scientific reports

OPEN

Identification and analysis of low light responsive yield enhancing QTLs in rice

Shamba Ganguly[1,2], K. Nimitha[1,2], Shoumik Saha[1], Nilanjan Sinha Mahapatra[1], Kriti Bhattacharya[1], Rimpa Kundu[1], Sebantee Ganguly[1], Poulomi Sen[1], Arup Kumar Saha[1], Shampa Purkayastha[1], Prabir Kumar Bhattacharyya[1], Tirthankar Biswas[1] & Somnath Bhattacharyya[1]✉

Rice is one of the major food crops grown globally. However, during the wet season, rice suffers significant yield loss due to reduced light intensity caused by overcast clouds when the light intensity is only around 450–500 µmol/m²/s, compared to 1400–1800 µmol/m²/s in summer. This reduction in light intensity leads to a decrease in seed yield, mainly by limiting tiller or panicle numbers. Yield and its attributing parameters were recorded in one hundred thirty RILs for four consecutive wet seasons in ambient light (AL) and low light (LL, 35% light-cut using white shade net). QTL analysis was performed using Inclusive Composite Interval Mapping (ICIM) with all the phenotypic data and 927 polymorphic SNPs identified by the 7 K Infinium chip. The study identified a large QTL influencing panicle numbers and yield exclusively in lowlight on chromosome 1 (qPNLL1.1, qGYLL1.1) in four consecutive seasons with LOD > 10 and PVE > 30%. The favourable alleles are from the tolerant parent, Swarnaprabha. Another grain yield improving QTL was identified on chromosome 6 (qGYLL6.1), with LOD > 3 in three consecutive seasons. In a diverse rice panel of one hundred seventeen genotypes with five different models, association analysis identified the associated marker for panicle numbers and grain yield in LL, which is also the left marker of the newly identified QTLs for the traits under LL condition. A shade-responsive gene, monoculm 2 (MOC2, LOC_Os01g64660) inside the QTL on chromosome 1, upregulated in the tolerant parent and its QTL-carrying RILs, whereas repressed in the susceptible one. Therefore, due to its significant additive effect and validation across various genotypes, the yield-improving QTL on chromosome 1 can be directly utilised in marker-assisted selection (MAS) for developing shade-tolerant rice. This can also help reduce the yield gap between wet and dry-season rice.

Rice (*Oryza sativa* L.) is the most important cereal crop, providing sustenance to most people worldwide. Its yield has almost reached a plateau in most of the rice-growing areas of the world. There is limited scope for expanding rice cultivation areas in rapidly developing countries like India and China. It is becoming challenging to increase the yield potential of rice by developing new genotypes through increasing intrinsic yield potential, as evidenced by the low genetic gain during the last two decades[1]. In several parts of India, China, and Bangladesh, rice, also called wet rice, is cultivated mainly in the rainy season. One of the main reasons for the low rice production in this region is the low intensity of light during the wet season, which results in low productivity of the crop[2–4]. Rice experiences significant yield loss due to low light intensity caused by seasonal overcast clouds and global dimming[5–7]. India's eastern and northeastern hill regions contribute to 48% of the total rice production from 58% of the area under rice cultivation[8]. In the wet season, light intensity is around 500 µmol/m²/s compared to 1400 µmol/m²/s in the summer (*Boro*) season. 400 µmol/m²/s PPFD reduces 30–50%net photosynthesis, ultimately reducing yield by 34–55%[2]. This reduced light intensity also hinders grain filling and quality through reduced amylose, starch content (6–37%), grain protein content and increased chalky grain numbers[9,10].

The yield is a complex trait influenced by source and sink strength and translocation efficiency between them. Panicles per plant, grains per panicle, and seed weight are the major sink strengths directly controlling rice yield. Rice is a widely researched crop, with numerous studies on its yield and component traits conducted under various conditions, including biotic and abiotic stresses[11,12]. Although a wide genetic variation is observed

[1]Crop Research Unit, Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, India. [2]These authors contributed equally: Shamba Ganguly and K. Nimitha. ✉email: bhattacharya.somnath@bckv.edu.in; somnathbhat@yahoo.com

among rice genotypes for shade tolerance ability, its genetic or physiological mechanism is not unveiled except in a few with inconclusive transcriptome analysis[8,9]. Many low-light-tolerant genotypes, including Swarnaprabha, Rudra, and Purnendu, have been reported earlier[13], but the tolerance mechanisms vary depending on the genotype. Furthermore, no universal shade tolerance mechanism is observed; genotype-specific plasticity significantly reduces yield loss in low light intensity[13]. However, low-light intensity grown rice showed reduced panicle number compared to other yield-attributing parameters in almost all the genotypes. No studies have been conducted to identify the genomic region controlling rice yield loss under reduced light intensity in rice-growing countries, including India. Improving the productivity of wet season rice is hindered by a poor understanding of the genetics of traits that alleviate yield and panicle number loss in low light intensity. Some tolerant genotypes maintain almost the same number of tiller and panicle in open and shade, while a few maintain equal grains per panicle and seed weight[2]. It is also unclear whether the QTLs previously identified in ambient light are involved in yield enhancement in low light. Genetic analysis of low-light tolerant genotypes, including identifying QTLs and genomic regions controlling yield loss in low-light conditions, can aid in the development of high-yielding, low-light tolerant varieties of rice, which can improve yield potentiality in the wet season by reducing the yield gap between the wet and summer rice.

Therefore, to identify QTLs related to low light (LL) tolerance in Swarnaprabha (SP), the study was conducted where a RIL population resulting from a cross between SP and IR64 (LL susceptible genotype) was used as a mapping population. The mapping used yield components like panicle number, yield per plant, test weight, panicle weight, seed per panicle for four seasons and genotyping data of a 7 K SNP array. The further association analysis of a panel of one hundred seventeen genotypes was used to validate the QTLs identified in the RIL population.

Results

Parental differences: Swarnaprabha vs IR64

The performance of Swarnaprabha (SP) and IR64 were compared both in the open field (Ambient light, AL) and under the white net (35% light cut, LL) for four consecutive wet seasons (*Kharif)*: 2019, 2020, 2021, and 2022 (Supplementary Table 2). The Mean grain yield of four years in Swarnaprabha (539 g/m²) was higher than IR64 (450.8 g/m²). The thousand seed weight (TW) of Swarnaprabha was higher in all four years and is the main reason for a marginally higher yield than IR64. In ambient light, the average number of panicles (PN) for the variety SP was 12.6, and for IR64 it was 11.1. In low light, SP had a 19% reduction in panicle number and a remaining PN of 10.2, while IR64, with a 35.7% reduction, had only 7.1 panicles. One hundred thirty RILs derived from SP x IR64 showed a wide range of variations with continuous frequency distribution for all the yield-attributing parameters (Supplementary Table 3). The mean values for all the traits in all four seasons are significantly reduced in LL compared to the AL condition (Table 1).

All five traits showed several extreme values, either larger or smaller than those of the parents, i.e., bi-directional transgressive segregation was observed for all the traits. Analysis of variance confirmed the significant variations among the RILs for all the traits (Supplementary Table 4). The interaction between the RILs and the years has the predominant contribution to the total variation of PN and GY on ambient conditions, where the highest contributions are by the PN and GY under LL. The effect of RILs and the RILs x years interaction contributed almost equally to the total variations in PW and GPP.

QTL mapping

2268 SNPs were considered good data based on the parameters described in the methodology, and 927 SNPs showed polymorphism between two parents, and more than fifty minor alleles were observed for all. So, finally, 927 SNPs were considered for linkage map construction. The polymorphic SNPs were evenly distributed throughout the genome (Supplementary Fig. 1). (Chromosome 1 contains 124 SNPs; chromosome 2 contains 71 SNPs; chromosome 3 contains 65 SNPs; chromosome 4 contains 105 SNPs; chromosome 5 contains 81 SNPs; chromosome 6 contains 75 SNPs; chromosome 7 contains 82 SNPs; chromosome 8 contains 81 SNPs; chromosome 9 contains 36 SNPs; chromosome 10 contains 57 SNPs; chromosome 11 contains 99 SNPs and on chromosome 12 contains 51 SNPs). This linkage map covers the 2024 cM linkage distance of the total rice genome with a marker density of 1 SNP per 2.2 cM of the rice genome. QTL mapping was carried out using the year-wise data separately, along with the mean data of all seasons. For each season, mapping was performed separately for AL and LL conditions. The linkage map construction was followed by QTL analysis using Inclusive Composite Interval Mapping (ICIM software, IciMapping, Version4.0) with mapping parameters, i.e., step (1 c M) 1.0 and PIN 0.00100. A minimum of LOD 3 was considered for being a putative QTL (Supplementary Fig. 2). Forty four putative QTLs for all the yield-attributing parameters were identified in ambient and low light (Table 2). Exclusively, a major QTL on chromosome 1 was identified in low light conditions for PN and GY (qPNLL1.1 and qGYLL1.1,), where Swarnaprabha contributes favourable alleles. The locus was between 35825579 bp and 37692801 bp with 3 SNPs, 1163456, SNP-1.37415410. and 1212517 inside the QTL. When the four-year mean value was considered, qPNLL1.1 explained 47% of the total phenotypic variance with LOD 19, where the additive effect was 1.1. As expected, the same locus also contains yield-enhancing QTL, qGYLL1.1, which explains 38.8% PVE at LOD 14.3. Another region on chromosome 6 between the marker interval of 5950897-5951985 and physical interval of 4641044-4680281 bp was identified for GY under LL (qGYLL6.1) with an average LOD 6.4 and PVE% of 10.3 in three consecutive seasons as well as from the mean data. The study also identified two TW-improving QTLs in lowlight located on chromosomes 1 and 7, where favourable alleles from both the parents SP and IR 64.

Trait	Season	Light	Mean ± sd	SE	Range	Kurtosis	Skewness	% Reduction
Per plant grain yield (g)	Wet-19	Ambient	16.8 ± 4.8	0.4	8.9–35.4	1.5	1	43.8
	Wet-19	Lowlight	9.5 ± 4.1	0.4	3.6–22.8	0.3	1	
	Wet-20	Ambient	20.3 ± 5.7	0.5	5.8–37.3	0.8	0.8	48.3
	Wet-20	Lowlight	10.5 ± 4.3	0.4	3.7–25.3	0.7	0.9	
	Wet-21	Ambient	26.5 ± 6	0.5	14.7–46.3	0.1	0.3	53.2
	Wet-21	Lowlight	11.7 ± 4.5	0.4	4.2–24.0	-0.3	0.6	
	Wet-22	Ambient	23.1 ± 5.9	0.5	10.5– 41.0	0.1	0.5	45.9
	Wet-22	Lowlight	12.6 ± 4	0.4	5.9–29.2	2	1.1	
	MEAN	Ambient	21.7 ± 4	0.4	13.4–34.8	0.8	0.8	48.2
	MEAN	Lowlight	11.3 ± 3.5	0.3	5.7–23.5	1.4	1.1	
Panicles/plant	Wet-19	Ambient	8.2 ± 2.2	0.2	4–15.3	0.6	11.3	28.1
	Wet-19	Lowlight	6.4 ± 1.8	0.2	3–11.3	0.6	8.3	
	Wet-20	Ambient	8.2 ± 1.8	0.2	4.3–13.3	0.6	9	34.4
	Wet-20	Lowlight	6.1 ± 2	0.2	2.4–12.1	0.7	9.7	
	Wet-21	Ambient	9 ± 1.9	0.2	3.7–14.7	0.1	11	40.6
	Wet-21	Lowlight	6.4 ± 1.9	0.2	3–12.2	0.7	9.2	
	Wet-22	Ambient	8.7 ± 2	0.2	4–15.3	0.3	11.3	33.8
	Wet-22	Lowlight	6.5 ± 1.7	0.2	3–12	0.8	9	
	MEAN	Ambient	8.5 ± 1.4	0.1	5.7–13.1	0.4	7.5	34.9
	MEAN	Lowlight	6.3 ± 1.6	0.1	3.8–11.2	1	7.4	
Per panicle weight (g)	Wet-19	Ambient	1.9 ± 0.5	0.1	0.7–3.8	0.7	3	15.8
	Wet-19	Lowlight	1.6 ± 0.6	0.1	0.7–3.6	0.7	2.9	
	Wet-20	Ambient	2.6 ± 0.9	0.1	0.8–6.3	1.2	5.6	30.8
	Wet-20	Lowlight	1.8 ± 0.5	0.1	0.7–3.7	0.8	3	
	Wet-21	Ambient	3.1 ± 0.8	0.1	1–6.5	0.7	5.5	32.3
	Wet-21	Lowlight	2.1 ± 0.6	0.1	0.8–3.5	0.1	2.6	
	Wet-22	Ambient	2.9 ± 0.9	0.1	1.2–7.1	1	5.9	27.6
	Wet-22	Lowlight	2.1 ± 0.6	0.1	0.8–3.9	0.6	3	
	MEAN	Ambient	2.6 ± 0.6	0.1	1.5–5.6	1.3	4.1	26.9
	MEAN	Lowlight	1.9 ± 0.4	0	1–2.9	0.3	1.8	
Grains/panicle	Wet-19	Ambient	78.7 ± 22.4	2	31.3–173.6	1	142.2	6.5
	Wet-19	Lowlight	73.6 ± 29.6	2.6	27.7–175	0.9	147.3	
	Wet-20	Ambient	107 ± 37.7	3.3	29.1–249.5	1	220.5	25.1
	Wet-20	Lowlight	80.1 ± 23.6	2.1	33–158	0.6	125	
	Wet-21	Ambient	126 ± 32.1	2.8	43.9–254.9	0.6	211	26.5
	Wet-21	Lowlight	92.6 ± 26.6	2.3	36.7–159.7	0.2	123	
	Wet-22	Ambient	118 ± 39.4	3.5	43.4–282.3	1	238.8	22.1
	Wet-22	Lowlight	91.9 ± 27.6	2.4	36.7–175.7	0.6	139	
	MEAN	Ambient	108 ± 24.8	2.2	59–221.7	1.1	162.8	21.7
	MEAN	Lowlight	84.6 ± 18.2	1.6	48.1–131.5	0.3	83.4	
Thousand seed weight (g)	Wet-19	Ambient	24.5 ± 2.2	0.2	16–30.2	− 0.3	14.2	9.0
	Wet-19	Lowlight	22.3 ± 2.5	0.2	16–27.8	− 0.2	11.8	
	Wet-20	Ambient	24.6 ± 2.2	0.2	16.2–30	− 0.3	13.8	8.9
	Wet-20	Lowlight	22.4 ± 2.4	0.2	16.3–27.4	− 0.2	11.1	
	Wet-21	Ambient	24.5 ± 2.2	0.2	16.1–30.1	− 0.3	14	9.0
	Wet-21	Lowlight	22.3 ± 2.7	0.2	15.9–28.3	0.1	12.4	
	Wet-22	Ambient	24.5 ± 2.3	0.2	15.9–30.3	− 0.3	14.4	8.6
	Wet-22	Lowlight	22.4 ± 2.5	0.2	16.8–28	− 0.1	11.2	
	MEAN	Ambient	24.5 ± 2.2	0.2	16.1–30.2	− 0.3	14.1	9.0
	MEAN	Lowlight	22.3 ± 2.5	0.2	16.2–27.9	− 0.1	11.6	

Table 1. Descriptive statistics of the major yield attributing parameters in 130 RILs and the percentage of reduction under low light compared to ambient light for each trait.

QTL	Trait	Ch	Left Marker	Position (bp)	RightMarker	Position (bp)	Effect	WET 2019 LOD	PVE (%)	Add	WET 2020 LOD	PVE (%)	Add	WET 2021 LOD	PVE (%)	Add	WET 2022 LOD	PVE (%)	Add	Mean of four seasons LOD	PVE (%)	Add
qPN_AL1.1	PN_AL	1	168485	5354306	170435	5408523	IR 64				3.4	11.8	0.6									
qPN_AL1.2	PN_AL	1	693369	20706894	707007	21106769	IR 64	6.5	15.5	1.0												
qPN_AL2.1	PN_AL	2	2362152	30956323	2383802	31914486	SP	3.8	8.7	0.7												
qPN_AL4.1	PN_AL	4	SNP-4.437072	438075	SNP-4.658734	659736	SP							2.9	10.0	0.6						
qPN_AL9.1	PN_AL	9	SNP-9.21772633	21773115	9865892	21885499	SP	4.9	11.5	0.8												
qPN_LL1.1	PN_LL	1	1163456	35825579	SNP-1.37415410	37416454	SP	16.3	41.4	1.2	14.3	38.5	1.2	10.1	30.1	1.1				19.2	47.7	1.1
qPN_LL1.1	PN_LL	1	SNP-1.37415410	37416454	1212517	37692801	SP										10.3	30.9	1.0			
qPN_LL3.1	PN_LL	3	2543372	2794807	2551624	3192451	IR 64	3.8	7.6	0.5	3.9	8.4	0.6							3.2	5.7	0.4
qGY_LL1.1	GY_LL	1	1163456	35825579	SNP-1.37415410	37416454	SP							10.7	19.0	2.3	4.5	14.3	1.3	14.3	38.8	2.1
qGY_LL1.1	GY_LL	1	SNP-1.37415410	37416454	1212517	37692801	SP	11.4	33.4	2.5	10.2	30.4	2.2									
qGY_LL5.1	GY_LL	5	id5013231	27289052	5747652	27531788	SP							3.0	4.6	1.2	3.8	11.7	1.3	4.6	10.3	1.1
qGY_LL6.1	GY_LL	6	5950897	4641044	5951985	4680281	SP				4.5	11.8	1.4	3.7	5.7	1.3	4.9	15.3	34.2	3.4	10.3	0.2
qPW_AL1.1	PW_AL	1	214137	6829682	223608	7155401	SP															
qPW_LL6.1	PW_LL	6	5950897	4641044	5951985	4680281	SP										2.6	8.9	0.2	3.5	12.0	0.1
qPW_LL6.2	PW_LL	6	5958262	4887859	5965570	5172991	SP															
qGPP_AL1.1	GPP_AL	1	214137	6829682	223608	7155401	SP							3.1	8.9	10.0	3.4	10.3	13.4	4.5	12.8	9.2
qGPP_AL4.1	GPP_AL	4	4230805	15944977	ud4001319	18706317	SP							3.6	10.5	11.3	3.1	8.8	12.9	4.1	11.4	9.0
qGPP_LL4.2	GPP_LL	4	SNP-4.33436480	33621598	SNP-4.33515854	33700973	SP										2.7	7.1	8.1			
qTW_AL1.1	TW_AL	1	1163456	35825579	SNP-1.37415410	37416454	SP				3.9	4.3	0.6									
qTW_AL1.2	TW_AL	1	SNP-1.37834974	37836018	1243398	38847770	SP	9.1	10.7	1.0										7.4	11.2	1.0
qTW_AL2.1	TW_AL	2	id2007797	19981768	2017530	20260555	SP	8.6	9.8	1.0				5.7	8.1	0.8	5.9	8.0	0.9	5.6	8.0	0.8
qTW_AL2.2	TW_AL	2	id2008866	2362152	2267750	27548893	SP	11.9	17.3	1.3												
qTW_AL2.3	TW_AL	2	2362152	30956323	2383802	31914486	IR 64	3.3	3.6	0.5				6.7	10.0	0.9	6.1	8.7	0.9			
qTW_AL3.1	TW_AL	3	id3003535	5983456	2628805	6366944	IR 64	6.7	7.4	0.8				6.1	8.8	0.8	6.4	8.9	0.9	4.8	6.8	0.7
qTW_AL3.2	TW_AL	3	SNP-3.9896907	98897972	id3005216	10093744	IR 64	7.2	8.1	0.8												

Continued

QTL	Trait	Ch	Left Marker	Position (bp)	RightMarker	Position (bp)	Effect	WET 2019			WET 2020			WET 2021			WET 2022			Mean of four seasons		
								LOD	PVE (%)	Add	LOD	PVE (%)	Add	LOD	PVE (%)	Add	LOD	PVE (%)	Add	LOD	PVE (%)	Add
qTW_AL4.1	TW_AL	4	4146180	13257606	4159544	13551673	IR 64	3.4	3.6	0.6												
qTW_AL5.1	TW_AL	5	4945316	4589783	SNP-5.5362675	5362698	SP	7.6	10.4	0.9	11.4	14.8	1.1	7.6	13.5	1.0	6.4	11.5	1.0	6.8	11.3	0.9
qTW_AL5.2	TW_AL	5	id5013231	27289052	5747652	27531788	SP	6.4	6.9	0.8												
qTW_AL7.1	TW_AL	7	6986098	1058718	6990305	1195861	IR 64	4.8	5.0	0.6												
qTW_AL8.1	TW_AL	8	8956374	24903648	8959207	25004745	SP							5.0	7.2	0.7	6.5	9.0	0.9			
qTW_AL8.2	TW_AL	8	8964581	25157676	id8006926	25209532	SP				5.0	5.4	0.7									
qTW_LL1.1	TW_LL	1	SNP-1.5867020	5868021	188269	5982772	SP	5.9	11.7	0.8	9.7	22.3	1.1	5.1	8.4	0.8	4.7	7.9	0.7	9.9	22.6	1.2
qTW_LL4.1	TW_LL	4	4075613	11331474	4119706	12589729	SP							3.7	6.1	0.7						
qTW_LL4.2	TW_LL	4	4075613	11331474	4119706	12589729	SP										3.6	6.3	0.7			
qTW_LL5.1	TW_LL	5	4814140	313688	4821710	632216	IR 64	5.3	8.8	0.8												
qTW_LL5.2	TW_LL	5	4987236	5789766	4993759	5956294	SP							9.2	11.7	1.1	8.5	15.1	1.1	8.9	11.3	1.1
qTW_LL5.3	TW_LL	5	4993759	5956294	4996358	6040749	SP										8.4	15.4	1.0			
qTW_LL5.4	TW_LL	5	SNP-5.6448200	6448261	wd5000542	6544781	SP	9.1	15.8	1.1												
qTW_LL7.1	TW_LL	7	6998977	1558687	SNP-7.1688372	1689372	IR64				4.4	5.1	0.8							4.1	4.8	0.8
qTW_LL7.2	TW_LL	7	7072228	4434376	7089136	5003396	IR 64	4.5	7.5	-0.7												
qTW_LL7.3	TW_LL	7	7910596	26898645	SNP-7_26972908	26973903	IR 64	5.2	8.5	0.8	11.2	15.3	1.3	8.3	15.4	1.2	8.1	15.5	1.1	11.8	16.2	1.4

Table 2. The trait-specific QTLs, their locations LOD, phenotypic variation explained (PVE), and additivity (Add) are given for season-wise (WET 2019, 2020, 2021, 2022) and the mean data. *GY* Per plat grain yield (g), *PN* Panicle number/plant, *PW* panicle weight, *GPP* Grains per panicle, *PW* panicle weight, *TW* Thousand-grain weight.

Validation of QTLs in an association panel

One hundred seventeen genotypes' yield and attributing traits reduced significantly in low light compared to AL (Supplementary Table 5). In the LOSS curve, the half decay distance of this association panel at the arbitrary nominal level of $r^2 = 0.10$ was found to be 403032 (Supplementary Fig. 3). The identified QTNs in at least two models are shown in Table 3. SNP 1163456 on chromosome 1 was associated with low light-responsive PN and GY in almost all four models (Table 3). In two models, another QTN (SNP 5951985), was identified for GY and PN on chromosome 6.

The regression analysis using the allelic variants of SNP 1163456 on chromosome 1 showed a significant deviation from zero for the lowlight-responsive GY but not in ambient light (Fig. 1). On the other hand, when PN was considered for the regression analysis, both lowlight ambient light responsive regression curves deviated significantly from zero but in contrasting directions.

Predicting shade-responsive genes inside newly identified QTLs

A stable QTL on chromosome 1, improving panicle and yield in lowlight (qPNLL1.1 and qGYLL1.1), was considered for further genome analysis. The marker interval between 1163456 and 1212517 was between the physical distance from 35825579 to 37692801nt. Exploring the Rice Genome Annotation Project database, ten transcripts were selected based on their expression in leaf, culm or shoot apical meristem region from RNA-Seq FPKM Expression Values, (Supplementary Table 6).

Relative transcript abundance of the selected genes in seven-day-old flag leaves (Table 4) and shoot apical meristem (Table 5) of the AL and LL-grown plants was compared through qRT-PCR. The analysis was first done in parental pairs, i.e., Swarnaprabha and IR64. In flag leaves, the relative expression of *MOC2, PsbS1* and *qSH1* genes were downregulated in shade in both the parents. On the other hand, in the apical meristem, *DUF668-1, AUX1,* and *qSH1* genes showed upregulation in the shade for both parents. However, *MOC2* was upregulated in shade-grown Swarnaprabha compared to ambient, but the repression was observed in IR64. The transcript availability of *DGP1* was higher in shade-grown IR64, but *MOC2* transcript availability in apical meristem was

Traits	QTN	Chr	Position	p-value	Min Allele	Add	Model
GY_LL	1163456	1	35825579	9.33E−09	0.37	−2.1	BLINK
	1163456	1	35825579	1.86E−09	0.37	−1.6	FarmCPU
	1163456	1	35825579	9.65E−06	0.37	1.7	IIIVmrMLM
	1163456	1	35825579	7.40E−06	0.37	−1.6	FASTmrMLM
PN_LL	1163456	1	35825579	2.10E−09	0.37	0.7	BLINK
	1163456	1	35825579	6.71E−07	0.37	0.5	FarmCPU
	1163456	1	35825579	5.46E−05	0.37	0.5	IIIVmrMLM
	1163456	1	35825579	0.00017	0.37	−0.42	mrMLM
	1163456	1	35825579	8.91E−05	0.37	−0.38	FASTmrMLM
PN_LL	465718	1	14375630	4.95E−07	0.19	1.2	BLINK
	465718	1	14375630	6.71E−07	0.19	1	FarmCPU
GPP_LL	SNP-2.25351027	2	25356897	1.35E−07	0.13	26.5	BLINK
	SNP-2.25351027	2	25356897	1.90E−05	0.13	20.34	mrMLM
TW_AL	SNP-3.16841419	3	16842546	1.40E−06	0.39	1.8	FarmCPU
	SNP-3.16841419	3	16842546	2.05E−07	0.39	2.1	IIIVmrMLM
GY_AL	SNP-4.3704513	4	3708908	2.95E−06	0.25	2.2	BLINK
	SNP-4.3704513	4	3708908	1.34E−06	0.25	1.8	IIIVmrMLM
GPP-LL	3956648	4	7825479	1.14E−04	0.43	9.1	mrMLM
PW_LL	3956648	4	7825479	2.03E−07	0.43	10.1	FASTmrMLM
	3956648	4	7825479	4.21E−06	0.43	14.2	mrMLM
GY_LL	5951985	6	4680281	2.07E−10	0.17	3	FarmCPU
PN_LL	5951985	6	4680281	5.59E−06	0.17	2.61	FASTmrMLM
	5951985	6	4680281	6.71E−07	0.17	0.5	FarmCPU
PW_AL	wd7002824	7	19590130	1.78E−08	0.10	0.7	BLINK
	wd7002824	7	19590130	3.10E−10	0.10	0.6	FarmCPU
TW_LL	9657687	9	14851680	2.31E−07	0.33	1.90	mrMLM
	9657687	9	14851680	8.31E−05	0.33	1.34	FASTmrMLM
TW_LL	id12002740	12	6819391	6.54E−05	0.14	−1.83	FASTmrMLM
	id12002740	12	6819391	6.69E−05	0.14	−1.42	mrMLM

Table 3. The GWAS-based QTNs identified in at least two models in low light (LL) and ambient light (AL), their chromosomal positions (Chr), minor allele frequency (MAF), and the additive effect. *GY* Per plat grain yield (g), *PN* Panicle number/plant, *GPP* Grains per panicle, *PW* panicle weight, *TW* Thousand-grain weight.
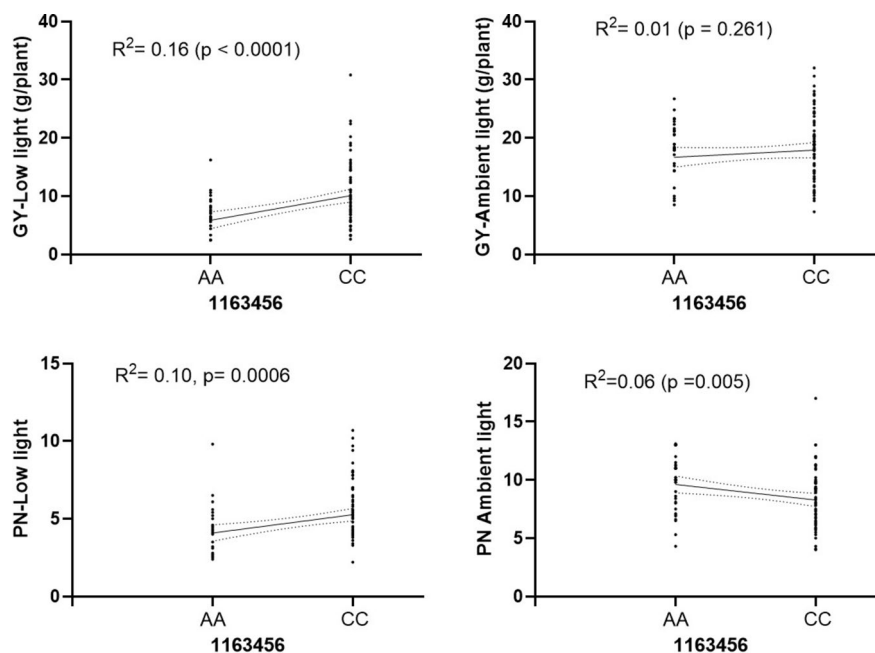
Fig. 1. The regression curve between allelic variants of the SNP-1163456 and grain yield (GY) and panicle number (PN) in low and ambient low light.

Gene	SP(AL)	SP(LL)	SP(AL) vs. SP (LL)	IR64(AL)	IR64(LL)	IR64 (AL) vs. IR64 (LL)	SP(AL) vs. IR64 (AL)	SP (LL) vs. IR64 (LL)
DGP1	0.51 ± 0.03	0.76 ± 0.18	ns	0.93 ± 0.07	0.15 ± 0.03	***	**	ns
DUF668-1	0.78 ± 0.32	1.52 ± 0.41	*	1 ± 0.27	0.84 ± 0.15	ns	ns	ns
MOC2	0.92 ± 0.19	0.49 ± 0.17	*	1.02 ± 0.18	0.34 ± 0.07	**	ns	ns
MPG1	0.8 ± 0.23	0.31 ± 0.52	ns	1 ± 0.47	1.12 ± 0.16	ns	ns	ns
NAC59	0.67 ± 0.16	1.99 ± 0.78	**	1 ± 0.19	0.96 ± 0.56	ns	ns	ns
OsAUX1	1.01 ± 0.23	0.73 ± 0.37	ns	1 ± 0.12	1.06 ± 0.11	ns	ns	ns
PP2C9	0.89 ± 0.29	1.61 ± 0.35	ns	1 ± 0.53	1.93 ± 0.28	ns	ns	ns
PSBS1,	0.79 ± 0.17	0.26 ± 0.11	**	1.02 ± 0.23	0.33 ± 0.03	**	ns	ns
qSH1	1.45 ± 0.02	0.63 ± 0.05	**	1 ± 0.1	0.51 ± 0.34	*	ns	ns

Table 4. Transcript abundance of selected genes in flag leaves of ambient light (AL) and lowlight (LL)-grown Swarnaprabha (SP) and IR64. The significant difference is indicated by * (<0.05), ** (<0.001) and *** (<0.0001).

Gene	Chr	SP(AL)	SP(LL)	SP(AL) vs. SP (LL)	IR64(AL)	IR64(LL)	IR64 (AL) vs. IR64 (LL)	SP(AL) vs. IR64 (AL)	SP (LL) vs. IR64 (LL)
DGP1	1	1.34 ± 0.24	2.74 ± 0.18	*	1 ± 0.09	16 ± 1.2	***	ns	***
DUF668-1	1	0.84 ± 0.16	5.02 ± 0.82	**	1 ± 0.2	3.61 ± 0.95	*	ns	ns
MOC2	1	0.94 ± 0.21	1.78 ± 0.37	*	1 ± 0.16	0.27 ± 0.03	*	ns	**
MPG1	1	0.52 ± 0.13	0.86 ± 0.35	ns	1 ± 0.2	1.25 ± 0.18	ns	*	ns
NAC59	1	0.16 ± 0.01	0.08 ± 0.01	ns	1 ± 0.14	0.08 ± 0	***	***	ns
OsAUX1	1	0.66 ± 0.13	1.67 ± 0.1	**	1 ± 0.14	1.45 ± 0.17	ns	ns	ns
PP2C9	1	1.56 ± 0.15	1.48 ± 0.45	ns	1 ± 0.12	2.11 ± 0.87	*	*	ns
PSBS1,	1	0.41 ± 0.07	0.47 ± 0.09	ns	1 ± 0.37	0.31 ± 0.04	**	*	ns
qSH1	1	0.74 ± 0.15	1.79 ± 0.16	**	1 ± 0.13	1.9 ± 0.34	**	ns	ns

Table 5. Transcript abundance in the apical meristem of selected genes from the yield and panicle improving QTL of chromosome 1. The significant difference is indicated by * (<0.05), ** (<0.001) and *** (<0.0001).
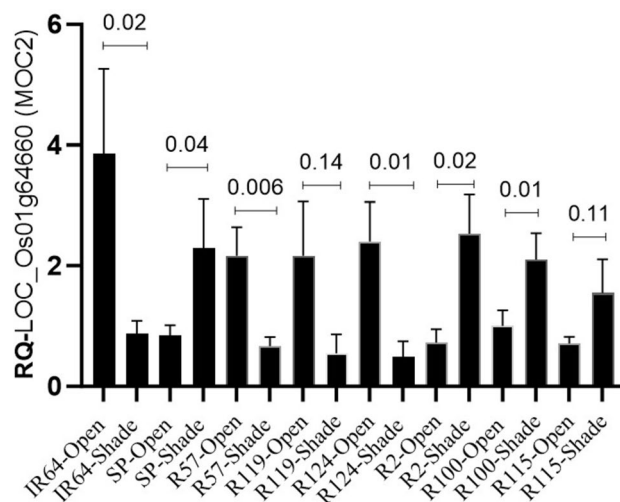
Fig. 2. The relative transcript abundance of the MOC2 gene in open and shade-grown apical meristem in IR64 and Swarnaprabha (SP) and three RILs, R57, R119 and R124. p-value of the t-test between open and shade-grown plants is given above.

Table 6. Types and position of the nucleotide polymorphism between SP and IR64 and its effect on amino acid (AA) modifications.

MSU ID	Gene	Size (nt)	Type of the polymorphism	Position (nt)	Genomic position	AA change	SP	IR64
LOC_Os01g62060	DGP1	1158	SNP	856	3rd exon	E-V	T	A
LOC_Os01g64660	MOC2	2830	InDel	1613	7th intron	–	2 bp deletion (AA)	AA
LOC_Os01g64660	MOC2	2830	SNP	2243	10th intron	–	C	A
LOC_Os01g64660 Promoter	MOC2	1000	InDel	777 bp Upstream	Upstream	–	GA insertion	
LOC_Os01g64660 Promoter	MOC2	1000	SNP	735 bp Upstream	Upstream	–	A	T
LOC_Os01g64660 Promoter	MOC2	1000	SNP	730 bp Upstream	Upstream	–	A	G

higher in Swarnaprabha compared to IR64. Three RILs with SP-carrying QTL showed upregulation in the shade compared to their open-grown, whereas downregulation was observed in RILs carrying IR64 alleles (Fig. 2).

A nucleotide sequence comparison of the *DGP1* gene between SP and IR64 identified an SNP in 856th nt on the 3rd exon, which has the potential to convert an amino acid Glu to Val. MONOCULM 2 (MOC 2, LOC_Os01g64660) carries two nucleotide polymorphisms between SP and IR64. There are two nucleotide indel at 1613 nt at the 7th intronic region of the gene and a single base pair polymorphism (C/A) at 2243 bp at the 10th intronic region (Table 6). There are also three polymorphisms in the upstream regulatory element of MOC2. A 2 bp in/del is observed in SP at 777 bp upstream of the gene. There is one SNP at – 735 bp upstream (A/T) and another at – 730 bp upstream (A/G) (Table 6).

Discussion

Rice is usually grown in the rainy season across the globe. In several countries, prolonged overcast clouds affect rice production during the growing season. In Eastern Indian states, the mean yield of summer rice is almost double that of wet rice (kharif)[14]. One of the reasons for a huge yield gap is the low light intensity during the main rice-growing season. Although lowlight responsive rice genotypes were identified, their analysis is yet to be deciphered[2,13]. Thus, the study aims to identify the robust QTLs and predicted genes from the QTL so that a breeder can use them in future rice breeding programs. Earlier reports identified that lowlight-grown rice reduces panicle numbers drastically[2]. So, based on the earlier observation, a contrasting photoperiod-insensitive parental pair, Swarnaprabha and IR64, is considered. The differences in yield and its attributing parameters between the two parents were minimal when grown under ambient light (AL). However, when grown in lowlight, GY (SP vs IR64:: 25% vs 48%) and PN (SP vs IR64:: 19% vs 36%) reduction were significantly less in SP compared to IR64. Thus, the RIL population (F9) developed from Swarnaprabha x IR64 was ideal for mapping the yield-enhancing traits in low light (35% light cut). In this study, the performance of rice genotypes in low light was assessed by covering the field with a white shade net (35% light cut), thus preventing the absorption of any specific colour from the natural sunshine. Also, shade-net covering was made by keeping a 1.5mt gap between the field and

the net, which confirms the insignificant differences in the relative humidity and temperature between open and shade-net-grown plants. The QTL mapping was conducted to minimize the environmental effect by taking the four-year's data for yield and its component characters in both AL and LL. The SNP orders perfectly match the order deduced from their physical position, which implies that the RIL population used in this study is unbiased and ideal for mapping any traits using the linkage map. The highest number of polymorphic SNPs was observed in chromosome 1, which supports the observation of most saturated markers in chromosome 1 in the meta-analysis[11].

Forty four putative QTLs were identified for the GY, PN and other yield components in both AL and LL conditions. Among them, the QTLs on chromosome 1 (qPN_LL1.1 and qGY_LL1.1) were identified between 35.82 to 37.69 Mbp for the trait of GY and PN in lowlight only, which means they are not found in AL. Additionally, GWAS by five different models also identified a QTN (1163456 on chromosome 1), associated with the grain yield and panicle numbers, which is exactly one of the flanking markers of the previously identified grain yield and panicle improving QTL (qPN_LL1.1 and qGY_LL1.1) under low light. As the Swarnaprabha alleles contribute favourable phenotypes followed by the confirmation of the same in a panel of rice genotypes by GWAS, the QTL with a high additive effect may be targeted for its yield and panicle loss alleviation in shade-grown rice. One previous report confirms that two closely located QTLs in the same region controlling PN in a cross between IR64 and O. *rufipogon* where favourable alleles come from IR64[15] in ambient light. Although no panicle-improving QTL in ambient light was identified, the allelic variation-based regression curve confirmed a panicle-improving QTL contributed by the IR 64-type allele in an association panel. Hittalmani et al.[16] reported approximately two closely located thousand seed weight controlling QTLs, one at 174 cM and another at 197 cM. In this study, one seed weight-controlling QTL (qTW_AL1.1) was identified in a similar location in wet-2020, where a favourable allele comes from SP. Here, a PN-controlling QTL located on chromosome 1, (qPN_AL1.1) at 56 cM, the region also carries a consensus grain yield and yield components QTL[11]. Only one QTL on chromosome 2 has been reported to control PN in rice by several earlier reports[11]; in this study, the same region identified qPN_AL2.1 in 2019 wet. Thus, the identified lowlight responsive PN and GY-improving QTL on chromosome 1 are newly reported QTLs. The study also identified panicle-number improving two QTLs on chromosome 1 in ambient light but far away from the qPN_LL1.1. A recent report confirms that the same region carries tiller number-improving QTLs in ambient light[17].

Another QTL on chromosome 6, qGY_LL6.1, from 4.6 to 4.7 Mbp, was identified to control panicle and grain yield under LL conditions. It was also stable across the seasons, controlling 8–12% of total PVE with 4–6 LOD. Reference[18] reported a panicle improving QTL on chromosome 6 near the lowlight responsive panicle and yield improving QTL, qGY_LL6.1, identified in this study. Associated QTN, SNP 5,951,985, was identified for panicle and grain yield on chromosome 6, which is also the right marker of previously identified QTL qGY_LL6.1 using SPxIR64 derived RIL population in this study. Thus, association mapping validates that the identified lowlight responsive QTLs are not only Swarnaprabha or IR64 specific. So, the identified PN and GY improving QTL on chromosome 1 and chromosome 6 can be recommended to introduce other high-yielding backgrounds to reduce the yield gap between wet and summer rice and to develop new varieties targeting the lowlight-prone areas. An association panel of one hundred seventeen genotypes further validated the identified QTLs. ANOVA analysis deciphered the significant contribution of light intensity to the total variation.

Although six genes are differentially expressed in low light-grown flag leaves, there are no significant differences between SP and IR64. However, eight genes expressed differentially between ambient and lowlight-grown apical meristem, and only *DGP1* and *MOC2* showed significant differences between SP and IR64. *DGP1* (LOC_Os01g62060) has previously been reported to have a role in photosynthesis regulation[19]. However, as RQ was higher in IR64 than SP, it was not considered for the probable reason, as the yield-enhancing favourable gene is coming from Swarnaprabha. However, sequencing of the *DGP1* gene showed an SNP (A_{SP}/T_{IR}), which can convert the Glutamate to Valine in the polypeptides. It can be targeted for allele-specific marker development to elucidate its role in controlling panicle number and photosynthesis in low light. As the Swarnaprabha is more efficient in tiller development under shade, *MOC2* (LOC_Os01g64660), within the identified QTL might be a yield and panicle-enhancing gene for the purpose. *MOC2,* also a Fructose 1–6 bisphosphatase gene, has previously been reported to have a role in controlling tiller outgrowth[20] and has shown upregulation in SP under LL inside the shoot meristem in this study. The earlier reports confirmed that the gene does not inhibit auxiliary bur formation. Rather, its absence promotes outgrowth deficiency. Higher expression and shade responsiveness in Swarnaprabha might have a role in preventing tiller number reduction in the shade. Nucleotide polymorphism in coding and 5'-UTR between SP and IR 64 gives scope for its deeper analysis in mitigating lowlight stress, particularly tiller development. Although *MOC1* is previously reported as a tillering gene in rice, located on chromosome 6[21], no QTLs were identified in any year in a similar location in this study, probably due to a lack of difference between the two parents.

Thus, the study identified two QTLs in chromosomes 1 and 6 as responsible for alleviating yield loss in shade-grown rice. It also predicted *MOC2* as a gene for their shade-responsive transcript upregulation in the tolerant and a few favourable RILs. Nucleotide polymorphism between Swarnaprabha and IR64 will help transfer the allele more easily and accurately in another rice background. However, further fine mapping of the QTL may lead to identifying the candidate gene, followed by allele mining in the vast rice repository and developing allele-specific markers. Therefore, due to the significant additive effect and validation across various genotypes, the low-light-responsive yield-improving QTL on chromosome 1 can be directly utilized by introducing the trait in other high-yielding genotypes through marker-assisted selection easily.

Methods

Field-phenotyping

A mapping population of 130 biparental RILs developed from a cross between Swarnaprabha x IR64 was used to evaluate the yield and its attributing parameters in open and shaded fields. Both are released varieties in India. The RILs were developed from F2 through single-seed descent. The F7-derived bulked seeds of each RIL were selfed for two additional generations, and they have been maintained at the Crop Research Unit of the university experimental farm. A panel of one hundred seventeen genotypes comprised of Indica landraces, released varieties, and aromatic and aus subtypes were evaluated similarly as described in RILs. One hundred thirty RILs were grown in both open (Ambient light) and shade (low light) with 3 replications following RBD design, considering a single line of 4 m as one replication. Twenty-six plants were grown per line with 20 cm between rows and 15 cm plant-to-plant following the recommended package of agronomic practices in four consecutive seasons, i.e. Kharif 2019 (S1), Kharif 2020 (S2), Kharif 2021 (S3), and Kharif 2022 (S4). Similarly, one hundred seventeen genotypes were evaluated in Kharif-2023 for association analysis. As described earlier, a white shade net (30% light cut) was used in the field to reduce the light intensity[2,13]. Temperature and photosynthetically active radiation (PAR) were recorded daily at 9 h, 13 h, and 16 h during transplanting to maturity in both open and shade (Supplementary Table 1). No significant temperature differences between open and shade-grown fields were noticed. The mean of ten plants in each row was considered for recording effective tiller number or panicle number (PN), yield per plant (PY), hundred seed weight (HSW), panicle weight (PW), and filled spikelet per panicle (SPP) for each replication. ANOVA analysis for partitioning of genotypic and light intensity across the seasons for yield and other parameters was performed by GraphPad Prism 10.2.3. The significant difference between different parameters of plants grown in open-field and shade was estimated using a paired t-test (nonparametric). Descriptive statistics for RILs and genotypes were also analysed using GraphPad Prism 10.2.3.

Genotyping

DNA was extracted from young leaves of one hundred thirty RILs, their parents and one hundred seventeen genotypes. For genotyping, Illumina's custom-designed 7 K iSelect array was used. 7 k array, also known as C7AIR, consisted of 7098 SNPs that represented an improved version of Cornell_6K_Array_Infinium_Rice (C6AIR) with additional content from the Rice Haplotype map project[22,23]. One hundred seventeen genotypes were genotyped with the same 7 K SNP chip as in the RIL population. After calling the data, 927 polymorphic SNPs between the parents were used for mapping. SNPs with a call rate of less than 90% and minor allele frequency of less than 40% were removed from the dataset (Supplementary Tables 7 and 8). The polymorphic SNPs were used for linkage mapping.

Construction of linkage map and QTL analysis

Integrated QTL software, IciMapping, Version 4.0, available from http://www.isbreeding.net[24] was used for linkage map preparation and QTL mapping. Based on the genotypic data of 130 RILs, the linkage map was prepared with the following parameters i.e. grouping with anchor only, ordering with input order and rippling by DIS (sum of adjacent distances) where genetic distances (cM) were calculated based on Kosambi mapping function which was further used for preparing the input file (BIP file) for QTL mapping. Before QTL mapping the test of normality of distribution at p > = 0.05 was performed following Kolmogorov–Smirnov test for goodness of fit. Mapping of QTLs was performed through Inclusive Composite Interval Mapping (ICIM) using the same software with mapping parameters i.e. step (cM) 1.0 and PIN 0.00100. For epistatic QTL analysis, ICIM-EPI function was used in the same software.

Validation of QTL through association analysis

In 2023-kharif, yield and its attributing parameters were measured further in a panel of one hundred seventeen genotypes comprising local landraces, released varieties, aus, and aromatic rice. All the genotypes were collected from our University's Crop Research Unit field gene bank. SNPs with a call rate of less than 95% were removed from the dataset, and 2928 good-quality SNPs were used to create a diversity tree using the neighbour-joining method, giving rise to 5 separate sub-groups. Linkage disequilibrium (LD) among the polymorphic markers was created by Tassel 5 with a sliding window size of 50 markers. To determine the decay of LD, obtained r^2 values were graphed against physical and genetic distances, and a LOESS curve was created. A previously used approach[25] was used to estimate LD decay distance and evaluated at the commonly accepted r^2 threshold of 0.2, as described earlier[26]. Genome-Wide Association Study (GWAS) was carried out in R studio using five different models, i.e., 'FarmCPU'[27] and 'BLINK'[28] in the GAPIT package, mrMLM[29] and FastmrMLM[30] in the mrMLM package, and 3VmrMLM[31] in the IIIVmrMLM package.

Relative transcript quantification

The leaves and meristematic tissues were collected from 8 to 10 days of flag leaves and 45 days of meristematic tissues of field-grown rice plants. Three independent plants were marked and considered as one replication. Collected tissues were washed properly with phosphate buffer saline and immediately placed in an RNA-protector solution. Good-quality RNA was extracted from the collected sample leaves following the RNeasy plant mini kit as described earlier[32], and RNase-free DNase treatment was conducted to avoid DNA contamination. Extracted RNA was immediately converted to cDNA using a high-capacity reverse transcriptase (Applied biosystem kit). qPCR was performed using cDNA as a template using gene-specific primer and tubulin, and relative transcript quantification was estimated following $\Delta\Delta CT$, as described earlier[33].

Data availability

References

1. Tester, M. & Langridge, P. Breeding technologies to increase crop productionin a changing world. *Science* **327**, 818–822 (2010).
2. Ganguly, S. et al. Identification and analysis of low light tolerant rice genotypes in field conditions and their SSR-based diversity in various abiotic stress tolerant lines. *J. Genet.* **99**(1), 1–9 (2020).
3. Singh, S. 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 (2005).
4. Yang, W., Peng, S., Laza, R. C. & Visperas, R. M. Yield gap analysis between dry and wet season rice crop grown under high-yielding management conditions. *Agron. J.* **100**, 1390–1395 (2008).
5. Janardhan, K. V., Murty, K. S. & Dash, N. B. Effect of low light during ripening period on grain yield and translocation of assimilates in rice varieties. *Indian J. Plant Physiol.* **23**, 163–168 (1980).
6. Nayak, S. K. & Murty, K. S. Effects of varying light intensities on yield and growth parameters in rice. *Indian J. Plant Physiol.* **23**(3), 309–316 (1980).
7. Shao, L. et al. The impact of global dimming on crop yields is determined by the source–sink imbalance of carbon during grain filling. *Glob. Chang. Biol.* **27**, 689–708 (2021).
8. Dutta, S. et al. Marker–trait association for low-light intensity tolerance in rice genotypes from Eastern India. *Mol. Genet. Genom.* **293**(6), 1493–1506 (2018).
9. Liu, Q. H., Xiu, W. U., Chen, B. C. & Jie, G. A. O. Effects of low light on agronomic and physiological characteristics of rice including grain yield and quality. *Rice Sci.* **21**(5), 243–251 (2014).
10. Panda, D. et al. Impact of low light stress on physiological, biochemical and agronomic attributes of rice. *J. Pharm. Phytochem.* **8**, 1814–2182 (2019).
11. Aloryi, K. D. et al. A meta-quantitative trait loci analysis identified consensus genomic regions and candidate genes associated with grain yield in rice. *Front. Plant Sci.* **13**, 1035851 (2022).
12. Wei, X. et al. A quantitative genomics map of rice provides genetic insights and guides breeding. *Nat. Genet.* **53**(2), 243–253 (2021).
13. Saha, S. et al. Rice (*Oryza sativa*) alleviates photosynthesis and yield loss by limiting specific leaf weight under low light intensity. *Funct. Plant Biol.* **50**(4), 267–276 (2023).
14. Murty, K. S. & Sahu, G. Impact of low-light stress on growth and yield of rice. In *Weather and Rice* (eds Murty, K. S. & Sahu, G.) 93–101 (International Rice Research Institute, 1987).
15. Septiningsih, E. M. et al. Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. *Theor. Appl. Genet.* **107**, 1419–1432 (2003).
16. Hittalmani, S. et al. Identification of QTL for growth and grain yield-related traits in rice across nine locations of Asia. *Theor. Appl. Genet.* **107**, 679–690 (2003).
17. Barnaby, J., McClung, A. M., Edwards, J. D. & Pinson, S. R. M. Identification of quantitative loci for tillering, root and shoot biomass at the maximum tillering stage in rice. *Sci. Rep.* **12**, 13304 (2022).
18. Zhuang, J. Y. et al. Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice. *Theor. Appl. Genet.* **105**, 1137–1145 (2002).
19. Zhang, C. et al. DEEP GREEN PANICLE1 suppresses GOLDEN2-LIKE activity to reduce chlorophyll synthesis in rice glumes. *Plant Physiol.* **185**(2), 469–477 (2021).
20. Koumoto, T. et al. Rice monoculm mutation moc2, which inhibits outgrowth of the second tillers, is ascribed to lack of a fructose-1, 6-bisphosphatase. *Plant Biotech.* **30**(1), 47–56 (2013).
21. Li, X. et al. Control of tillering in rice. *Nature* **422**, 618–621 (2003).
22. Morales, K. Y. et al. An improved 7K SNP array, the C7AIR, provides a wealth of validated SNP markers for rice breeding and genetics studies. *PLoS One* **15**(5), 1–14 (2020).
23. Thomson, M. J. et al. Large-scale deployment of a rice 6 K SNP array for genetics and breeding applications. *Rice* **10**(1), 1–13 (2017).
24. Meng, L., Li, H., Zhang, L. & Wang, J. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J.* **3**(3), 269–283 (2015).
25. Hill, W. G. & Weir, B. S. Variances and covariances of squared linkage disequilibria in finite populations. *Theor. Popul. Biol.* **33**(1), 54–78 (1988).
26. Vos, P. G. et al. Evaluation of LD decay and various LD-decay estimators in simulated and SNP-array data of tetraploid potato. *Theor. Appl. Genet.* **130**, 123–135 (2017).
27. Liu, X., Huang, M., Fan, B., Buckler, E. S. & Zhang, Z. Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genet.* **12**(2), 1005767 (2016).
28. Huang, M., Liu, X., Zhou, Y., Summers, R. M. & Zhang, Z. BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *Gigascience* **8**(2), 154 (2019).
29. Wang, S. B. et al. Improving power and accuracy of genome-wide association studies via a multi-locus mixed linear model methodology. *Sci. Rep.* **6**, 19444 (2016).
30. Tamba, C. L. & Zhang, Y. M. A fast mrMLM algorithm for multi-locus genome-wide association studies. *bioRxiv* https://doi.org/10.1101/341784 (2018).
31. Li, M. et al. A compressed variance component mixed model for detecting QTNs and QTN-by-environment and QTN-by-QTN interactions in genome-wide association studies. *Mol. Plant* **15**, 630–650 (2022).
32. Das, D. et al. A perfect PCR-based codominant marker for low grain-arsenic accumulation genotyping in rice. *Ecotoxicol. Environ. Saf.* **212**, 111960 (2021).
33. Das, N., Bhattacharyya, S. & Bhattacharyya, S. Maiti MK Expression of rice MATE family transporter OsMATE2 modulates arsenic accumulation in tobacco and rice. *Plant Mol. Biol.* **98**(1–2), 101–120 (2018).

Acknowledgements

Author contributions
SG and NK designed the experiment and analyzed the data. SS, NSM, KB, RK, SG, PS, AKS, SP, PKB, and TKB performed all experiments along with SB. SB wrote the manuscript with the assistance of KB and NK.

Competing interests
The authors declare no competing interests.

Ethical approval
All the genotypes used in this study are either released varieties or indigenous rice of Bengal maintained in the University's Crop Research Unit field gene bank, where permissions or licenses are not required as they were not used for commercial purposes. All experimental procedures were conducted in accordance with the guidelines.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-71593-y.

Correspondence and requests for materials should be addressed to S.B.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.