CONTRIBUTION OF PHYTOCHROME A IN THE REGULATION OF SINK CAPACITY, STARCH BIOSYNTHESIS, GRAIN QUALITY, GRAIN YIELD AND RELATED TRAITS IN RICE

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Abstract

Low light (LL) intensity is a major abiotic constraint for grain yield in rice during wet season. Phytochromes principally perceive and respond to the red (R) and far-red (FR) region of light and regulate several aspects of photomorphogenesis. Most phytochromes are responsive under R-light and de-activated under FR-light, but phytochrome A (phyA) is biologically functional under both light conditions and plays an important role in the entire life cycle of the plant. Field experiment was conducted on the effect of LL intensity and subsequent exposure to natural light on *japonica* rice cultivar *Akitokomachi* (WT) and its phytochrome A mutant (phyA) to understand the role of phyA in the regulation of sink capacity, starch biosynthesis, grain quality, grain yield and related traits during wet season of 2018. Leaf soluble protein, photosynthetic CO_2 assimilation rate, leaf non-structural carbohydrate content, sink capacity, grain quality traits such as hulling percentage, milling percentage, head rice recovery, water uptake, volume expansion, alkali spreading value, gel consistency, grain breadth, grain starch content, amylose content and yield attributes such as grain yield, panicle fertility, 1000-grain weight, panicle per plant, spikelets per panicle and grains per panicle along with the expression of starch biosynthesizing enzymes such as such as ADP-glucose pyrophosphorylase (AGPase), starch synthase III (SSIII) and granule bound starch synthase I (GBSSI) were reduced significantly in *phyA* than WT plants. However, % of grain chalkiness, length and amylopectin content increased in the phyA than WT plants. This study indicates the possible role of phyA in the regulation of source-sink communication, starch biosynthesis and grain quality in rice. Presumably, by participating in the control of several starch synthesizing pathways in the grains, phyA play a crucial role in the regulation of grain quality of rice which depends on the availability of the light intensity during grain-filling stage.

Key words: Grain quality, Low light, Photosynthesis, Phytochrome A, Sink capacity, Starch biosynthesis, Rice, Yield.

Introduction

Rice (*Oryza sativa* L.) is the staple food of more than half of the world population. It is cultivated over an area of 156.1 mha with a production of 476 Mt (Maraseni *et al.,* 2018). The growth and development of plants depend on various abiotic factors such as light, temperature, humidity, etc. Light is an important factor that not only regulates the photosynthetic rate and yield but also the respective content and quality of several macromolecules in crop plants (Liu *et al.*, 2017; Chen *et al.*, 2019). In South-East Asia, 60 to 70% of rice is grown during wet season (June-December) when the light intensity is 40 to 60% less than that of dry (January-May) season (Venkateswarlu *et al.*, 1977). Solar radiation is limited during the wet season and the average daily duration of bright sunshine is only 2-3 hours per day. Therefore, low light (LL) stress coupled with fluctuating light intensity due to overcast sky during the wet season is one of the major constraints for rice production in Asian, African and Latin American countries (Murty *et al.*, 1987).

Various stages of photomorphogenesis in plants are driven by specific irradiance requirements which influences yield. This could be attributed to the primary role of light in driving the net assimilation rate through

modulating the structure and function of the photosynthetic apparatus. Moreover, radiation signals adjust plant growth and development to the prevailing environmental conditions. Recently, the average amount of solar radiation reaching the earth's surface has been reported to diminish substantially (Diaz-Torres *et al.*, 2017). Several studies have previously reported that LL stress during grain filling stage negatively impairs photosynthetic rate, grain quality and yield (Yamori *et al.,* 2016). In crop plants, the physiological basis of dry matter production is dependent on the source-sink concept, where the source is the potential capacity for photosynthesis and the sink is the potential capacity to utilize the photosynthetic products. Previous studies indicated that when LL is imposed during grain-filling stage, the source-sink ratio is markedly altered (Benard *et al.*, 2015). If the sink is small, the yield cannot be high and even if the sink is large, the yield cannot increase with the limitation in the source capacity. Thus, it is accepted, that source-sink relation plays a key role in the integration of physiological processes in the plant adaptation to growth conditions. Under LL, more photosynthates are directed to the formation of leaf blade. Consequently, its translocation to roots and reproductive organs decreases, resulting in an unfavourable alteration in the stoichiometry of source-sink communications that ultimately impairs yield (Venkateswarlu *et al.,* 1987). Thereupon, the total soluble sugar, starch, protein, chalkiness and several other grain quality traits are affected to various degrees in response to LL resulting in the production of inferior quality rice (Ren *et al*., 2003). Grain quality is an integration of various physical and chemical kernel attributes providing a certain category to rice that decides customer's demands (Zhao *et al.,* 2015). Low-quality rice is devalued in the global market for their poor appearance, milling, cooking, eating and nutritional quality (Peng *et al.,* 2014). Therefore, breeders and producers set-prime insistence on the maintenance of rice quality. Rice grain quality is regulated by several environmental factors (Gong *et al.,* 2017) and grain starch content largely contribute to it (Sreenivasulu, 2019). Our own previous studies showed that LL results in the depletion of amylose and starch contents of rice grain (Kumar *et al.*, 2018). However, the mechanism by which LL stress decreases starch content of rice grain is not fully understood. From these standpoints, studying the inûuence of LL on source-sink relations and its final impact on the grain quality and yield related traits of rice causes a special interest.

Phytochromes are a class of photoreceptors that principally perceive and respond to the red and far-red region of light and regulate several aspects of plant growth and development. In rice genome, three genes encode for phytochromes: phytochrome A (PhyA), PhyB and PhyC (Kay *et al.,* 1989). Phytochromes are involved in numerous physiological processes, such as seed germination, seedling development, photosynthesis, flowering and shade avoidance (Galvao *et al.,* 2015). PhyA particularly ensures regulation of plant photomorphogenesis under LL condition of dense canopies higher far-red enriched environment (Cassal *et al.,* 1997). This process is believed to have contributed to the evolutionary advantages of plants. Apart from this it has also unique and overlapping roles with phyB in the regulation of photosynthesis in rice. Light-regulated overexpression of an Arabidopsis phyA gene in rice has been reported to alter plant architecture and increases grain yield (Garg *et al.,* 2006). By regulating the photosynthetic process, the phytochrome system also influences the source-sink relationship, grain quality and yield. Moreover, efficient CO_2 assimilation strategies under dynamic light environments have been hypothesised to contribute to optimize yield loss in rice (Sekhar *et al*., 2019). However, we still lack a clear understanding of how LL tolerance in rice is mediated through the optimization of photosynthetic dynamics and the various photoreceptor-mediated cell signalling pathways involved in it. Much research has been conducted to study the influence of the light on the biochemical traits and grain starch synthesis in rice, mostly on the starch accumulation, while the background role of phytochromes, especially phyA, in the regulation of starch synthesizing enzymes and grain quality has not been reported. Therefore, in the present study, field experiments were conducted under low light and normal light conditions using *japonica* cultivar Akitakomachi and its phyA mutant (phyA) for sink capacity, grain quality, physiological characteristics and yield traits. The aim of this study was to unravel the possible role of phytochrome A in the regulation of sink capacity, grain starch content and quality in rice. Answers were sought to (a) impact of LL stress on amylose content and amylopectin distribution in rice grains; and (b) the possible role of phyA in the maintenance of photosynthesis in the source organ and the resultant communication with the sink organ that ultimately influences the grain quality, grain yield and related traits in rice.

Materials and methods

Plant material and Light treatment

Field experiments were conducted in the experimental plots of ICAR-National Rice Research Institute (NRRI) Cuttack, Odisha, India (20.4625_N, 24.85.8830_E) during the wet season (WS) of 2018. The

seeds of rice cultivar, Akitakomachi (WT) and its phytochrome A mutant (phyA) were purchased from Rice Genome Resource Centre, National Institute of Agrobiological Sciences, Japan. Seeds were shown in pots and 25 days old seedlings were grown in randomized complete block design (RCBD) with three replications of each, in a plot size of 10×10 m^2 (spacing 15 cm $\times$ 20 cm). At the active tillering stage, one set of each WT and phyA plants were subjected to the low light (LL) treatment (350 µmol quanta m^2 s^{-1}) using agro-shade nets matted on a wooden frame, while the other set was grown under the open condition with 100% natural light (NL) intensity (1400 µmol quanta m^2 s^{-1}). The recommended dose of N$_2$, P$_2$O$_5$ and K$_2$O (80:40:40) fertilizers were applied. Recommended agronomic practices were followed.

Measurement of photosynthetic active radiation (PAR)

Relative availability of photosynthetic active radiation (PAR) under LL and NL conditions were estimated using radiometer (LI-1500 LICOR, USA) thrice per day (9.00 AM, 12.00 PM and 4.00 PM of Indian Standard Time, IST) during WS of 2018. Five replicas under each condition were maintained for accurate PAR measurement.

Estimation of gas exchange

The net CO$_2$ assimilation rate (Pn) of the flag leaf during 50% anthesis was analysed using a LI-6400XT portable photosynthesis system (LI-COR, Inc., USA). The measurements were recorded for both WT and phyA exposed to NL and LL conditions. The data are averaged of recordings from six flag leaves from each replicate. Pn was determined in WS of 2018 under NL and LL conditions at a CO$_2$ concentration of 380 µmol m^{-2}s^{-1}. The PAR was set at 1200 µmolm^{-2}s^{-1} for the NL and 300 µmolm^{-2}s^{-1} for LL, which was provided by a 6400-2B LED light source.

Estimation of leaf soluble protein

Protein content of the leaves was measured according to Lowry (1951). Six biological replicates were prepared for analysis for each season. 1g leaf sample was ground in 10ml of Na-phosphate buffer in a mortar pestle and allowed to centrifuge for 15 minutes at 10000 rpm. The supernatant was collected for protein estimation. 0.1 ml sample extract was pipetted out in test tube and volume was made up to 1 ml with distilled water. 5 ml of solution C was added to each test tubes and mixed well. Test tubes were allowed to incubate at room temperature for 10 minutes. Then 0.5ml of FCR reagent (2N) was added and mixed well and allowed to incubate in dark for 30 min. Absorbance was measured at 660 nm and amount of protein present in sample is estimated from the standard graph and the values were expressed in mg/g FW.

Estimation of leaf carbohydrate

Non-structural carbohydrates (total soluble sugar, reducing sugar and non-reducing sugar) in leaves were estimated.

• *Estimation of leaf total soluble sugar:* Leaf total soluble sugar was analysed following the method of Dubios *et al.,* (1951). 100 mg of the fresh flag leaf collected during anthesis were crushed in a motar using liquid nitrogen and allowed to boil using 10 ml of 80% ethanol (v/v) in 15-ml polypropylene tube for 30 min. The homogenate was centrifuged at 10,000 rpm for 15 minutes to get clear supernatant. This process was repeated for two more times. All the three supernatants were pooled in a conical flask and dried on boiling water bath at 100°C for 10 minutes. The contents of the flask were dissolved 50 ml distilled water. An aliquot of 0.5 ml was taken in a test tube and diluted to 1 ml with distilled water, followed by addition of 4ml of anthrone reagent. These tubes were placed in boiling water bath for 8 minutes. The absorbance was read after cooling the tubes at 630 nm (Genesys 20, Thermo Spectronic). The TSS content was calculated and expressed in µg glucose equivalent/g fresh weight.

• *Estimation of leaf reducing sugar:* Reducing sugar was estimated following the method of Miller (1959). 0.1 gm fresh flag leaf was homogenised separately in 80% hot ethanol and centrifuged at 5000g for 15 min at room temperature. The supernatant was evaporated by keeping in water bath at 800C and sugars were dissolved by adding 10 ml distilled water. 1 ml of solution from both the samples were taken in separate tubes and 3 ml of DNSA reagent was added, then boiled for 5 min in a water bath. After boiling 1 ml of Rochelle salt solution was added. The tubes were cooled to room temperature and measured the intensity of dark red colour at 530 nm and calculated the concentration of reducing sugars from glucose standard graph.

• *Estimation of leaf non-reducing sugar:* Non-reducing sugar was estimated following method of Hedge and Hofreiter (1962). Flag leaves were hydrolysed separately by keeping in water bath for 3 hrs, with 2.5 N HCl (5 ml) and was neutralised with Na$_2$CO$_3$ after cooled it to room temperature. 0.1 ml extracts were taken in separate test tubes and makeup to volume 1 ml with distilled water and 4 ml of anthrone was added. The tubes were kept for boiling for 5 min. colour intensity was measured at 630 nm. Non reducing sugars concentration was calculated from glucose standard graph.

Estimation of grain quality parameters

Well dried paddy samples from each plot weighing 100 g were hulled using mini 'Satake Rice Medium' and the weight of brown rice was recorded. The hulled brown rice samples were milled and head rice recovery was estimated. Hulling (%), milling (%) and head rice recovery (%) were calculated following method of Bhonsle *et al.,* (2010). Percentage of chalkiness was estimated by considering ten de-husked rice grains which were placed on lightbox and visually identified. The grains with more than 50% of chalkiness were weighed and the percentage of chalkiness was calculated. Six milled rice grains were taken in Petri plates and 10 mL of potassium hydroxide (19.54 g of potassium hydroxide dissolved in one litre) was added to the sample and its alkali spreading was estimated following Juliano *et al.,* (1993). To estimate the gel consistency (GC), milled rice grains were ground to a fine powder using mortar and pestle and sieved using 1 mm sieve. The degree of disintegration and the transparency of paste dissolved out of the kernels were evaluated using a 7-point scale. The volume expansion ratio and elongation ratio were calculated by Juliano *et al.,* (1993). Water uptake was calculated using 2g of samples by following Subudhi *et al.,* (2012). Grain starch content was estimated using α-amylase, amyloglucosidase, glucose oxidase plus peroxidase (GOPOD) and 4-aminoantipyrine reagents obtained from Megazyme (Total starch assay kit K-TSTA-100A, Megazyme International, Ireland Limited, Bray Business Park, Bray, co, Wicklow, Ireland) as per Kumar *et al.,* (2018). The grain amylose and amylopectin content were also determined as described by Kumar *et al.,* (2018). Grain images were taken to the nearest using microscopy (Keyence-VH500, Japan).

Real-time expression analysis of grain filling genes

To understand the pattern of differential gene expression related to starch biosynthesis in grains under NL and LL in WT and phyA plants, three genes, ADP-glucose pyrophosphorylase (AGPase), starch synthase III (SSIII) and granule bound starch synthase I (GBSSI) were studied in the milky stage of grains. Total RNA was extracted from the milky grains using RNEasy Plant Mini Kit (Qiagen, USA) following the manufacturer's protocol. First-strand cDNA synthesis was conducted using 5 mg of total DNase-treated RNA using primer script 1st strand cDNA synthesis kit (Takara Clontech, Japan). The primers with Tm 58°C to 62°C and a length of 19-mer to 22-mer were designed for above three genes using the Primer Blast software at the NCBI site (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers designed were specific to *O. sativa* subsp. *Japonica* and amplified fragments ranging from 100 to 250 bp (Supplementary Table 1). qRT-PCRs were performed (Sekhar *et al.,* 2015).

Yield and yield components

After maturity, seeds from ten plants from each plot were harvested to record yield and yield related components such as fertility percent, 1000-grain weight, panicles/plant, spikelets/panicle, grains/panicle and yield per plant during WS of 2018. Before taking the observations, grains were dried to a standard moisture content of 13.5%.

Statistical analysis

The experiments were carried out in three biological as well as three technical replicates. Standard statistical (Mean, SD, significance), principal component analysis (PCA) and correlation analysis were done for different yield and related traits under NL and LL conditions by using PAST3.0 software. Heatmap was also generated using the Clustvis a web-based software.

Results

Photosynthetic active radiation (PAR)

Measurement of PAR under LL and NL intensity above canopy of WT and phyA was recorded (supplementary Table 1) during three times of the day (9.00 am, 12.00 pm and 4.00 pm of Indian Standard Time (IST), UTC + 05:30) after LL simulation to confirm the LL stress and NL intensity.

Leaf soluble protein

Leaf soluble protein (LSP) was reduced significantly

Table 1: Sink capacity attributes to monitor the source-sink communications. Number of spikelets per cm^2 of flag leaf, number of grains per cm^2 of flag leaf, grain yield per cm^2 of flag leaf and sink capacity of *Oryza sativa*. *cv* Akitakomachi (WT) and phyA plants, estimated under natural light (NL) and low light (LL) conditions.

	Spikelets/cm^2 leaf				Grains/cm^2 leaf				Yeild/cm^2 leaf				Sink Capacity			
	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL
Mean	0.91	1.53	0.91	1.00	1.14	0.25	0.74	0.15	0.13	0.08	0.11	0.03	55.16	7.64	26.86	3.72
STD	0.04	0.03	0.03	0.02	0.04	0.01	0.03	0.01	0.01	0.00	0.01	0.01	8.98	0.45	2.74	0.74
P-VALUE at 0.05	0.02		0.00		0.01		0.02		0.07		0.00		0.10		0.07	

Table 2: Yield attributes. Grain yield (g/plant), spikelet fertility(%), number of panicles/plant, 1000 grain weight(g), number of grains per panicle and number of spikelets per panicle of *Oryza sativa.cv* Akitakomachi (WT) and phyA grown under natural light (NL) and low light (LL) conditions.

|	Yield/plant(g)				Grain Fertility(%)				1000 Grain Weight(g)				Panicles/plant				Spikelets/panicle				Grains/panicle			
	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL	WT-NL	Phy A-NL	WT-LL	Phy A-LL
Mean	7.57	5.37	5.67	2.45	0.02	0.01	0.02	0.01	0.04	0.00	0.00	0.01	8.43	4.12	7.10	2.93	88.33	30.00	56.50	18.83	62.23	19.07	37.37	9.37
STD	0.33	0.16	0.19	0.13	0.65	0.17	0.42	0.10	0.06	0.05	0.06	0.02	0.28	0.46	0.14	0.42	0.14	0.21	0.00	0.21	0.07	0.21	0.07	0.07
P-VALUE at 0.05		0.07		0.03		0.12		0.03		0.11		0.67		0.09		0.06		0.00		0.00		0.00		0.00

under LL as compared to NL (supplementary Table 1) both in WT and *phyA* genotypes. Reduction was found to be 15.28% and 18.48%, respectively in WT and phyA genotypes, respectively. phyA showed a significant reduction in LSP in both NL and LL conditions as compared to WT. Reduction was found to be 40.96% and 43.19% under NL and LL, respectively (Fig. 1a, Supplementary Table 2).

Photosynthetic CO$_2$ assimilation

Photosynthetic CO$_2$ assimilation (Pn) was reduced significantly under LL as compared to NL both in WT and phyA genotypes. Reduction was found to be 15.49% and 22.68%, respectively in WT and phyA genotypes. phyA showed a significant reduction in Pn in both NL and LL conditions as compared to WT. Reduction was found to be 8.08% and 15.90% under NL and LL, respectively (Fig. 1b, Supplementary Table 3).

Accumulation of non-structural carbohydrates in leaves

The non-structural carbohydrates (NSC) accumulated in the flag leaf of rice as photo assimilates markedly decrease under LL stress. Total soluble sugar (TSS) was reduced significantly under LL as compared to NL both in WT and phyA genotypes. Reduction was found to be 25.17% and 27.87% in WT and phyA plants, respectively. phyA showed a significant reduction in TSS in both NL and LL conditions as compared to WT. Reduction was found to be 19.21% and 22.12% under NL and LL, respectively (Fig. 2a). Reducing sugar (RS) was reduced significantly under LL as compared to NL both in WT and phyA genotypes. Reduction was found to be 10.63% and
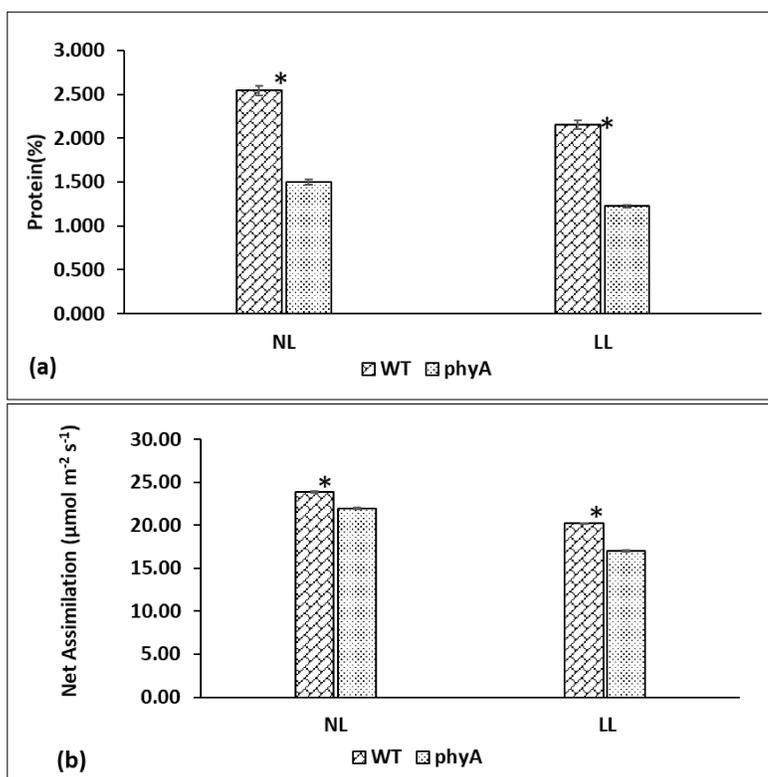

Fig. 1: Flag leaf protein content and net assimilation rate during flowering stage.

13.98% in WT and phyA plants, respectively. phyA showed a significant reduction in RS in both NL and LL conditions as compared to WT. Reduction was found to be 14.45% and 17.66% under NL and LL, respectively (Fig. 2b). Non reducing sugar (NRS) was reduced significantly under LL as compared to NL both in WT and phyA plants. Reduction was found to be 48.47% and
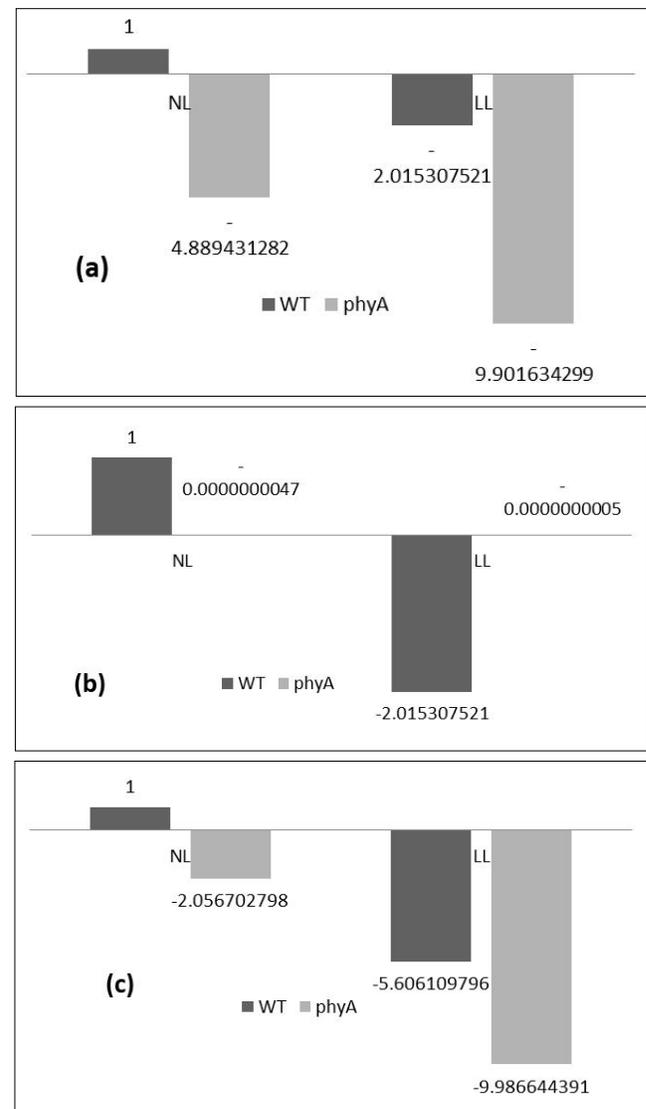
47.92% in WT and phyA genotypes, respectively. phyA showed a significant reduction in NRS in both NL and LL conditions as compared to WT. Reduction was found to be 17.32% and 16.45% under NL and LL, respectively (Fig. 2c, Supplementary Table 4).

Characteristics of sink-capacity attributes

Different types of grain-leaf ratio reflecting source-sink characteristics are listed in table 1. Number of spikelets per unit leaf area (S/LA), number of grains per unit leaf area (G/LA), grain yield per unit leaf area (GY/LA) and sink capacity (SC) was reduced significantly under LL as compared to NL both in WT and phyA plants. Reduction was found to be 25.62%, 22.08%; 34.89%, 40.48%; 11.47%, 54.97% and 51.313%, 51.322% for the above traits in WT and phyA plants, respectively. phyA
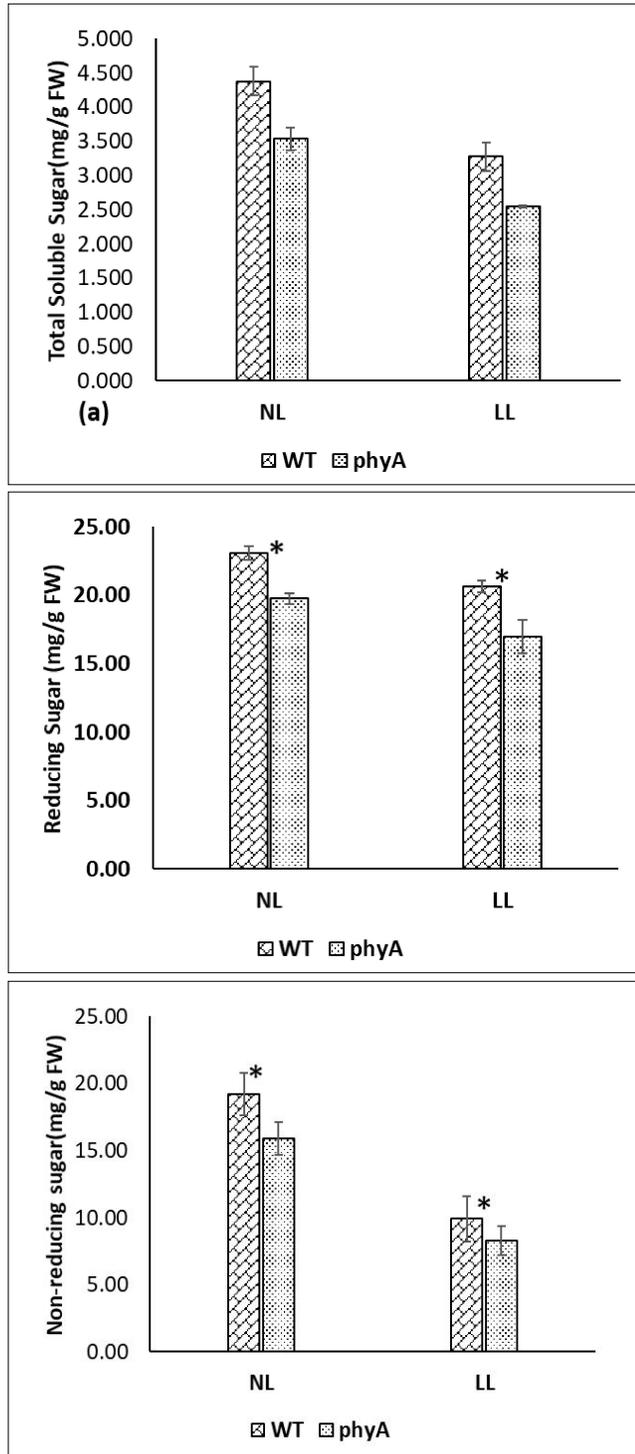
Fig. 2: Non-structural carbohydrates accumulated in the Flag leaf.

Fig. 3: Real time expression of ADP-glucose pyrophosphorylase (AGPase), starch synthase III (SSIII) and granule bound starch synthase I (GBSSI) genes through qRT-PCR.

showed a significant reduction in S/LA, G/LA, GY/LA and SC in both NL and LL conditions as compared to WT. Reduction was found to be 72.46%, 71.15%; 78.16%, 80.03%; 38.74%, 68.84% and 86.156, 86.158% in phyA for the above traits under NL and LL, respectively (Supplementary Fig. 1).

Reduction in the real-time expression of starch biosynthesizing genes

To further check the efficiency of starch accumulation in the grains WT and phyA, we investigated the expression pattern of starch biosynthesizing genes such as AGPase, SSIII and GBSSI during the grain filling stage through quantitative real-time PCR analysis. The data suggested that the primary process of biosynthesis of starch was optimum during grain filling stage (Fig. 3). Under LL condition, the expression of all the starch biosynthesizing genes in both the plants were downregulated. This was comparatively more downregulated in phyA than WT plants. However, under NL condition, all the related genes in phyA were comparatively downregulated than WT plants.

Reduction in grain quality

Different grain quality attributing traits are given in fig. 4. The percentage of hulling (HP), percentage of milling (MP), head rice recovery (HRR), water uptake

(WU), volume expansion (VE), alkali spreading value (ASV), gel consistency (GC), kernel breadth (Kb), grain starch percentage (GSP) and grain amylose content (GAC) were reduced significantly under LL as compared to NL both in both WT and phyA genotypes. Reduction was found to be 4.98%, 4.31%; 4.33%, 4.06%; 4.22%, 3.14%; 13.64%, 27.63%; 25.34%, 36.08%; 41.98%, 41.56%; 17.31%, 18 %; 25.03%, 24.06%; 5.89%, 5.68% and 19.89%, 32.91% for the above traits in WT and phyA plants, respectively. phyA showed a significant reduction in HP, MP, HRR, VE, ASV, GC, GSP and GAC in both NL and LL conditions as compared to WT plants. Reduction was found to be 3.73%, 3.06%; 5.29%, 5.03%; 4.22%, 3.14%; 27.07%, 37.56%; 27.36%, 26.83%; 3.85%, 4.65%; 4.07%, 3.86% and 16.40%, 29.99% in phyA for the above traits under NL and LL, respectively. Comparison of grain image shows a depletion of grain appearance quality under LL which was more affected in the case of phyA than WT grains (Fig. 5). Percentage of grain chalkiness (PC), kernel length (Kl) and grain amylopectin content (APC) were increased significantly under LL as compared to NL both in WT and phyA genotypes. Increment was found to be 28.44%, 80.81%; 8.26%, 3.59% and 6.15%, 8.10% for the above traits in WT and phyA plants, respectively. phyA showed a significant increment in PC, Kl and APC both under NL
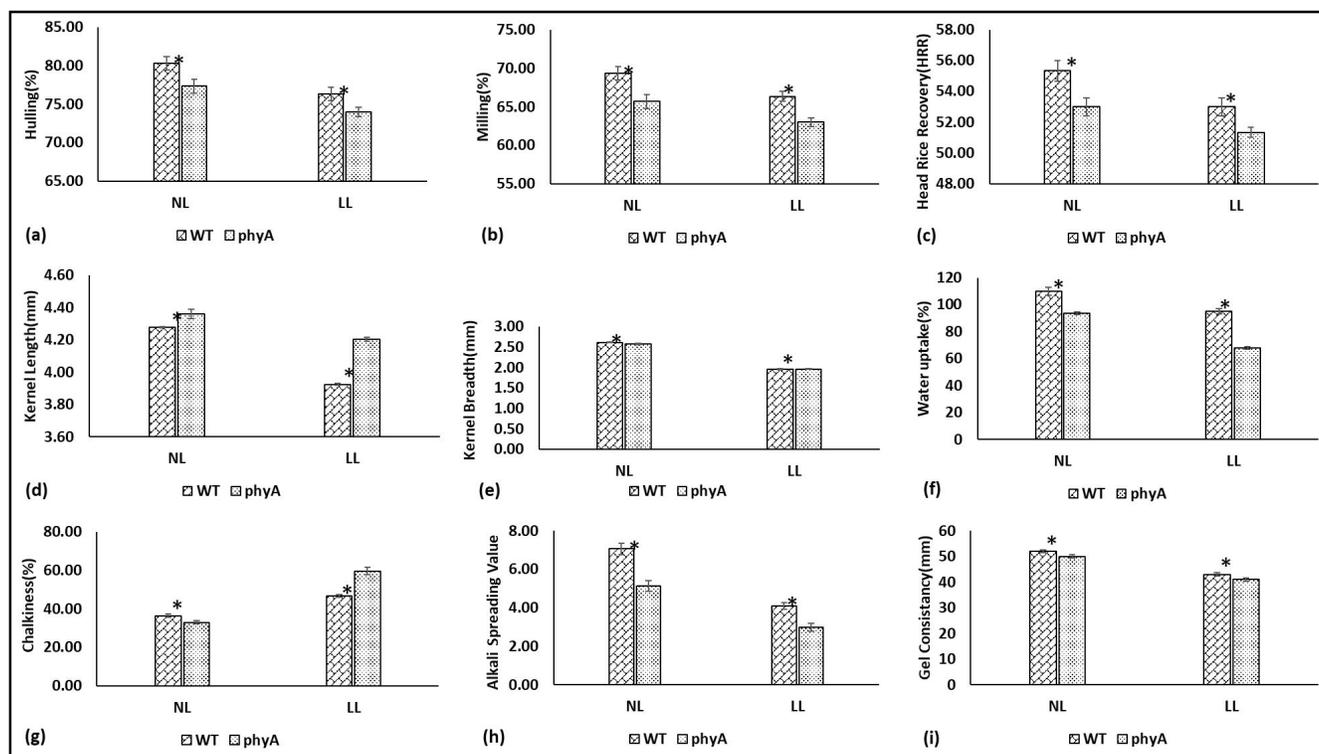
Fig. 4: Grain quality attributes. (a) Grain hulling percentage (%), (b) grain milling percentage (%), (c) head rice recovery, (d) kernel length (mm), (e) kernel breadth (mm), (f) Water uptake(%), (g) Percentage chalkiness (%), (h) alkali spreading value and (i) gel consistency (mm) of *Oryza sativa*. cv Akitakomachi (WT) and phyA grains, grown under natural light (NL) and low light (LL) conditions. Each data point is the average of six replicates and the error bars represent SE.

and LL conditions as compared to WT plants. Increment was found to be 9.17%, 27.86%; 1.95%, 7.14% and 5.07%, 7% in phyA for the above traits under NL and LL, respectively (Fig. 6). Additionally, phyA showed a significant reduction in Kb in NL (1.28%) condition as compared to WT. However, no difference was observed in Kb under LL (Supplementary Table 5).

Reduction in yield attributes

Yield attributes were listed in table 2. Grain yield/plant (GY), grain fertility percent (PF), 1000-grain weight (GW), number of panicles per plant (PP), number of spikelets per panicle (SP) and number of grains per panicle (GP) were reduced significantly under LL as compared to NL both in WT and phyA plants. Reduction was found to be 25.11%, 54.35%; 8.29%,17.87%; 10.16%, 10.33%; 15.81%, 28.74%; 36.04%, 37.22% and 39.96%, 50.87% under NL and LL in WT and phyA plants for the above traits respectively. phyA showed a significant reduction in GY, PF, GW, PP, SP and GP in both NL and LL conditions as compared to WT. Reduction was found to be 29.07%, 56.76%; 10.83%, 20.14%; 6.38%, 6.56%; 51.19%, 58.69%; 66.04%, 66.67% and 69.36%, 74.93% in phyA under NL and LL for the above traits, respectively (Supplementary Fig. 2).

Principal component analysis and Heat Map

Two treatments (NL and LL) and twenty-eight parameters for grain quality sink capacity, net assimilation rate, leaf non-structural carbohydrates, flag leaf protein content and yield component traits for WT and phyA rice plants were analysed using principal component analysis (PCA). Principal component analysis was used
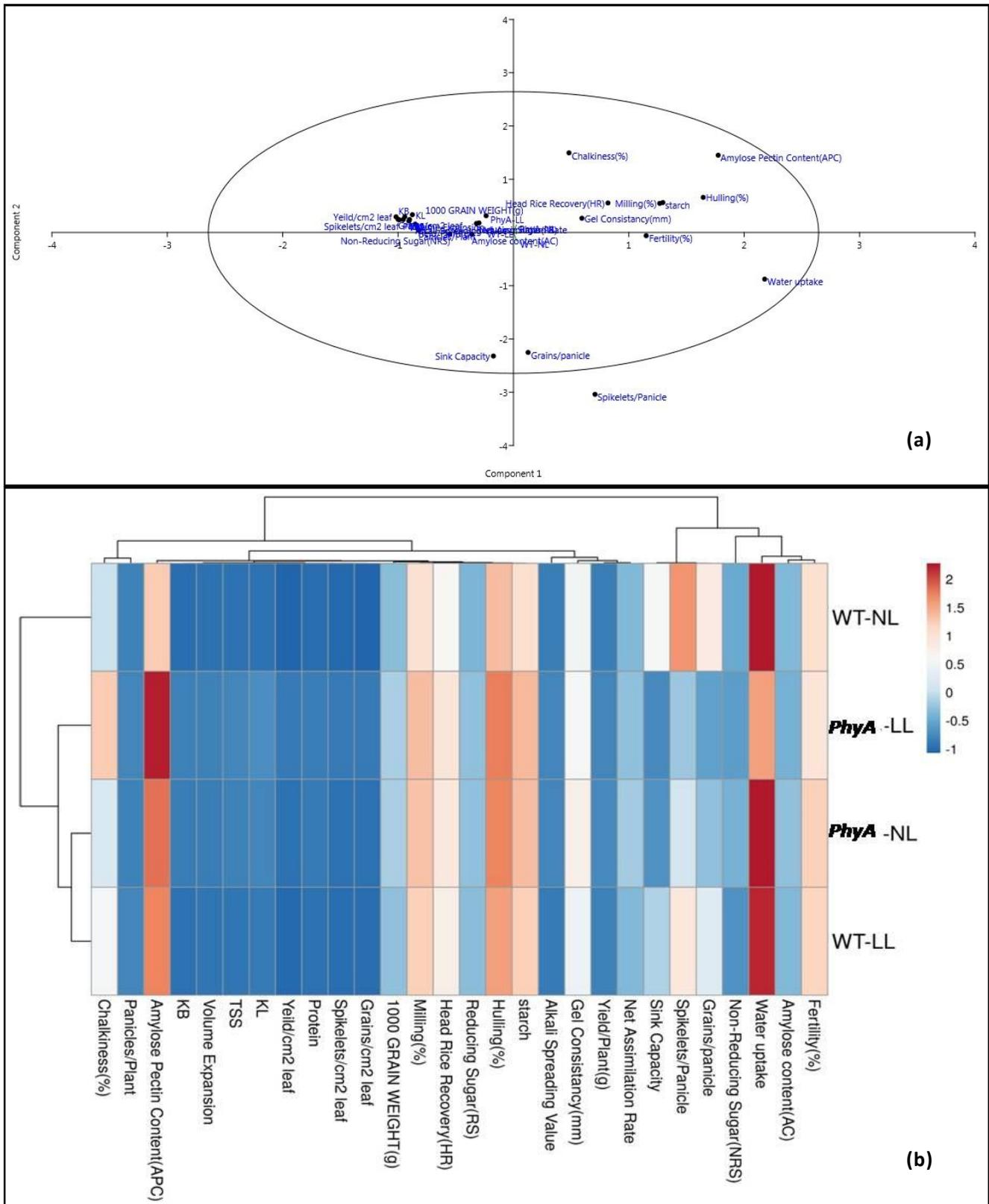
to establish the patterns and interrelationships existing between the two genotypes (WT and phyA) under NL and LL with their quantitative and qualitative traits (Fig. 7a). The first three principal components explained a total of 100% variability in all traits. The analysis of eigen vectors gave the information of qualitative traits for percentage of variation to the first three principal components, which were 84.37%, 13.17% and 2.46% under both NL and LL conditions (Supplementary Table 1-5). In PCA biplot, the two genotypes (WT and phyA) under NL and LL conditions along with twenty-eight parameters are equally distributed in the four quadrants under both NL and LL conditions. In the biplot ordinance plot, WT for both NL and LL conditions came under second quadrant while phyA for NL and LL conditions
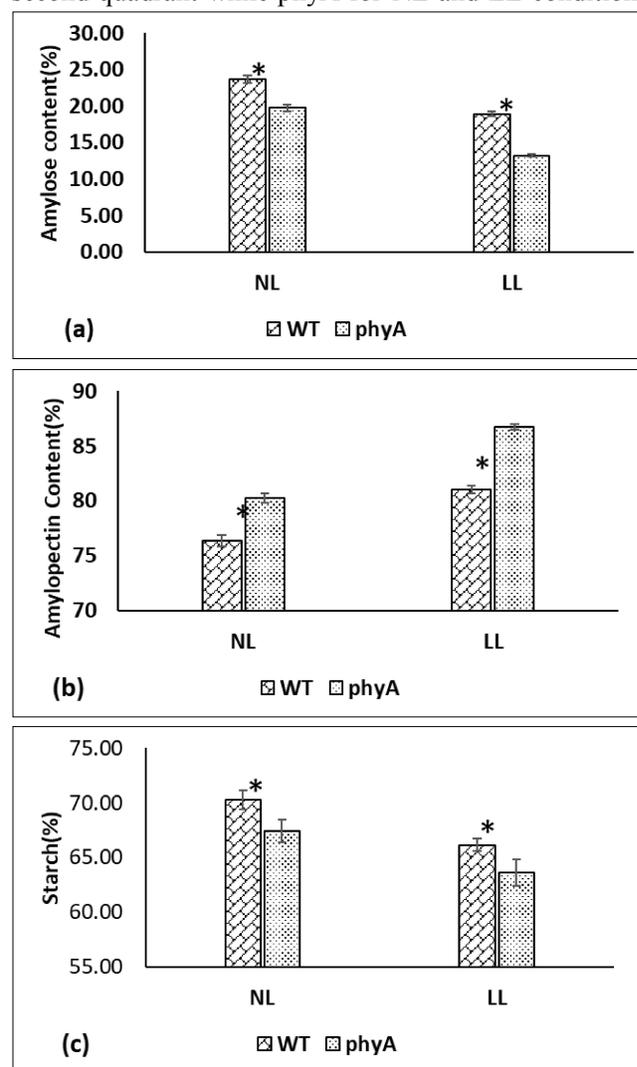
Fig. 6: Grain carbohydrate content. (a) Grain amylose content, (b) grain amylopectin content (%) and (c) grain starch content of *Oryza sativa*. cv Akitakomachi (WT) and phyA grains, grown under natural light (NL) and low light (LL) conditions. Each data point is the average of six replicates and the error bars represent SE.

Fig. 5: Comparison of chalkiness between WT and phyA grown under NL and low light conditions. (a) WT-NL, (b) phyA-NL), (c) WT-LL and (d) phyA-LL. Bar = 3mm.

Fig. 7: (a) Two-dimensional diagram of 27 traits related to grain quality, sink capacity and grain yield in rice under LL and NL conditions in WT and phyA describing the role and interrelationship of phyA in rice grain starch synthesis resulting from principal component analysis (PCA). (b) Hierarchical Heat map clustering for 27 traits related to grain quality, sink capacity and grain yield in rice under LL and NL conditions in WT and phyA describing the role and interrelationship of phyA in rice grain starch synthesis.

came under third quadrant. Heat map has been successfully generated by unit variance scaling is applied to rows. Both rows and columns are clustered using correlation distance and average linkage. Under LL condition, amylopectin showed maximum association with phyA while WT showed weak association with amylopectin (Fig. 7b).

Character Association Analysis

The estimates of correlation coefficient are presented in fig. 8. WT and phyA showed strong positive significant correlation both under NL and LL conditions. Starch content showed strong positive significant correlation with 1000-grain weight (0.999), TSS (0.999), NRS (0.950), Pn (0.986), grain yield (0.955) and grain amylose content (0.955) while negative correlation with grain amylopectin content (0.953). Pn showed a positive and highly significant association with 1000-grain weight (0.989), leaf TSS (0.980) and grain starch content (0.986) while it was found to be negatively correlated with grain amylopectin content. Grain yield showed positive and highly significant association with Pn (0.953), percent fertility (0.998), TSS (0.963) and grain starch content (0.955).

Discussion

Light is an abiotic factor that is particularly important for crop growth and development (Yang *et al.,* 2017). In agricultural production, crops often suffer from mutual low light (LL) stress under close-planting or intercropping conditions (Li *et al.,* 2014; Yang *et al.,* 2017) that reduces the amount of PAR and changes the spectral composition of light (Park and Runkle, 2017). The spectral irradiances of different wavelengths significantly decrease under LL conditions relative to those under NL conditions. Previous studies reported that agronomic net-shade commonly used in studying the impact of LL on plants affects the light quality by decreasing red(R)/far-red(FR) ratio and increasing the proportion of blue light significantly (Chen *et al.,* 2019). Reports have also suggested the significant role of proportion and composition of spectral components, specifically red, blue, far-red and infra-red lights, in a certain environment to play a crucial role in photomorphogenesis (Chang *et al.*, 2008). The role of various phytochromes in the processes of growth and photomorphogenesis has been largely studied (Whitelam and Devlin, 1997; Kim *et al.*, 2005; Carvalho *et al,.* 2016; Kaiserli and Chory, 2016). It is also known that different types of phytochrome are involved in the mechanisms of adaptation to environmental stress factors (Carvalho *et al.,* 2011; Markovskaya *et al.,* 2016; Rusaczonek *et al.,* 2015). On the other hand, little is known about the contribution of PHYA in the maintenance of source-sink communication in rice that further influences grain yield and quality.

Leaf is the principal organ of photosynthesis in plants. The expression and activation of various photosynthesis related enzymes in the leaves are regulated by light (Zucker, 1972). In rice, flag leaf is the chief place of photosynthesis that significantly contribute photo-assimilates to sink organs, thereby influencing panicle development and grain yield. Rubisco is the predominant protein in leaves of C_3 plants and may contribute up to 50% to the soluble leaf proteins (Spreitzer and Salvucci, 2002) and 20-30% of total leaf nitrogen. Regulation of *rbc*L and *Rbc*S genes by light is a phenomenon that is probably shared by all plant species (Wang *et al.*, 2001). In our study, we found a reduction in the soluble protein content of flag leaf under LL in comparison to control both in WT and phyA. However, phyA showed a significant decrease in soluble protein level in comparison to WT under both light regimes. This suggests the possible role of phyA in the regulation of light mediated protein synthesis which possibly played an important role in the maintenance of Rubisco pool in the flag leaves of rice. Previous reports have suggested the involvement of a phytochrome controlled mechanism in the synthesis of Calvin cycle enzymes such as Rubisco and NADP-linked glyceraldehyde-3-phosphate dehydrogenase (Krahmer, 2018). Fomina *et al.,* (2018) reported the role of phytochrome in the distribution of various photosynthetic, respiratory and photorespiratory enzymes in the leaves of C3 plants. In crop communities, photosynthesis is the basis of crop growth and yield and depends on the density and quality of light that reaches chloroplasts (Yang *et al.,* 2014). Shade conditions are characterized by changes in light density and quality with decreased R/FR ratio (Chen *et al.,* 2019). In addition, leaves under LL have lower contents of electron transfer carriers and ribulose-1, 5-bisphosphate carboxylase/oxygenase than those under NL condition (Slattery, 2018). Therefore, LL stress decreases the photosynthetic capacities of leaves during crop growth and development. In our previous work, we have suggested the accelerated expression of CAB, LRP, SBPase, MT15, TF PCL1 and Photosystem I and II complex related genes in the LL tolerant plants that assisted them to preserve the efficiency of photosynthetic processes even under LL that eventually helped in the maintenance of grain yield (Sekhar *et al.*, 2019). Additionally, Carlson *et al.,* (2019) found that phyA participates in the regulation of carbon flux through major primary metabolic pathways, such as glycolysis, beta-oxidation and the tricarboxylic acid (TCA) cycle. In our study, compared to WT, phyA has a significantly

decreased Pn both under NL and LL conditions (Fig. 1). According to previous reports, changes in the phytochrome system lead to a rapid regulation of the expression of genes encoding photosynthetic proteins. Phytochromes further interact with stress signalling components and, as a consequence, perform the fine regulation of the photosynthetic processes and chloroplast stability towards changing environment (Kreslavski *et al.,* 2018). This result suggested the possible involvement of PhyA in the expression of various light modulated photosynthetic genes exclusively required to be upregulated under LL stress in order to lower the yield loss. Both PhyA and PhyB are suggested to play a regulatory role in CO_2 fixation and non-photochemical quenching of chlorophyll (Chl) fluorescence (Rusaczonek *et al.,* 2015). Recently phyB has been reported to enhance plant growth, biomass and grain yield in field-grown maize (Wies *et al.,* 2019). However, PhyB often acts together with PhyA (Casal, 2000). It is found that Phy-sensitive genes encode a number of key photosynthetic proteins, enzymes of antioxidant system as well as enzymes of flavonoid and photosynthetic pigments biosynthesis. Therefore, many researchers are focused at studying the role of phytochrome systems in the development of biological resistance in plants in response to various abiotic environmental stressors. Thus, our results suggest that phyA might be contributing to photosynthetic efficiency of plants by moderating an efficient rubisco pool in the source organs.

The decline in photosynthetic rate under LL stress concomitantly reduced the total NSC concentration in source organ thus relatively decreasing, the net photo-assimilate pool that is further thought to quantitatively lower the transportation of sucrose to the sink organs. TSS mobilises from the leaf during grain filling stages can become an important source of assimilate for grain yield in rice (Liu *et al.,* 2006). Leaf TSS accumulation is influenced by available light intensity (Zhang *et al.*, 2018). In our study, we found a decrease in the accumulation of TSS, RS and NRS in the flag leaf of phyA in comparison to WT both under NL and LL conditions. Under LL, this was more pronounced. A direct positive correlation between photosynthesis, NSC accumulation, grain weight and yield were found (Fig. 8). This suggests that a reduction in Pn in the source organ of leaves directly influences the yield output which is possibly regulated by PHYA signalling. According to the previous reports, high TSS in the flag leaf is considered to be a potentially useful attribute for improving grain weight and yield in rice (Zhang *et al*., 2019). The carbohydrates produced during
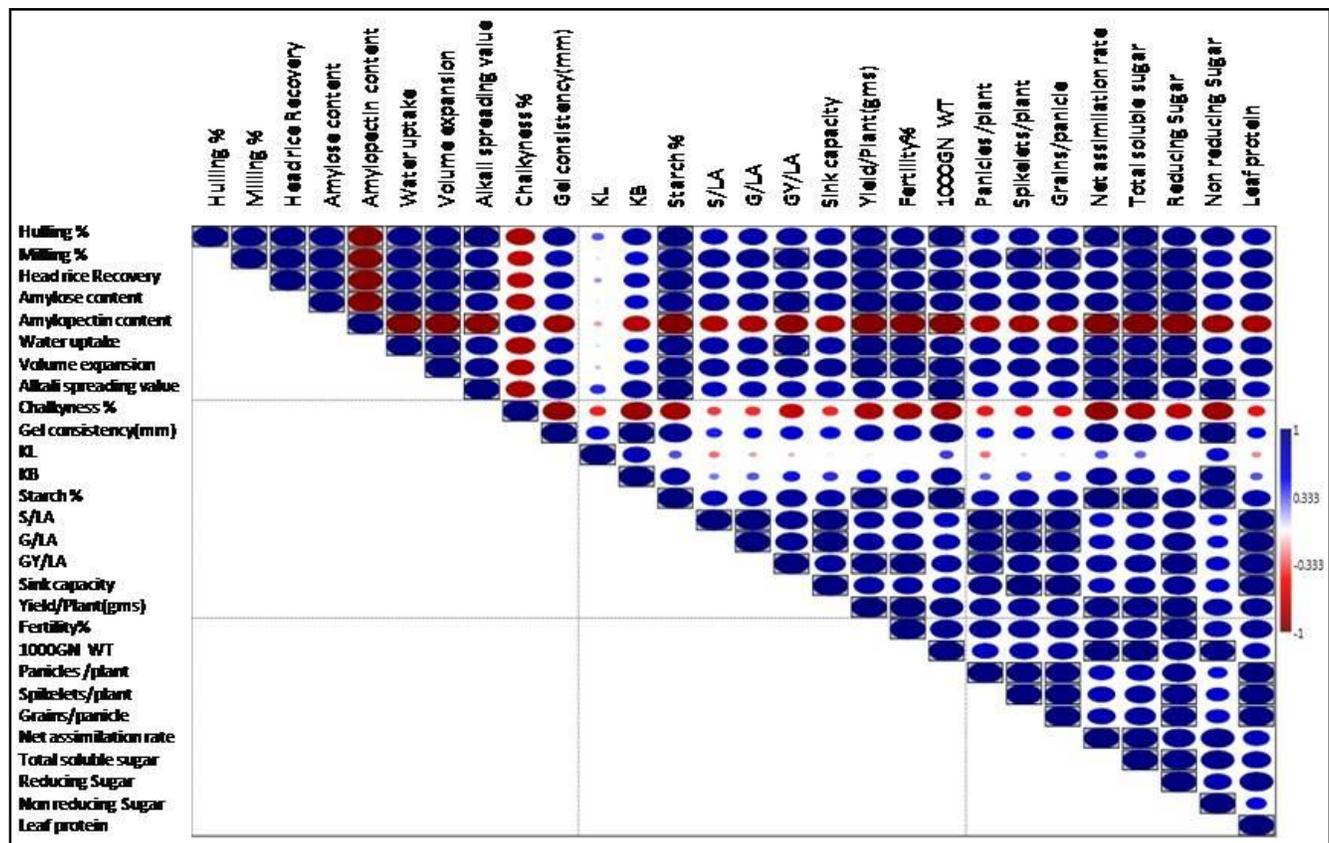

Fig. 8: Correlation map clustering for 27 traits related to grain quality, sink capacity and grain yield in rice under LL and NL conditions in WT and phyA describing the role and interrelationship of phyA in rice grain starch synthesis.

photosynthesis is loaded to the phloem in the form of sucrose which plays pivotal roles in development, stress response and yield formation, mainly by generating a range of sugars as metabolites to fuel growth and synthesize essential compounds (Liu *et al.,* 2006). The coefficients of variation of yield sink per unit area could be employed to understand the real characteristics of source-sink among different combinations (Wei *et al.,* 2018). Sink capacity of phyA was significantly reduced than WT both under NL and LL conditions which was prominent in latter case than former (Table 1). Under LL, leaves try to adapt to shade environments and lower rates of assimilate production by altering chloroplast protein and pigment composition to optimize light capture and light use efficiency. Conversely, in response to high assimilate availability sink capacity is further enhanced. Inability to establish new sink capacity results in accumulation of assimilate in leaves. Plants that can readily increase sink size suffer less from feedback inhibition. Therefore, a reduction in sink capacity of phyA is thought to negatively influence the photosynthetic rate of the source organ.

After pollination, the seed develops to be the major site of starch depository. The starch synthesis in seed starts from sucrose translocated from leaf cells. In photosynthetic cells, triose phosphates, the glycolytic products of starch, exported from chloroplast, serve as the starting materials for the sucrose synthesis in cytosol. Therefore, a reduction in Pn would further decline the sucrose reserve in the source that would negatively influence starch biosynthesis in the grain. Starch biosynthesis in the grains is catalysed by four enzyme classes: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE) and starch debranching enzyme (DBE) (Liu *et al.,* 2006). Previously, the activities of AGPase and starch branching enzymes were significantly correlated with starch accumulation rate and starch synthesis and they are key enzymes during starch synthesis course in rice grains (Umemoto *et al.,* 2002). Low light during the grain-filling stage results in a decreased supply of carbohydrates to grains as well as a decrease in starch synthase activity in grains, which directly inhibits grain filling and enhances the occurrence of chalky rice (Liu *et al.,* 2006). The starch synthesis amount declined under the LL condition. We investigated the real-time gene expression of three major starch synthesizing genes in rice- AGPase, GBSS I and SSIII at the milky stage of grain filling. AGPase catalyses the synthesis of ADP-glucose and pyrophosphate from ATP and glucose 1-phosphate, which plays a significant role in starch biosynthesis, GBSSI is mainly expressed in the endosperm and catalyses the

extension of long glucan chains primarily in amylose whereas SSIII synthesizes the linear chains of amylopectin (Yu *et al.,* 2019). In our study, we have found downregulation of all the above starch biosynthesis genes significantly in phyA than WT under LL stress and also under normal light. This has further negatively influenced starch accumulation and 1000-grain weight in grain of phyA genotype than WT genotype both under LL stress and normal light. A positive correlation between starch biosynthesis and grain filling in rice has been previously reported (Panigrahi *et al.,* 2019). An intricate interplay between phytochromes and various phyto-hormones during the entire process of fruit development and ripening has been observed (Gramegna *et al.,* 2019), thus providing an evidence regarding the regulation of fruit quality by fruit-localized phytochromes. However, the clear roles of phytochromes in rice grain development are not yet properly understood. Our data suggested the possible role of phyA in this process. Amylose content (AC) is considered to be the single most important characteristic for predicting rice cooking and processing behaviours. In high yielding varieties, AC ranged from 17.86% to 24.75%. Most consumers prefer rice with intermediate AC ranged between 20% to 25% (Valladares *et al.*, 2012). When rice is grown under LL for 32 days (starting from the initial heading stage), brown rice, milled rice and head rice yield as well as grain amylose content decrease (Liu *et al.,* 2006). A sharp downregulation of the expression of major starch biosynthesis genes under LL could be attributed to decrease in amylose content in rice grain under LL, which was more pronounced in phyA than WT plants. Therefore, we speculate that changes in the light intensity may influence phytochrome A mediated physicochemical metabolism within plants during the formation of grains and has an impact on its quality attributes. Cooking and eating qualities of rice have long been associated with amylose content (Yu *et al.,* 2019). The structure of the amylopectin molecule, in particular, appears to influence viscoelastic properties of rice which influences the cooking quality. Amylopectin content was found to increase in the grains from the LL grown plants. Starches from chalky kernels contained less amylose (more amylopectin) and shorter branch-chain amylopectin. We found an increase in AC content comparatively more in WT which was directly correlated with the chalkiness. Congruent to above finding, in our study, we observed a decrease in starch and amylose content in grain with an increase in amylopectin content. PhyA grains had increased chalkiness under LL than WT grains which could be attributed to its lower amylose and higher

amylopectin accumulation. The rice varieties having minimum amount of chalkiness is considered as a preferred grain character. Grain shape and endosperm opacity are major attributes that determine the appearance quality. The greater amount of chalkiness in the grain indicates that it is more prone to grain breakage during milling, which results in lower HR recovery (Santos *et al.,* 2019). HR indicates that weight of whole grains obtained after industrial processing and is the proportion of the intact grain in the milled rice. It was reported that the quality rice variety should have HR value at least 70%. HR value depends on the grain type, chalkiness, cultivation practices and drying condition. Therefore, as chalkiness was more in *phyA* under LL, its HRR was concomitantly found less than WT. We found a direct correlation between the expression level of AGPase, GBSSI and SSIII along with the starch and amylose content of grains in phyA and WT under LL and normal light condition. This suggests that a reduction in photo-assimilate in the source organ of phyA, decreases its sink capacity that further reduces the expression of starch biosynthesizing genes in the grains enhancing chalkiness, sterility percent and diminishing 1000-grain weight and yield. LL conditions during the grain-filling stage result in poor appearance and milling qualities of rice grains (Ren *et al.,* 2003). The de-hulling of rice is one of the important post-harvest processes. If the hulling percentage is high, then the recovery of rice is also increased. The eighty percent or more are the desirable hulling characteristics for rice. In our study, we have found a significant decrease in the hulling percent under LL, which was more pronounced in phyA than WT. Kernel length and breadth decreased under LL stress. However, the KL of phyA grains was found to be more than that of WT both under LL and control. Recently, phytochrome-interacting factors-Like protein *OsPIL1* has been reported as a positive regulator of grain length (Todaka *et al.,* 2012). Three *OsbHLH* genes with antagonistic effects on grain size were identified: PGL1, PGL2 and APG (*OsPIL16*). PGL1 and PGL2 are positive grain length regulators and APG belongs to the *OsPIL* family, which has functionally antagonistic effects on PGL1 and PGL2 as a negative grain length regulator (Heang and Sassa, 2012). Hence, it is suggested that in the absence of PHYA in the mutant lines, APG fails to regulate the expression of PGL1 and PGL2, which further participate in the increase in grain length. Water uptake, volume expansion ratio and alkali spreading value deceased under LL stress, which was more pronounced in phyA than WT. Previous reports have established a positive correlation of AC with water uptake, volume expansion ratio and alkali spreading value. This indicates that high amylose rice varieties will absorb more water and will produce a greater volume of cooked material. Gel consistency decreased under LL and was found to be correlated with AC. Furthermore, a depletion in photosynthetic rate in the source organ followed by sink-capacity and the expression of major enzymes involved in starch biosynthesis have been found primarily responsible for the reduction of yield in phyA than WT genotype.

Fertility *per cent* comprehensively reflects the status of coordination between source and sink. Fertility *per cent* of grans of *phyA* was lower than WT, indicating that the relation between source and sink was basically inapposite in former than latter. Lower fertility percent in phyA also indicated that source use efficiency is lower or source potential is inefficient, which is probably related to lower dry matter production after heading, or lower matter translocation efficiency during the late growth period. As a whole, the yield sink per unit leaf area of phyA was higher than that of WT. This has resulted in a significant reduction in yield under LL stress in phyA than WT plants.

Conclusion

The present work on the analysis of the relative net assimilation rate, sink capacity, differential expression of starch synthesizing genes and its effect on the grain quality in phyA and WT plants has provided a comprehensive insight into the possible role of phytochrome A in the regulation of source-sink communication which concomitantly modulates grain filling rate and yield in rice. Change in light intensity results in an alteration it its spectral components which further affects photosynthetic yield and transportation of photo-contracted compounds from source to sink organs, finally depleting the grain quality and yield in rice. The study clearly suggested that the expression of certain starch synthesizing genes was significantly down-regulated under LL in the grains of phyA genotype than WT genotype which further increases sterility percent, decreases 1000-grain weight and yield in former than latter. The details of the phytochrome A mediated signalling mechanism involving in the interplay between the scope of sink production, grain quality and yield potency are however, yet to be perceived. The results also specified that the reduction in light intensity during rice grain filling stage is a significant cause of yield reduction and the alteration in light quality may be a principal factor causing the decline in grain quality. This study indicates the potent role of phytochrome A in the regulation of light-dependent growth attributes in rice that could be further studied through biotechnological interventions.

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