PCA) revealed that the first three most informative components accounted for 64% variance among the 110 genotypes rice under low-light conditions. For the first principle component (PC1), panicle weight (0.45), ear bearing tillers (0.43) and grain yield (0.38) were the major contributors in low-light condition (Table 2). For PC2, spikelet fertility (0.49) was the major contributor under low-light condition. Number of tillers per plant (0.56), ear bearing tillers (0.27) and SPAD index (0.21) were the contributors to PC 3 under low light condition.

PCA indicated that grain yield, number of effective panicles per plant, spikelet fertility, ear bearing tillers and panicle weight were the major traits in relation to low-light intensity (Fig. 2a). Outlier genotypes like MeghaRice1, Pusa Sugandha, Mahisugandh, PS-4, Kunti, Danteshwari, Tenwadhan, Kalanunia and Bardogi dhan were identified (Fig. 2b) and included in the genotyping panel (supplemental data 2).

In silico identification of target genomic regions for response to light intensity

To narrow down number of genes for marker–trait association, trait classification tool available in Oryzabase database was used. A total of 10,223 genes distributed across twelve chromosomes of rice associated with eight trait classes (vegetative organ, leaf panicle, leaf, biochemical character, chlorophyll, seed, tolerance and resistance and spikelet) were identified. The number of genes was narrowed down to 90 by selecting only functionally annotated genes (supplemental data 4). For many of the key traits identified, QTLs are already reported. For example, 39 QTLs for leaf area, 40 for

Table 1 Pearson correlation coefficient among functional traits in 30% low-light condition during kharif-2015

Variables	PH	TN	EBT	EP	SI	BY	SF	PL	PW	SW	GY	SLW
TN	−0.109											
EBT	0.216	0.689										
EP	0.249	0.392	0.635									
SI	−0.179	0.02	−0.025	0.395								
BY	0.369	0.393	0.575	0.184	−0.426							
SF	−0.049	−0.063	−0.121	0.392	0.58	−0.518						
PL	0.022	0.062	0.163	−0.175	−0.261	0.387	−0.547					
PW	0.417	0.279	0.578	0.501	−0.085	0.611	−0.09	0.176				
SW	0.062	−0.086	−0.043	−0.093	−0.058	−0.149	−0.121	−0.033	−0.1			
GY	0.461	0.09	0.404	0.591	0.156	0.305	0.222	−0.015	0.787	−0.04		
SLW	0.039	0.118	0.139	0.285	0.156	0.034	0.195	−0.082	0.116	0.006	0.163	
HI	0.14	−0.2	−0.078	0.359	0.52	−0.402	0.6	−0.35	0.033	0.044	0.463	0.05

Correlation coefficients in bold face are significant at 5% level of significance

PH plant height, TN number of tillers per plant, EBT ear bearing tillers, EP number of effective panicles, SI SPAD index, BY biological yield, SF spikelet fertility, PW panicle weight, SW seed weight, GY grain yield, PL panicle length, SLW specific leaf weight and HI harvest index

Table 2 Principle component analysis (PCA) performed on quantitative traits in this study

	Ambient light (control)			Low light (treatment)		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigen value	3.505	3.048	1.912	3.652	3.169	1.528
Variability (%)	26.959	23.449	14.706	28.091	24.378	11.752
Cumulative %	26.959	50.408	65.114	28.091	52.469	64.221
Top three traits contributing to PCA	No. of effective panicles per plant (0.43)	Biological yield (0.48)	Ear bearing tillers (0.64)	Panicle weight (0.45)	Spikelet fertility (0.49)	No. of tillers per plant (0.56)
	Harvest index (0.43)	Panicle weight (0.45)	No. of tillers per plant (0.61)	Ear bearing tillers (0.43)	Harvest index (0.45)	Ear bearing tillers (0.27)
	Spikelet fertility (0.43)	Panicle length (0.42)	No. of effective panicles per plant (0.17)	Grain yield (0.38)	SPAD index (0.42)	SPAD index (0.21)

Eigen values and percentage variability explained by the three most informative principal components (PC1, PC2 and PC3) and the top three morphological traits contributing towards them during kharif 2015

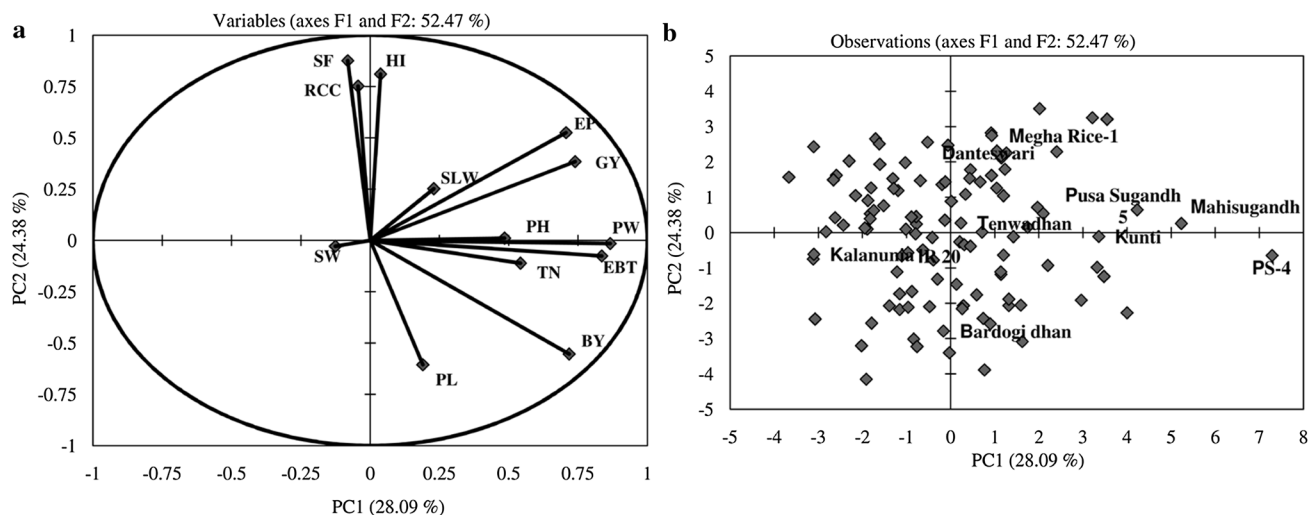


Fig. 2 Principle component analysis under low (70% of ambient) light condition showing distribution of thirteen traits (a) and 110 genotypes (b) with respect to the two principal components (PC1 and

PC2). Values in parentheses indicate percentage variation explained by the principal component. Significant traits are highlighted in bold and contrasting genotypes are identified

chlorophyll, 11 for nitrogen assimilation, 3 for ROS scavenging, 21 for spikelet, flower, glume, awn and 553 QTLs for seed related traits are already reported. However, only four reported QTLs (CQE24, CQE47, CQAS47 and CQAS51) overlap (within one Mb) with three genes (*SHM1*, *Type I chlorophyll alb-binding protein* and *D11*, respectively) (Table 3). To test the feasibility of strategy, at least 2 genes per trait were targeted leading to a set of twenty potential rice candidates. The selected twenty genes were annotated using in silico tools available in Gramene and TIGR databases. When these twenty genes were mapped, five genomic regions spanning chromosomes 1 (23.6–30 Mb), 2 (17.3–27.1 Mb), 6 (23.5–30.8 Mb), 7 (22.4–23.3 Mb) and 9 (18.4–19.4 Mb) having more than one candidate genes

(Fig. 3) were identified. These five regions covering thirteen CG were targeted for polymorphism survey using SSR and CG markers. A total of 45 reported HvSSR markers and CG markers targeting five genes lagging growth and development (*LGDI*), pinhead1 (*PNH1*), increased leaf angle 1 (*ILAI*), leaf panicle 2 (*LP2*) and chlorophyll *alb* binding protein 2r (*CAB2R*) were used (Table 3) (Fig. 3).

Marker polymorphism

Initial marker survey was conducted on a panel of eight contrasting rice genotypes (viz., Swarnaprabha, IR 8, Danteswari, Kuntti, Megha Rice-1, Pusa Sugandh-5, Bardogi Dhan and Kalanunia). Out of these genotypes,

Table 3 Details of twenty rice candidate genes having role in low light intensity tolerance

Main trait	Sub trait	Gene name	Gene symbol	MSU (LOC_ Os ID)	Chr. no.	Nucleotide length (bp)	Predicted protein length (aa)	Number of QTLs reported for this sub trait	QTL in flanking region of the gene
Vegetative organ	Vegetative organ	Lagging growth and development	<i>LGDI</i>	LOC_ Os09g32540	9	2901	967	0	0
	Leaf area	Pinhead1	<i>PNHI</i>	LOC_ Os06g39640	6	2922	974	39	0
	Leaf area	Increased leaf angle 1	<i>ILAI</i>	LOC_ Os06g50920	6	1695	565	39	0
	Leaf Panicle	Receptor kinase, putative, expressed	<i>LP2</i>	LOC_ Os02g40240	2	3006	1002	0	0
Leaf	Chlorophyll	Chlorophyll <i>alb</i> binding protein 1r	<i>CAB1R</i>	LOC_ Os09g17740	9	798	266	40	0
	Chlorophyll	Chlorophyll <i>alb</i> binding protein 2r	<i>CAB2R</i>	LOC_ Os01g41710	1	786	262	40	0
Biochemical character	Chlorophyll	Chlorophyll-deficient mutant 1	<i>SHM1</i>	LOC_ Os03g52840	3	1542	514	40	CQ E24
	Chlorophyll	Chlorophyll <i>a</i> degrading enzyme	<i>CHL</i>	LOC_ Os10g28370	10	1110	370	40	0
	Chlorophyll	Phytochrome B	<i>PHYB</i>	LOC_ Os03g19590	3	3516	1172	40	0
	<i>N</i> Assimilation	NADH-dependent glutamate synthase 1	<i>GLT1</i>	LOC_ Os01g48960	1	6504	2168	11	0
	ROS scavenging	Repressor of silencing 1B	<i>ROS1B</i>	LOC_ Os02g29230	2	4911	1637	3	0
Pigment	Chlorophyll	PSI type II chlorophyll <i>alb</i> -binding protein		LOC_ Os07g38960	7	792	264	40	0
	Chlorophyll	PSI type III chlorophyll <i>alb</i> -binding protein		LOC_ Os02g10390	2	810	270	40	0
	Chlorophyll	Type I chlorophyll <i>alb</i> -binding protein		LOC_ Os01g52240	1	798	266	40	CQE47
	Chlorophyll	Type III chlorophyll <i>alb</i> -binding protein PSII Cab type III		LOC_ Os07g37550	7	801	267	40	0
	Chlorophyll	Photosystem I subunit	<i>PSAG</i>	LOC_ Os09g30340	9	426	142	40	0

Table 3 (continued)

Main trait	Sub trait	Gene name	Gene symbol	MSU (LOC_ Os ID)	Chr. no.	Nucleotide length (bp)	Predicted protein length (aa)	Number of QTLs reported for this sub trait	QTL in flanking region of the gene
Tolerance and resist- ance	Chlorophyll	Photosystem I subunit	<i>PSAG</i>	LOC_ Os09g30340	9	426	142	40	0
Yield and productiv- ity	Spikelet, flower, glum, awn	Floral organ Number 1	<i>FON1</i>	LOC_ Os06g50340	6	2985	995	21	0
	Seed	Dwarf Shin- kaneaikoku	<i>D11</i>	LOC_ Os04g39430	4	1443	481	553	CQAS47 CQAS51
	Seed	Receptor- like cyto- plasmic kinase 192	<i>RLCK192</i>	LOC_ Os05g51400	5	1308	436	553	0

The trait, sub trait, locus ID, length of the nucleotides, predicted protein length, QTLs reported for the trait under study and QTLs lying in the 1 Mb flanking region of the gene are given

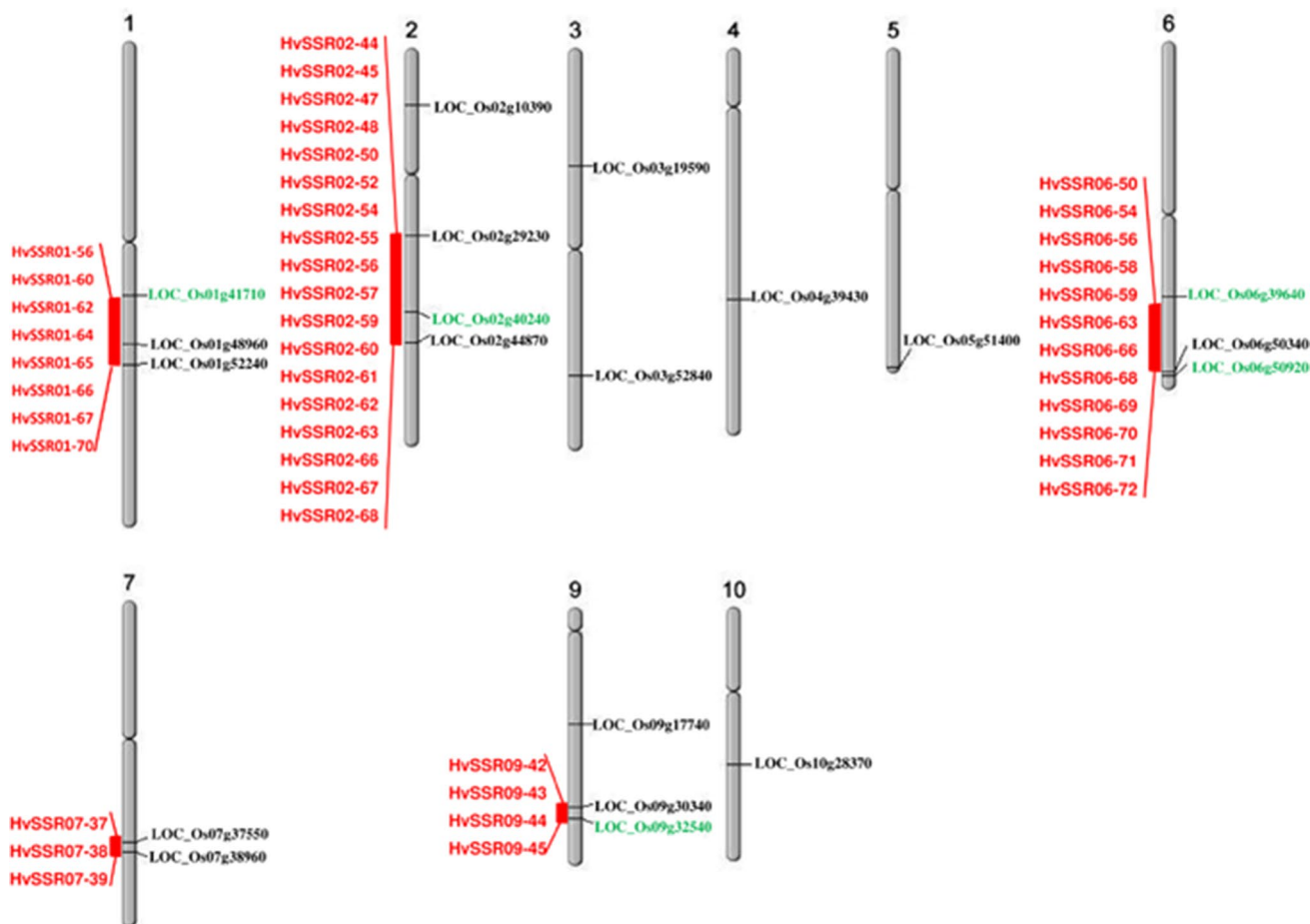


Fig. 3 Rice chromosomes showing potential regions for low-light intensity response. Map developed using MapTool Oryzabase database showing the 20 genes identified which have direct or indirect effect in response to low light (green colour indicates the gene

selected to design candidate gene based primers), 45 HvSSR and genic primers scattered in the specific selected regions of the chromosomes of rice (red). (Color figure online)

Swarnaprabha, Danteswari, Kunti, Megha Rice-1 are tolerant whereas IR 8, Pusa Sugandh-5, Bordogi Dhan and Kalanunia are susceptible to low-light intensity. A set of 45 HvSSRs (supplemental data 5) and 28 CG markers (supplemental data 6) when surveyed on this panel of 8 genotypes revealed 15 and 2 polymorphic markers from the two categories, respectively. The 15 polymorphic HvSSRs were distributed on chromosomes 1, 2, 6, 7 and 9. These markers when surveyed on panel of 48 gave a minimum 2 (HvSSR01-65, HvSSR01-67, HvSSR02-44, HvSSR02-54, HvSSR02-66, HvSSR06-56, HvSSR06-69 and HvSSR09-45) and a maximum of 3 alleles (HvSSR01-66, HvSSR02-50, HvSSR02-62, HvSSR06-54, HvSSR07-38 and HvSSR09-43) (Fig. 4).

Twenty-eight CG primers targeting five genes when run on a panel of eight genotypes, revealed CAU-CG-ILA1-3 and CAU-CG-RK-3 as polymorphic markers (data not shown). Though QTLs for different traits on the five targeted chromosomes are reported, none of them lie within 1 Mb region of the polymorphic markers identified in our study.

Marker–trait association

Marker-trait association studies based on t test for difference between phenotypic means of different allelic groups and marker–trait regression analysis revealed seven HvSSR and one gene-based markers to be associated with three key traits viz., grain yield, spikelet fertility and biological yield (Fig. 5). For all the other traits tested, no marker–trait association was observed.

Markers HvSSR01-66 and HvSSR02-54, lying on chromosomes 1 (28.1 Mb) and 2 (21.8 Mb), respectively, were significantly associated with grain yield per plant under low light. CG marker CAU-CG-ILA1-3 also showed significant association (Fig. 5). The R^2 values revealed that markers HvSSR01-66 and HvSSR02-54 explained 6.7 and 22.4% of the variation in grain yield under low light, respectively, whereas, R^2 value for marker CAU-CG-ILA1-3 was found to be non-significant (Fig. 5a).

Markers HvSSR02-52 (Chr # 2, 20.9 Mb position), HvSSR06-56 (Chr # 6, 25.6 Mb position), HvSSR06-69 (Chr # 6, 285 Mb position) and HvSSR09-45 (Chr # 9, 19.7 Mb

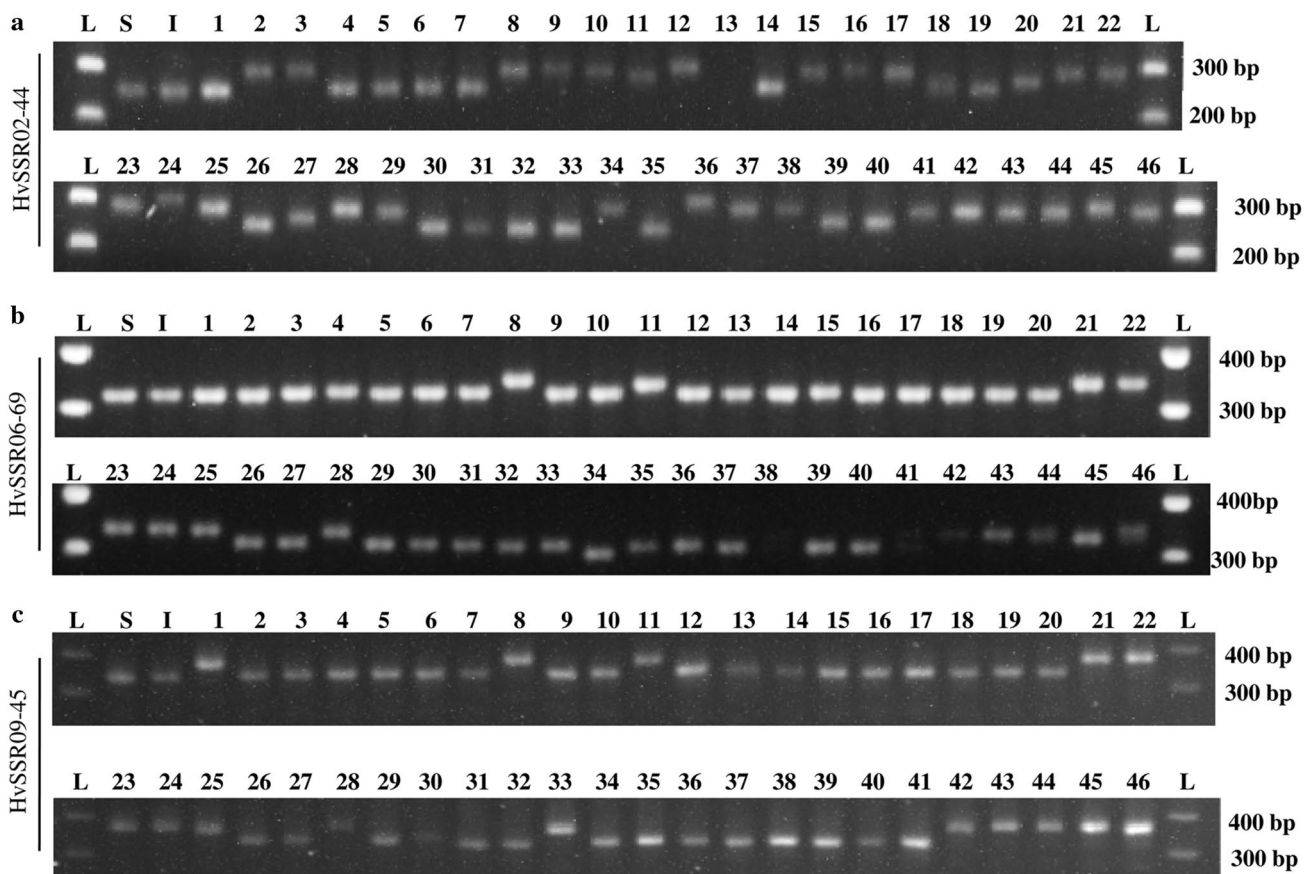


Fig. 4 Amplification profile of SSR markers **a** HvSSR02-44, **b** HvSSR06-69, **c** HvSSR09-45 on a panel of 48 rice genotypes, showing contrasting response to low-light intensity. All amplified frag-

ments were resolved on 2% agarose gel. L-100 bp DNA ladder, S-Swarnaprabha, I-IR-8 and 1–46 represent rice genotypes as mentioned in supplemental data 2

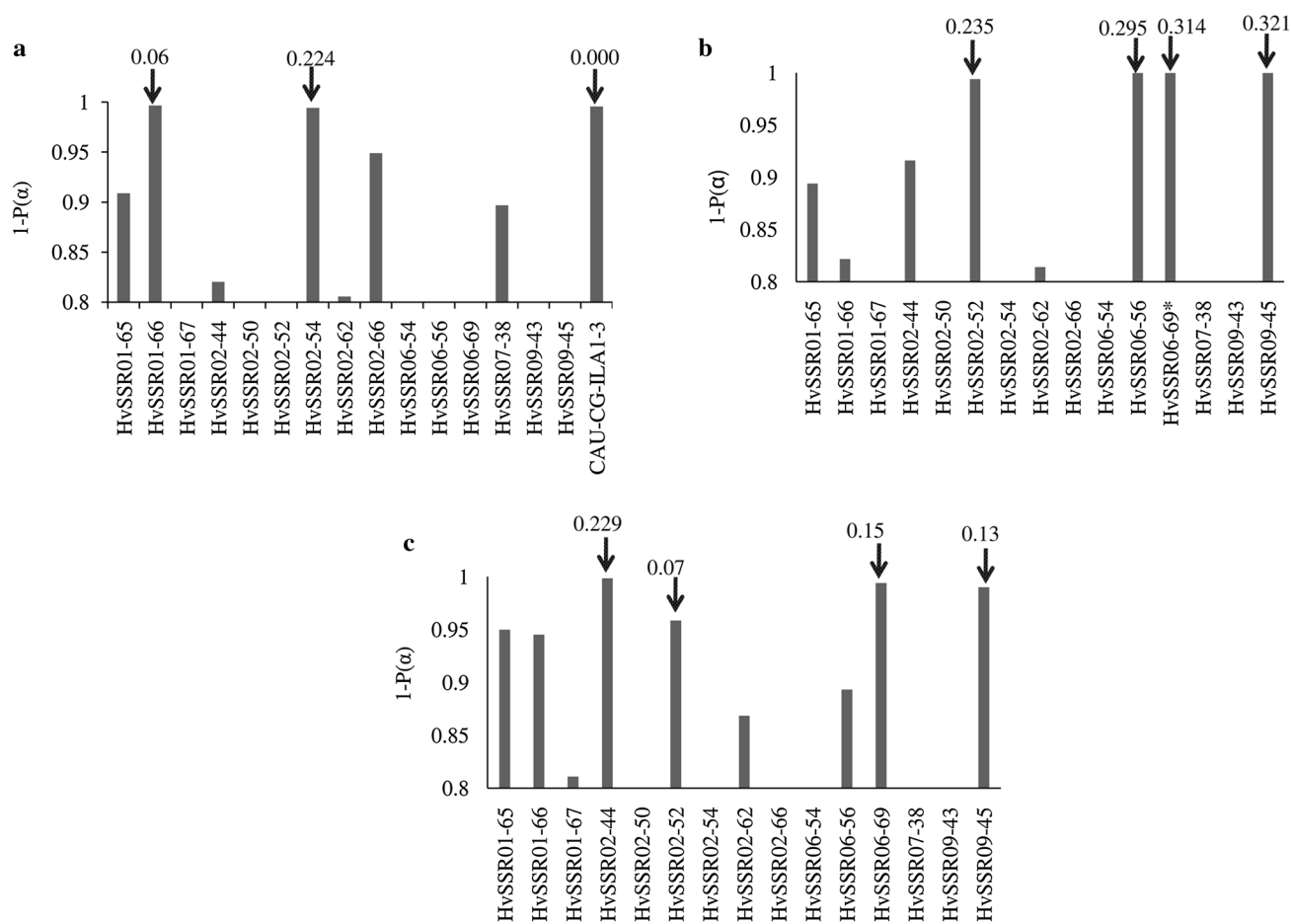


Fig. 5 Association of markers with grain yield per plant (**a**), spikelet fertility (**b**) and biological yield (**c**) in a panel of 48 rice genotypes. Assuming additive gene action, t test probability of the two homozygous genotypic groups of each marker locus being different for each trait was calculated using t test. Y -axis represents 1 minus the proba-

bility of type I error. Bold arrow: marker significantly associated with the trait. R^2 values for all the significantly associated markers showing the proportion of phenotypic variation explained in a panel of 48 contrasting genotypes are shown on top of the arrows

position) showed significant association with spikelet fertility under low light (Fig. 5b). The R^2 values for markers indicated that HvSSR02-52, HvSSR06-56, HvSSR06-69 and HvSSR09-45 explained 23.5, 29.5, 31.4 and 32.1% of the variation in spikelet fertility under low light, respectively.

For biological yield, markers viz., HvSSR02-44 (Chr # 2, 16.9 Mb position), HvSSR02-52, HvSSR06-69 and HvSSR09-45 showed significant association with R^2 values of 22.9, 7, 15.4 and 13%, respectively (Fig. 5c).

Discussion

Light intensity is a crucial parameter that affects photosynthesis and ultimately, grain yield in rice. However, no gene or marker directly associated with improved performance under low-light intensity under field conditions has been identified till date. Using an integrated approach of field

screening, in silico and wet lab validation, we have identified a set of genotypes, traits, genes and markers which can be used for future studies on low-light tolerance in rice. The aim of the study was to identify genotypes, genes and markers for improved performance under low-light intensity, so that better strategies can be designed and applied for marker-assisted breeding programmes.

Identification of tolerant genotypes and key phenotypic traits playing a role in low-light intensity tolerance

It has been suggested that solar radiation in tropics is one of the major climatic factors limiting grain yield in rice (Barmudoi and Bharali 2016). In our study, phenotyping of 110 genotypes under low-light intensity revealed that low light causes a general increase in plant height under low-light condition and a general decrease in panicle weight,

panicle length, no. of effective panicle, spikelet fertility, grain yield per plant, harvest index and 100 grain weight. Specific leaf weight also showed significant decrease under low-light condition. Grain yield reduction under low light stress was mainly attributed to decreased spikelet fertility and decreased number of grains per panicle. It has been previously suggested that low irradiance treatments in rice significantly diminished grain yield, particularly during the reproductive and ripening phases (Singh 2005). All the above observations are in confirmative with the previous observations made by Murchie et al. (2005), Liu et al. (2014), Sridevi and Chellamuthu (2015) and Barmudoi and Bharali (2016).

While comparing the difference in biochemical traits under ambient and low-light intensity, it was observed that the chlorophyll *a* content decreased under low-light condition, but the chlorophyll *b* content and total chlorophyll content was significantly increased. Previously, it has been suggested that varieties that are tolerant to low light exhibit higher chlorophyll *b* and lower chlorophyll *a/b* ratio in their leaves when compared with those that perform poorly in low light (Liu et al. 2014). In our study, the tolerant genotypes viz. like IRCTN 91-84, IRCTN 91-94, Rhylo Red, PS-4, Mahisugandh, Danteswari, Kunti and Megha Rice 1 can be harnessed further to incorporate low-light intensity tolerance in other rice genotypes showed higher chl *b* content under low light intensity.

Yield related traits like GY, BY, PN/plant and SF were identified as key traits influenced by genotype \times light interaction. Principal component analysis also identified characters like grain yield per plant, panicle weight, spikelet fertility and biological yield as important for distinguishing tolerant and susceptible genotypes. Previous studies in rice have also identified yield-related traits as significantly influenced by low light (Uga et al. 2003; Rashid et al. 2008). Our data suggests that chlorophyll content plays a major role in governing yield-related parameters as chlorophyll content showed positive correlation with spikelet fertility and grain yield. Similar correlation has been reported previously (Restrepo and Garcés 2013).

Candidate genes for low-light intensity tolerance

A large number of markers and QTLs are reported in rice. For example, mapping for low-light intensity tolerance has led to the identification of 43 QTLs and 21 QTLs are reported for spikelet fertility (<http://archive.gramene.org/ctl/>). We wanted to identify CG underlying low-light intensity tolerance, one of the key traits of relevance especially for Eastern and North-Eastern part of India. Using an in silico approach, a set of 90 rice genes were shortlisted which can be candidates for genome wide association study (GWAS). Furthermore, eight SSR markers tightly linked to three key

yield-related traits viz. GY, BY and SF have been identified. It is well known that QTL/genes for yield and adaptability to stress are often co-located (Swamy et al. 2011).

For grain yield under low light, one marker viz., HvSSR02-54 lying at position 21.8 Mb on chromosome 2 was found to be significantly associated (Fig. 6). HvSSR02-54 marker lies very close to an *ent-kaurene synthase*, chloroplast precursor gene (Os02g36210) (within 1 kb region). Studies on kaurene synthase-like gene family (*OsKSL*) in rice has suggested role of the members of this family in plant defence response (Xu et al. 2007; Tenzuka et al. 2015). Like many other abiotic stresses, there is cross-talk between light and other stresses (Liu et al. 2012; Petrillo et al. 2014). In fact, it is now proven that nuclear architecture and chromatin structure in plants undergoes dynamic changes in response to light (Perella and Kaiserli 2016).

For spikelet fertility, markers HvSSR02-52, HvSSR06-56, HvSSR06-69 and HvSSR09-45 lying on positions 20.9, 25.8, 28.5 and 19.7 Mb on chromosomes 2, 6 and 9, respectively, were significantly associated (Fig. 6). Within 6 kb of HvSSR02-52 lies a *NAC* gene (Os02g34970). Previously, a member of the rice *NAC* transcription factor family named *OsNAP*, has been associated with leaf senescence (Zhou et al. 2013). *OsNAP* overexpressing transgenic plants display accelerated leaf senescence at the grain-filling stage, but *OsNAP* RNAi transgenic plants show delayed leaf senescence. An *ACT* domain containing gene (Os02g34990) also lies close to the marker HvSSR02-52 (9 kb). This gene is reported to be differentially expressed during inflorescence and seed development. Previously, in *Arabidopsis* *ACT* domain-containing novel family has been reported to be differentially regulated by plant hormones, salt and cold stress, and light/dark treatment (Hsieh and Goodman 2002). Within 1 kb of HvSSR06-56 marker lies a *phytosulfokines 1* precursor gene (Os06g42680). In rice, this gene is expressed during inflorescence and seed development, and in iron and phosphorous interaction. Within 6.7 kb flanking region of HvSSR06-56 marker also lies a *bZIP* transcription factor domain containing gene (Os06g42690). The role of *Arabidopsis* *bZIP* HY5 protein in light response is well documented (Chattopadhyay et al. 1998). In rice, a set of unigenes and metabolites having a role in light-specific pathway has been identified (Lakshmanan et al. 2015) and these biomolecules could be potential targets for future work on low-light intensity. Within 10 kb of HvSSR06-69 lie three expressed proteins, Os06g47030 (8.5 kb), Os06g47050 (5.9 kb) and Os06g47060 (less than 1 kb). Near HvSSR06-69 (7.8 kb) also lies a *Ty3-gypsy* gene (Os06g47080) which is involved in response to bacterial leaf streak (Xu et al. 2011). Within 1.5 kb of HvSSR09-45 marker on chromosome 9 lies a *CCT/B-box zinc finger* domain containing gene (Os09g33555; OsBBX28) already associated with photoperiod (Fig. 6) (Huang et al. 2012). It has been suggested

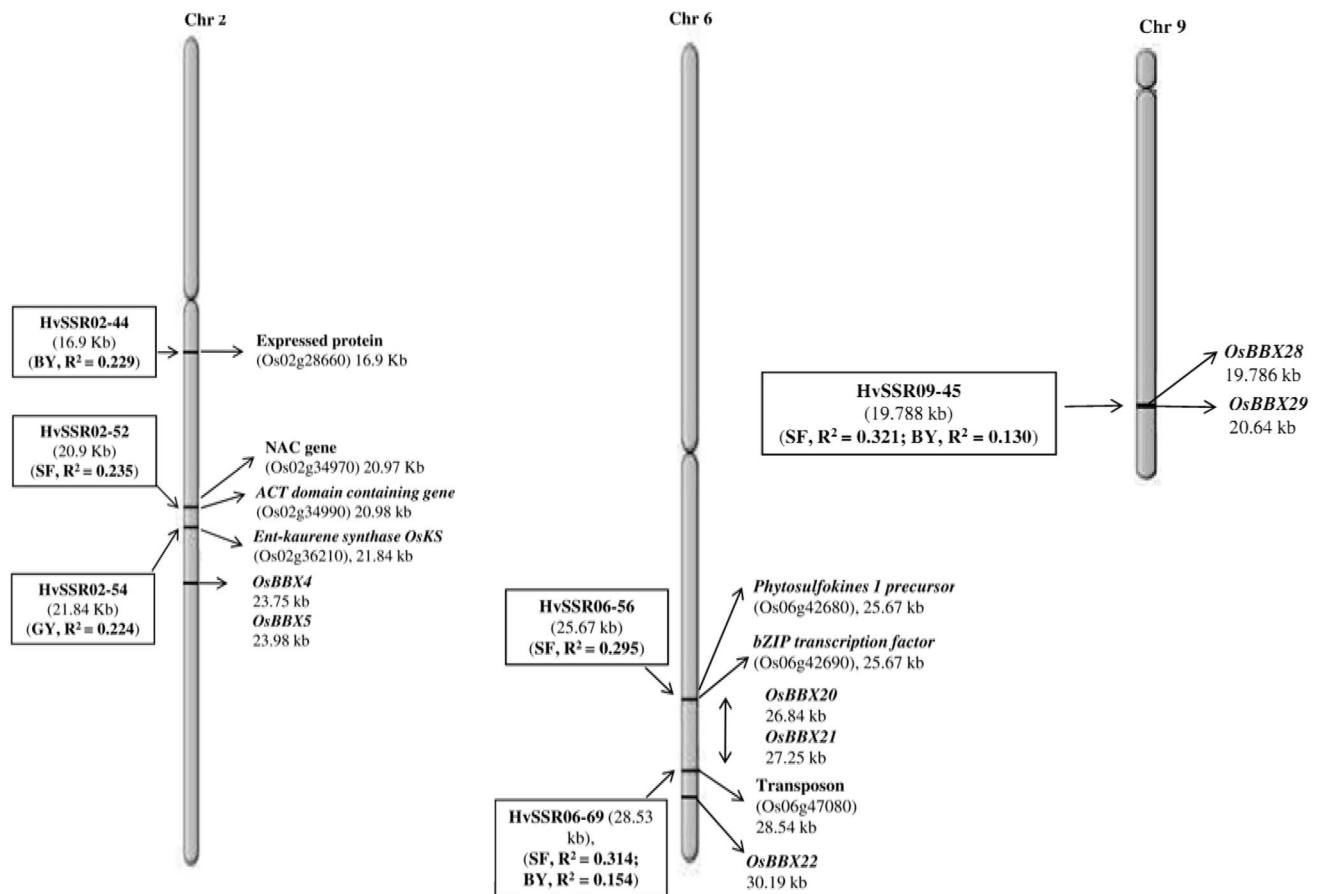


Fig. 6 Map showing the genic regions and markers identified for low-light intensity tolerance on rice chromosomes 2, 6 and 9

that out of the 30 *OsBBX* genes of rice, *OsBBX16* and *OsBBX29* can be regulated by light (Huang et al. 2012). The study also suggests that genes like *OsBBX21* and *OsBBX28* have obtained unique expression pattern during evolution. On the other hand, *OsBBX20* has redundant expression pattern when compared to its paralogs. Our marker data hints at role of *BBX* genes like 20, 21, 28 and 29 in low-light intensity (Fig. 6). These additional genes will serve as potential genic targets. Other *OsBBX* genes like *OsBBX4* and *OsBBX5* lying on chromosome 2 can also be explored. While this manuscript was in preparation, role of *AtBBX21* in enhancing photosynthesis under high-irradiance conditions in potato has come to light (Crocco et al. 2018). The contrasting role of *AtBBX21* and *AtBBX24* in photomorphogenesis by regulating *HY5* has also come to light (Job et al. 2018).

The marker–trait association revealed that three markers are significantly associated with biological yield namely, *HvSSR02-44*, *HvSSR06-69* and *HvSSR09-45*. Within 1 kb region of *HvSSR02-44* marker lies *Os02g28660*, the expression of which is associated with inflorescence and seed development (Fig. 6).

The CG primers used in this study were designed to amplify a minimum size of 750 bp to reduce the total number of primers required to span the entire gene. This strategy might have to be modified by designing primers with smaller amplicon size. Alternatively, the previous amplified products may be resolved using higher resolution agarose and/sequencing reactions to eliminate the probability of missing out smaller size polymorphisms/SNPs.

In conclusion, this study identified eight low-light intensity tolerant rice genotypes (IRCTN 91-84, IRCTN 91-94, Rhylo Red, PS-4, Mahisugandh, Danteswari, Kunti and Megha Rice 1). A set of 90 genes has been short-listed which can be targeted for future GWAS for studying light regulation in rice. Six markers tightly linked to yield related traits and seven candidate genes have been identified for MAS in response to low-light intensity. In this study, our phenotypic data suggests that the tolerant genotypes can be harnessed further to incorporate low-light intensity tolerance in other rice genotypes. These markers and the flanking/underlying genes will form the basis of future work to understand the low-light intensity tolerance mechanism. Furthermore, the markers identified

after further validation can be used for marker-assisted breeding programmes.

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Author contributions SSD performed the field evaluation and molecular experiment and wrote the manuscript. GP and JP performed the field experiment. CA and ArP supervised the field experiment. MR and AvP carried out the data analysis and interpretation. WT designed the molecular experiment, primers and revised the manuscript. MR conceptualised and designed the field and molecular experiment.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no conflicts of interest.

References

- Adhya TK, Singh ON, Ghosh A (2008) Rice in Eastern India: causes for low productivity and available options. *J Rice Res* 2(1):1–5
- Barmudoi B, Bharali B (2016) Effects of light intensity and quality on physiological changes in winter rice (*Oryza sativa* L.). *Int J Environ Agric Res* 2(3):65–76
- Chattopadhyay S, Ang LH, Puente P, Deng XW, Wei N (1998) *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. *Plant Cell* 10:673–683
- Crocco CD, Holm M, Yanovsky MJ, Botto JF (2010) AtBBX21 and COP1 genetically interact in the regulation of shade avoidance. *Plant J* 64(4):551–562
- Crocco CD, Ocampo GG, Ploschuk EL, Mantese A, Botto JF (2018) Heterologous expression of AtBBX21 enhances the rate of photosynthesis and alleviates photoinhibition in *Solanum tuberosum*. *Plant Physiol*. <https://doi.org/10.1104/pp.17.01417>
- Dkhar F, Rai M, Tyagi W (2014) Looking beyond PsTOL1: marker development for two novel rice genes showing differential expression in P deficient conditions. *J Genet* 93(2):573–577
- Flexas J, Loreto F, Medrano H (2012) Terrestrial photosynthesis in a changing environment: a molecular, physiological and ecological approach. Cambridge University Press, Cambridge
- Franklin KA (2008) Shade avoidance. *New Phytol* 179(4):930–944
- Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57(12):1332–1334
- Hsieh MH, Goodman HM (2002) Molecular characterization of a novel gene family encoding ACT domain repeats proteins in *Arabidopsis*. *Plant Physiol* 130(4):1797–1806
- Huang J, Zhao X, Weng X, Wang L, Xie W (2012) The rice B-Box zinc finger gene family: genomic identification, characterization, expression profiling and diurnal analysis. *PLoS One* 7(10):e48242
- Jiao Y, Ma L, Strickland E, Deng XW (2005) Conservation and divergence of light regulated genome expression patterns during seedling development in rice and *Arabidopsis*. *Plant Cell* 17:3239–3256
- Job N, Yadukrishnan P, Bursch K, Datta S, Johansson H (2018) Two B-box proteins regulate photomorphogenesis by oppositely modulating HY5 through diverse C-terminal domains. *Plant Physiol* 176(4):2963–2976
- Lakshmanan M, Lim SH, Mohanty B, Kim JK, Ha SH, Lee DY (2015) Unravelling the light-specific metabolic and regulatory signatures of rice through combined in silico modeling and multiomics analysis. *Plant Physiol* 169(4):3002
- Leivar P, Quail PH (2011) PIFs: pivotal components in a cellular signalling hub. *Trends Plant Sci* 16(1):19–28
- Li J, Terzaghi W, Deng XW (2012a) Genomic basis for light control of plant development. *Protein Cell* 3(2):106–116
- Li L, Ljung K, Breton G, Schmitz RJ, Pruneda-Paz J, Cowing-Zitron C, Cole BJ, Ivans LJ, Pedmale UV, Jung HS, Ecker JR, Kay SA, Chory J (2012b) Linking photoreceptor excitation to changes in plant architecture. *Genes Dev* 26(8):785–790
- Liu J, Zhang F, Zhou J, Chen F, Wang B, Xie X (2012) Phytochrome B control of total leaf area and stomatal density affects drought tolerance in rice. *Plant Mol Biol* 78(3):289–300
- Liu QH, Wu X, Chen BC, Ma JG, Gao J (2014) Effects of low light on agronomic and physiological characteristics of rice including grain yield and quality. *Rice Sci* 21(5):243–251
- Lorrain S, Allen T, Duek PD, Whitelam GC, Fankhauser C (2008) Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J* 53(2):312–323
- Murchie EH, Hubbart S, Chen YZ, Peng SB, Horton P (2005) Acclimation of photosynthesis to high irradiance in rice: gene expression and interactions with leaf development. *J Exp Bot* 56(411):449–456
- Murray HG, Thompson WF (1980) Rapid isolation of high molecular weight DNA. *Nucleic Acids Res* 8(19):4321–4325
- Perrella G, Kaiserli E (2016) Light behind the curtain: photoregulation of nuclear architecture and chromatin dynamics in plants. *New Phytol* 212:908–919
- Petrillo E, Godoy Herz MA, Barta A, Kalyna M, Kornbliht AR (2014) Let there be light: regulation of gene expression in plants. *RNA Biol* 11(10):1215–1220
- Pfalz J, Liebers M, Hirth M, Gr€ubler B, Holtzegel U, Schr€oter Y, Dietzel L, Pfannschmidt T (2012) Environmental control of plant nuclear gene expression by chloroplast redox signals. *Front Plant Sci* 3:257
- Rashid M, Cheema AA, Ashraf M (2008) Numerical analysis of variation among basmati rice mutants. *Pak J Bot* 40(6):2413–2417
- Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Plant Cell* 5:147–157
- Schmoll M, Tian C, Sun J, Tisch D, Glass N (2012) Unravelling the molecular basis for light modulated cellulose gene expression—the role of photoreceptors in *Neurospora crassa*. *BMC Genom* 13:127
- 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(2):36–37
- Singh H, Deshmukh RK, Singh A, Singh AK, Gaikwad K, Sharma TR, Mohapatra T, Singh NK (2010) Highly variable SSR markers suitable for rice genotyping using agarose gels. *Mol Breed* 25:359–364
- Sridevi V, Chellamuthu VM (2015) Impact of weather on rice—a review. *Int J Appl Res* 1(9):825–831
- Swamy BPM, Vikram P, Dixit S, Ahmed HU, Kumar A (2011) Meta-analysis of grain yield QTL identified during agricultural drought in grasses shows consensus. *BMC Genom* 12(1):319
- Tenzuka D, Ito A, Mitsunashi W, Toyomasu T, Imai R (2015) The rice ent-KAURENE SYNTHASE LIKE 2 encodes a

- functional ent-beyerene synthase. *Biochem Biophys Res Commun* 460(3):766–771
- Uga Y, Fukuta Y, Ohsawa R, Fujimura T (2003) Variations of floral traits in Asian cultivated rice (*Oryza sativa* L.) and its wild relatives (*O. rufipogon* Griff.). *Breed Sci* 53:345–352
- Venkateswarlu B, Visperas RM (1987) Solar radiation and rice productivity. IRRI Research Paper Series
- Wang Z, Zhang T, Xing Y, Zeng X, Wang L, Liu Z, Shi J, Zhu X, Ma L, Li Y, Ling Y, Sang X, He G (2016) *YGL9*, encoding the putative chloroplast signal recognition particle 43 kDa protein in rice, is involved in chloroplast development. *J Integr Agric* 15(5):944–953
- Xu M, Wilderman PR, Morrone D, Xu J, Roy A, Margis-Pinheiro M, Upadhyaya NM, Coates RM, Peters RJ (2007) Functional characterization of the rice kaurene synthase-like gene family. *Phytochemistry* 68(3):312–326
- Xu MR, Cruz CMB, Fu BY, Zhu LH, Zhou YL, Li ZK (2011) Different patterns of gene expression in rice varieties undergoing a resistant or susceptible interaction with the bacterial leaf streak pathogen. *Afr J Biotechnol* 10(65):14419–14438
- Zhou Y, Huang W, Liu L, Chen T, Zhou F, Lin Y (2013) Identification and functional characterization of a rice NAC gene involved in the regulation of leaf senescence. *BMC Plant Biol* 13:132