J.Natn.Sci.Foundation Sri Lanka 2024 53 (1): 33 - 43
DOI: http://dx.doi.org/10.4038/jnsfsr.v53i1.12325

RESEARCH ARTICLE

Genetics and Plant Breeding

Coincidence of inductive photoperiod and early vegetative phase leads to early flowering and increased yield in selected Sri Lankan traditional rice varieties

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Submitted: 15 August 2024; Revised: 13 December 2024; Accepted: 06 January 2025

Abstract: Despite the nutritional and environmental benefits, Sri Lankan traditional rice (SLTR) is under-cultivated due to the photoperiod sensitivity in most varieties. This experiment was carried out to determine the effect of photoperiod on the days to flowering (DF) and number of spikelets per first panicle (SP) of selected SLTR varieties *Masuran* (5530*)*, *Heras (*6412*)* and *Hathe pas dawase wee (*4237), by exposing to different photoperiods and developing a gene network model. Three week old plants were exposed to time controlled sunlight for three photoperiods of 11 hour and 45 minutes representing short-day photoperiod (SD), 12 hours, and 12 hours and 05 minutes in the photoperiod chamber, and to the prevailing natural photoperiod, which ranged from 12 hours and 14 minutes to 12 hours and 02 minutes, and 12 hours and 15 minutes to 12 hours and 03 minutes, in two locations in a completely randomized design with three replicates. DF and SP were recorded. The ANOVA revealed that DF and SP of each accession were significantly affected by photoperiod ($p < 0.05$). The SD resulted in significantly reduced DF in three accessions. The increased DF reduced the SP in accessions 5530 (r = -0.51) and 6412 (r = -0.53) according to the correlation analysis ($p < 0.05$). The photoperiod effect on SP was explained through quadratic regression models, which indicated an optimum photoperiod for the highest SP ($p < 0.05$). Gene network model analysis suggested distinct interactions among pleiotropic genetic factors in response to photoperiod, and tested rice accessions. *Hd1* and *Ghd7* interactions mediated late-flowering under long-day conditions, while the absence of the *Ghd7* pathway mitigated the delayed flowering response. *DTH8*-mediated suppression of *Ehd1* and *Hd3a* under long day conditions led to delayed flowering and a simultaneous increase in SP. Alternatively, the *Ehd1-RFT1* pathway facilitated early flowering under short-day conditions in the absence of *Hd1*. Plant responses and suggested gene interactions provide valuable insights for manipulating DF and yield in breeding SLTR.

Keywords: Days to flowering, gene network model, increased yield, photoperiod, Sri Lankan traditional rice.

INTRODUCTION

Rice grown in Asia is a major component of food security in today's context (Zhao *et al.,* 2020). However, the rice sector will face significant challenges in meeting the future demand, driven by the projected global population growth from 8.9 to 10.6 billion by 2050 (United Nations, 2019). Rice production is mainly dependent on the rice varieties, environmental factors, and inputs. Response to the environmental clues of photoperiod and temperature are common among most of the rice varieties, which are critical for the transition from vegetative phase to initiation of flowering. The temperature perception pathway and photoperiod pathway converge for the initiation (Vicentini *et al.,* 2023). The effect of photoperiod and temperature

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on the early vegetative phase of a Sri Lankan traditional rice core collection has been reported in previous studies. The average number of days to reach the fifth leaf stage in the core collection was significantly lower under a high temperature regime of 36.9 ± 0.4 ^{0}C compared to the low temperature regime of 34.0 ± 1.0 ^{0}C, irrespective of the photoperiod (Rathnathunga & Geekiyanage, 2017a; Rathnathunga *et. al*., 2019). According to Vergara and Chang (1985), rice is a facultative short-day plant that flowers early under short day exposure, while some rice accessions can flower under long-day conditions as well. During the photoperiod sensitive phase, the rice plant must receive the photoperiod required for flowering initiation: the critical photoperiod is dependent on the variety and determines the time of transition from the vegetative phase to flowering initiation. When a rice plant does not meet the critical photoperiod, it remains in the vegetative phase. The shortest time to initiate flowering is achieved when plants are exposed to the optimum photoperiod. The requirement for shorter day lengths to promote faster flowering is typically fulfilled with day lengths shorter than 13.5 hours (Itoh *et al.*, 2010). Extremely photoperiod sensitive varieties of Sri Lankan traditional rice remain in the vegetative phase if they are not exposed to the critical photoperiod in the latter part of the *Maha* season under natural field conditions (Rathnathunga *et al.*, 2016 a). The optimum photoperiod is an important phenomenon in the ecological adaptation of rice varieties for climate change resilience (Vergara & Chang, 1985).

The genetic basis of the photoperiod effect on days to flowering (DF) of rice has been intensively studied. Specific pathways are assigned to the flowering time quantitative trait loci, some of which behave in accordance with Mendelian laws (Hori *et al.,* 2016). Identification of a key flowering time gene in rice named *Heading date 1* (*Hd1*) of the CCT Family transcription factor was a major breakthrough in flowering time studies (Yano *et al*., 2000). *Hd1* is activated by *OsGI* and *Hd1* promotes the floregin *Hd3a* under short-day conditions (Kojima *et al*., 2002; Hamaya *et al.,* 2003). The *rice flowering locus T 1 (RFT1)* encodes a mobile flowering signal that induces floral transition under short-day environments (Komiya *et al.*, 2008). In addition, *Early heading date 1* (*Ehd1)*, *B* type response regulator is rice-specific and reported to be a mediater of the key flowering time pathway network under both short-day and long-day conditions (Vincentini *et al.,* 2023). Strong repressors of *Ehd1* under long-days include *grain number*, *plant height and heading date 7* (*Ghd7*, also known as *Hd4* and *Ghd8*) (Xue *et al.,* 2008) and *DTH8* (also known as *Hd5* or *Ghd8*) (Wang *et al.,* 2021), which affect DF and yield related agronomic traits. Interactions of non-functional *Hd1* with *Ghd7* and *DTH8* are established to explain the time of flowering (Sun *et al.*, 2022). Under long-days, *Hd1* represses flowering (Yano *et al.,* 2000). Flowering repression of *Hd1* under long-days is through repressing *Ehd1,* which indicates the importance of non-functional *Hd1* and *Ehd1* in artificial selection of rice for wider environments (Wei *et al.,* 2015). In addition, the presence of unique non-Circadian clock genes of rice, which act as master switches of transition from the vegetative phase to the reproductive phase, such as *Rice Indeterminate 1* (*RID1*), also play an important role in floral transition (Wu *et al.*, 2008). The effect of photoperiod on flower opening time in rice was recently studied. The variability in flower opening time is reported to be significantly lower in photoperiod sensitive cultivars compared to photoperiod-insensitive cultivars for flowering initiation (Deb, 2024).

Sri Lankan traditional rice germplasm is a diverse collection of accessions for variation in flowering time, yield and several agronomic characters (Rathnathunga *et al.,* 2014; Padukkage *et al.,* 2015 ; Pushpakumari *et al.,* 2016: Rathnathunga *et al.*, 2016a). Early maturing rice accessions are grouped as short-day sensitive and long-day sensitive for flowering initiation (Padukkage *et al.,* 2017). Diverse genes are reported to be responding to short-day and long-day conditions in early flowering sub-populations of *indica* and *japonica* rice (Han *et al.,* 2016). In a study using temperate *japonica* rice grown in Northeast China through molecular markers and flowering time records, *DTH2* and *Hd18* were identified as the major flowering promoters. *Hd2, Hd4*, and *Hd5* were identified as the major flowering repressors. Furtherm ore, *Hd6* and *Hd16* were identified as minor flowering repressors, and *Hd17* was minor flowering promoter (Li *et al.,* 2018). CRISPR/Cas9-mediated multiplex genome editing for manipulating flowering time in rice was reported (He *et al*., 2024). Flowering time genes of *Hd2, Ghd7*, and *DTH8* were edited in rice, and the mutant lines of the above genes flowered earlier without a significant yield loss. The successful development of homozygous mutant lines with early flowering and no yield reduction provides a foundation for breeding rice varieties with shorter growth periods and greater adaptability for rice breeding applications.

Several attempts have been made to determine the effect of the number of DF on grain yield. Early flowering during an inductive short-day season increased the grain yield of *Ma wee* accessions under field conditions, whereas delayed flowering of these accessions in a non-inductive season reduced the yield (Pushpakumari *et al.,* 2017). On the other hand, several studies reported a significant positive correlation between DF of different

rice accessions and their grain yield (Pushpakumari & Geekiyanage, 2014; Pushpakumari *et al.,* 2016; 2024; Rathnathunga & Geekiyanage*,* 2017b; Jayasinghe *et al.*, 2023). Furthermore, in several other studies, variation in DF among accessions of the same rice variety within a single growing season was positively correlated with vegetative growth while negatively correlated with grain yield in the varieties of *Kalu heenati* (Pushpakumari & Geekiyanage, 2015), *Sudu wee* (Rathnathunga & Geekiyanage, 2016), *Hondarawala* (Rathnathunga *et al*., 2016b) and the varieties of *Suduru samba* and *Pachchaperumal* (Rathnathunga & Geekiyanage, 2017b). Additionally, a positive correlation was found between plant height and yield in selected *Ma wee* accessions (Jayasinghe *et al.*, 2023).

These research findings highlight the necessity of investigating the genetic basis of the response to inductive photoperiod exposure during the photoperiod-sensitive phase of rice to manipulate yield. Based on the above reports, we hypothesize that there is genetic variation in Sri Lankan traditional rice varieties in response to inductive photoperiod exposure during the photoperiod-sensitive phase, which could be used to manipulate yield.

Therefore, the present study was conducted to determine the effect of photoperiod, through exposure to time controlled-natural sunlight during a defined period of vegetative phase on DF and yield under Sri Lankan conditions. Additionally, the study aimed to identify the gene network associated with photoperiod sensitivity and yield manipulation, in order to utilize the generated knowledge for future breeding purposes.

MATERIALS AND METHODS

Plant material

Three traditional rice accessions (accession 5530 of variety *Masuran*, accession 6412 of variety *Heras,* and accession 4237 of variety *Hathe pas dawase wee*) were selected based on the seasonal variation of flowering time during the *Yala* (in 2019) and *Maha* (in 2018/2019) seasons (unpublished data).

Experimental location, design and duration

The study was carried out at two locations in Ibbagamuwa, Kurunegala District, Sri Lanka in the agro-ecological zone IL1b (7.3500° N, 80.4333° E) from August 2021 to February 2022, and Vantharumoolai of the Batticaloa District, Sri Lanka, in agroecological zone DL2b (7.7178° N, 81.6894° E) from August 2022 to February 2023. The pots were filled with a mixuture of mud and sandy loam soil in the Ibbagamuwa experimental site, and with sandy soil in the Vantharumoolai experimental site. Pots with germinating seeds were exposed to sunlight. The exposure of plants to sunlight was restricted by a black cover for three photoperiod regimes of 11 h and 45 min, 12 h, and 12 h and 05 min from the 19th and 21st day of seed establishment at Ibbagamuwa and Vantharumoolai respectively, for a period of 30 ds. The choice of photoperiods−11 h and 45 min, 12 h, and 12 h and 5 min–was based on the natural variations in photoperiods under Sri Lankan conditions for plant exposure for one month during the early vegetative phase. The 11 h and 45 min photoperiod corresponds to the naturally prevailing short-day condition for about one month in the *Maha* season, which is supposed to facilitate traditional rice flowering behaviour under short day conditions in Sri Lanka. The chosen photoperiods of 12 h, and 12 h and 5 min are realistic representations of the day-neutral and long-day conditions that prevail during this period, ensuring that the experiment closely reflects the natural environmental conditions for the rice plants during the early vegetative phase. Such subtle differences were considered necessary to identify sensitivities in flowering regulation genes at the basic vegetative stage. After the treatment, the plants were exposed to photoperiods available naturally without any restriction. The control plants were exposed to photoperiods available naturally without any restriction for light exposure throughout the period. The experiment was laid out with three replicates in a completely randomized design.

Plant data collection and analysis

The DF and number of spikelets per first panicle (SP) of each accession under 4 photoperiods were recorded according to the descriptors for rice characters (Rathnathunga *et al.,* 2014; Padukkage *et al.,* 2015; Pushpakumari *et al.,* 2016). and mean values were calculated. The DF and SP data were separately analyzed through two way-ANOVA for significant effects of three imposed photoperiods, three rice accessions and their interaction. This was followed by Tukey's honestly significant difference (HSD) post-hoc test to identify significant differences in the DF and SP of the different treatments. Secondly, an analysis of simple main effects was conducted to explore the interaction effects further and to understand the impact of either variable (photoperiod or accession) at each level on the other variable. Following this, Fisher's least significant difference (LSD) test was employed to make pairwise comparisons.

A correlation analysis was carried out for the relationship between the DF and SP, while a regression analysis was carried out to determine the effect of photoperiod on SP for each accession at each location. Both methods of analysis were performed using IBM SPSS Statistics software (version 23).

Gene network model generation

Information on genes explaining the correlation between flowering and SP was collected from KEGG (https://www.genome.jp/kegg/pathway.html) and STRING (https://string-db.org/) databases and prior literature. The network table files were created out of these details. Network data were imported into the Cytoscape interface. Interaction parameters were defined by specifying columns of data containing the source interaction, target interaction, and interaction type. Network models were generated using Cytoscape 3.8. In the generated network, nodes (or vertices) represent genes, while edges represent relationships of interactions. The generated network was analyzed to decipher gene interactions.

RESULTS AND DISCUSSION

Natural photoperiod variation at two experimental sites

The natural photoperiod was experienced by rice plants from the first to the 18th and 21st day of seed germination, ranging over time from 12 hours and 21 minutes to 12 hours and 14 minutes, and 12 hours and 23 minutes to 12 hours and 15 minutes, in Ibbagamuwa and Vantharumoolai during the periods from 15.08.2021 to 02.09.2021 and 10.08.2022 to 31.08.2022, respectively. During the 30-day period of photoperiod treatments, the natural photoperiod variation was from 12 hours and 14 minutes to 12 hours and 02 minutes, and 12 hours and 15 minutes to 12 hours and 03 minutes in Ibbagamuwa and Vantharumoolai, respectively. After the 30-day treatment period, all plants were again exposed to sunlight without restriction. Plants experienced a reducing natural photoperiod during the experimental period, which ranged at the end of treatment at 12 hours and 02 minutes and 12 hours and 03 minutes, to 11 hours and 44 minutes and 11 hours and 45 minutes, on the 100th day in Ibaagamuwa and Vantharumoolai, respectively (Figure 1).

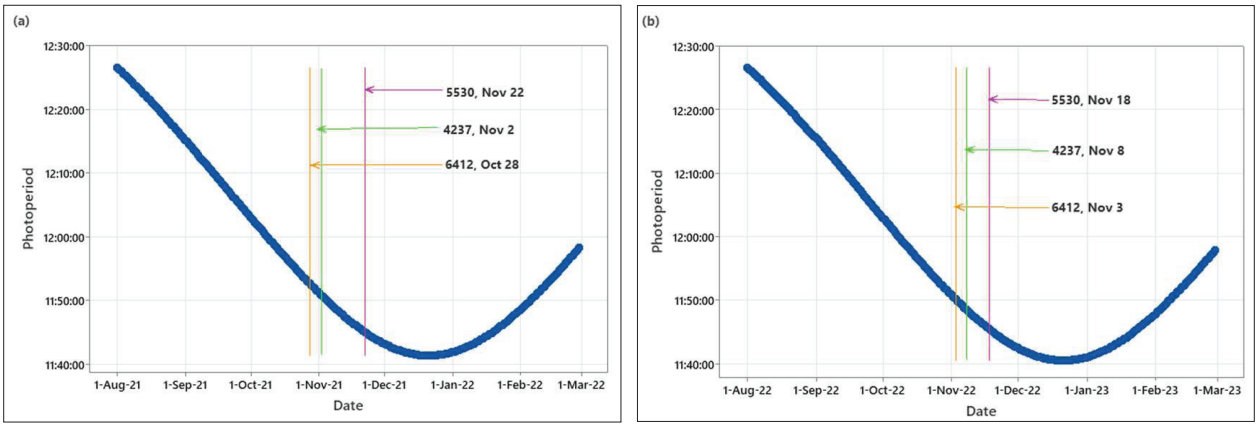

Figure 1: Natural variation of photoperiod during the experiment and flowering time of three accessions in Ibbagamuwa, Kurunegala (a) and Vantharumoolai, Batticaloa (b).

Effect of photoperiod on days to flowering

Exposure to photoperiod during the early vegetative phase affected the number of DF of three accessions significantly at each location. Three accessions responded at different levels to photoperiod. There was a significant interaction between rice accession and photoperiod for DF (Table 1). Simple main effect analysis revealed that the photoperiod of 11 hours and 45 minutes significantly produced the least number of DF in the 3 accessions of 5530 (76.33 ± 0.51 and 74.33 ± 1.67), 6412 (58.67 ± 0.50 and 62.33 ± 0.69), and 4237 (64.33±0.61 and 67±0.88) at Ibbagamuwa and Vantharumoolai, respectively (Supplementary Table S1 and Figure 2). Photoperiods of 12 hours, and 12 hours and 5 minutes during the treatment period, had influenced the DF positively, which is evident through regression analysis. Furthermore, exposure to the natural photoperiod which

was a long day condition for the 18th day and 21st day of seed germination in Ibbagamuwa and Vanthuramoolai, respectively, increased the DF significantly (Figure 1 and Figure 2).

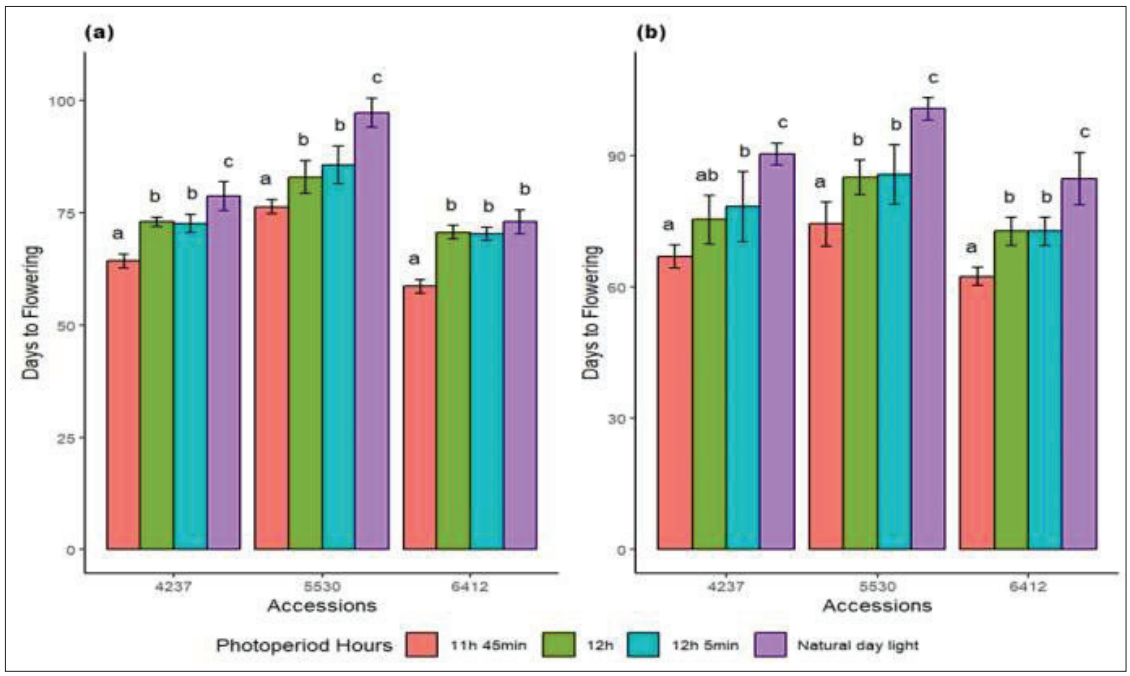

Figure 2: Effect of photoperiod on days to flowering of the three selected accessions at two locations in Sri Lanka. (a) Ibbagamuwa, (b)Vantharumoolai. Significantly different values of each accession at each location are given in different letters.

Table 1: Analysis of Variance for the effect of photoperiod and rice accession on days to flowering and number of spikelets per first panicle

Variables	F value					
	Ibbagamuwa			Vantharumoolai		
	Photoperiod	Accession	Photoperiod x Accession	Photoperiod	Accession	Photoperiod x Accession
DF	66.541*	159.791*	3.483*	39.829*	25.033*	0.177
SP	901.639*	166.374*	108.500*	727.498*	1049.494*	130.762*

** F value is significant at $p < 0.05$*

There was a variation in DF among three accessions under similar conditions of each photoperiod, suggesting their genetic diversity in responding to similar growing conditions. The linear regression relationships for the two locations under the tested photoperiods did not overlap in accessions 4237 and 6412, while those of 5530 overlapped at two locations under the tested photoperiod conditions (Figure 3).

The photoperiod response curves have been used by Chandraratna (1955) to determine the optimum photoperiod at the minimum number of DF. During this experiment, the photoperiod of 11 hours and 45 minutes was tested as it is close to the lowest photoperiod available under natural conditions in Sri Lanka. According to the regression analysis, short day conditions are favourable for early flowering (Figure 3). Therefore, the optimum

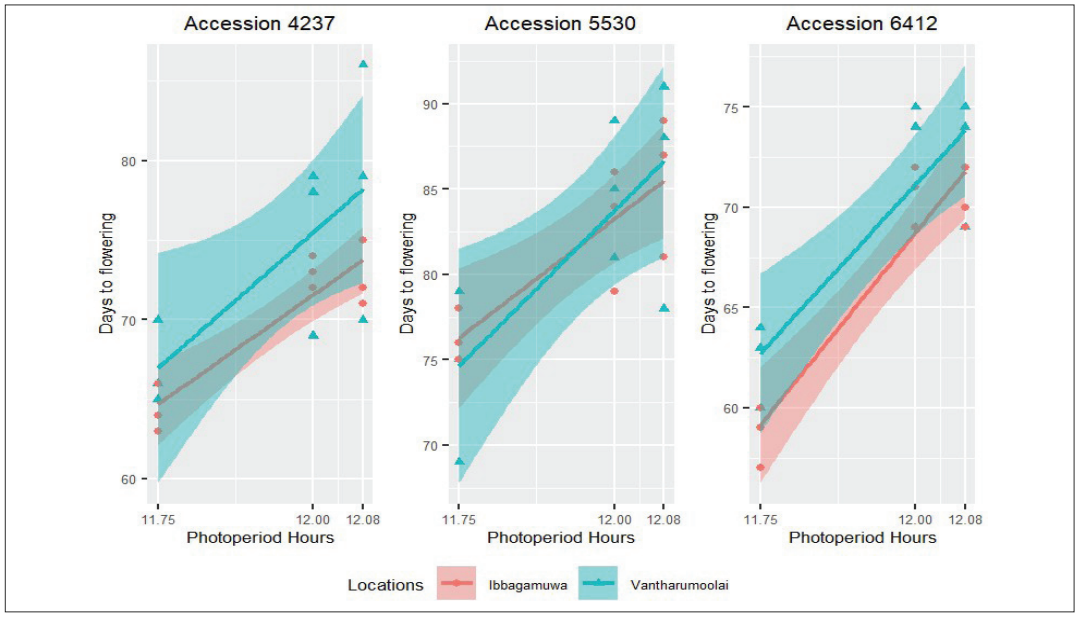

Figure 3: Effect of photoperiod on days to flowering of the three accessions based on the regression analysis (Accession 4237; DF = - 103.5 + 14.66 Photoperiod, R-Sq = 89.3% (Ibbagamuwa), DF = - 131.7 + 17.34 Photoperiod, R-Sq = 48.8% (Vantharumoolai), Accession 5530; DF = - 85.60 + 14.14 Photoperiod, R-Sq = 65.1% (Ibbagamuwa), DF = - 144.3 + 19.10 Photoperiod, R-Sq = 58.0% (Vantharumoolai), Accession 6412; DF = - 175.2 + 20.43 Photoperiod, R-Sq = 94.3% (Ibbagamuwa), DF = - 142.2 + 17.87 Photoperiod, R-Sq = 80.6% (Vantharumoolai).

inductive photoperiod of all 3 accessions for early flowering should be 11 hours and 45 minutes, which resulted in the lowest DF under tested conditions or photoperiods less than that. Accession 4237 was tested in a previous experiment by Padukkage *et al.* (2017) for DF under 8 hours, 12 hours and 14 hours of light throughout the plant life span. Accession 4237 flowered early under 8 hours of light over day-neutral conditions and long-day conditions of 14 hours of light. However, eight hours of sunlight exposure is not naturally available under tropical conditions in Sri Lanka. Early flowering at 11 hours and 45 minutes for a period of 1 month in the early vegetative phase, which is only 15 minutes less than day-neutral conditions, creates an interesting question to reveal the genetic factors that are sensitive to such a marginal photoperiod difference under tropical conditions.

Effect of photoperiod on number of spikelets per the first panicle

Photoperiod and rice accession were significant factors affecting the SP at both locations according to the analysis of variance (Table 1). Accessions 5530 and 6412 produced the highest number of spikelets per first panicle under the photoperiod of 11 hours and 45 minutes at Ibbagamuwa (214.6 ± 0.9 , 145 ± 3) and Vantharumoolai (220 ± 4, 150.6 ±1.4) respectively. The SPs of accession 4235 under 12 hours and 12 hours and 05 minutes were not lower than that under 11 hours and 45 minutes, while the natural photoperiod had the significant effect of lowering the number of spikelets according to the single main effect analysis (Supplementary Table S2).

Relationship between days to flowering and number of spikelets per first panicle

The correlation analysis revealed that the number of DF is negatively correlated with SP in accessions 5530 and 6412 with a correlation coefficient of 0.51 and 0.53 respectively at the 0.05% level of significance (Table 2).

Table 2: Correlation between days to flowering and the number of spikelets per first panicle

Accession	Correlation coefficient (r)	*p* value
4237	0.1878	0.4555
5530	-0.5168	0.0280
6412	-0.5341	0.0224

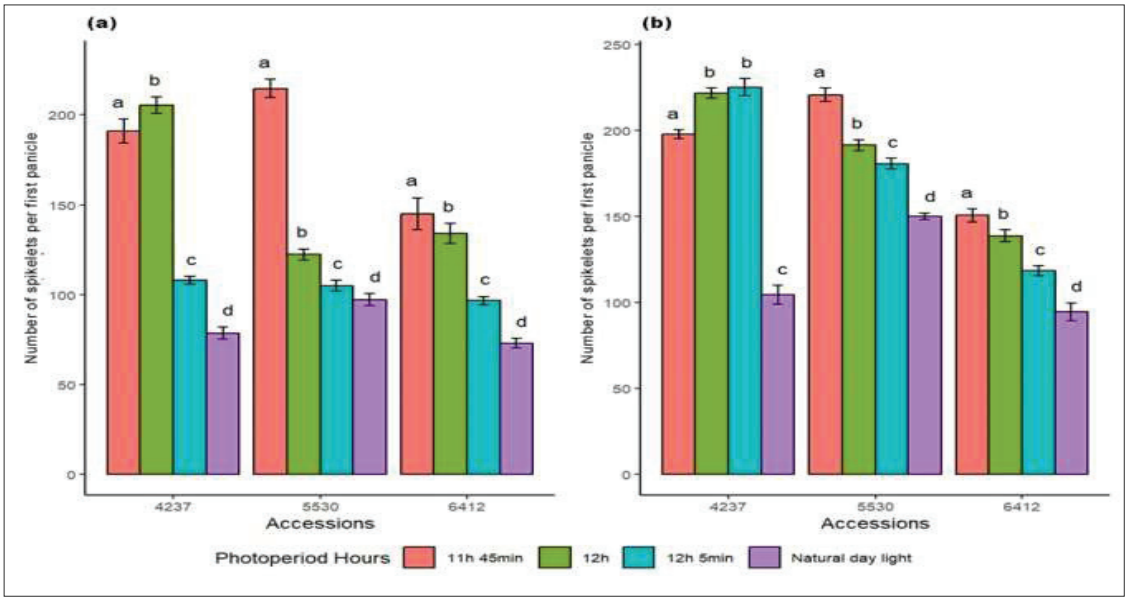

Figure 4: Effect of photoperiod on number of spikelets per first panicle of the three selected accessions at two locations in Sri Lanka (a) Ibbagamuwa, (b) Vantharumoolai. Significantly different values of each accession at each location are given in different letters.

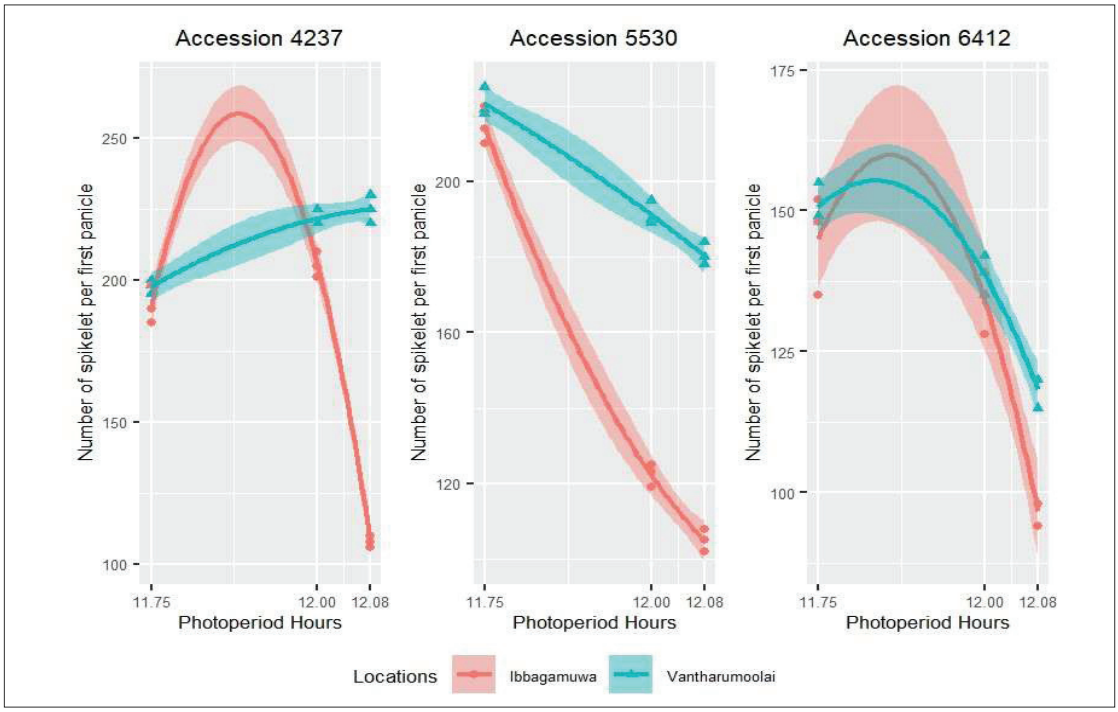

Figure 5: Relationship between photoperiod exposure and number of spikelets per first panicle at two locations. (Ibbagamuwa: Accession 6412: SP = -38806 + 6820 Photoperiod - 298.0 Photoperiod ² (R^2 = 99.6%); Accession 5530: SP = -38806 + 6820 Photoperiod - 298.0 Photoperiod ² (R^2 = 99.6%); Accession 4237: SP = -451862 + 77127 Photoperiod - 3288 Photoperiod ² (R^2 = 99.2%); Vantharumoolai: Accession 6412: SP = -87730 + 15019 Photoperiod - 641.4 Photoperiod ² (R^2 = 95.8%)., Accession 5530: SP = -35809 + 6200 Photoperiod - 266.7 Photoperiod² (R^2 = 97.4%)., Accession 4237: SP = 4972 - 856 Photoperiod + 38.4 Photoperiod ² (R^2 = 94.4%).

Long-aged rice varieties are considered to be high yielding over short-aged rice varieties. Previous studies on the relationship between DF of different traditional rice accessions and SP under similar field conditions had reported positive correlations between DF and SP (Rathnathunga *et al.*, 2016a; Rathnathunga & Geekiyanage, 2016; Jayasinghe *et al.*, 2023). According to a photoperiod chamber experiment, yield was reduced in the rice varieties *Sulai*, *Kohu ma wee*, and *Devaraddili* under long-day conditions (Geekiyanage *et al*., 2012). In contrast, timing of planting affects DF and SP in selected *Ma wee* accessions, where the extended DF of the same accession due to seasonal effects reduced the yield (Pushpakumari *et al.,* 2017).

Chandrarathna (1955) considered the minimum photoperiod for the minimum DF as the optimum photoperiod without considering the effect of DF on the yield. During this study, we developed the regression models of SP and photoperiod for each accession to determine the optimum photoperiod for the highest SP (Figure 5). The optimum photoperiod for the highest SP can be deduced through the regression curve. Accessions 5530 and 6412 produce the maximum SP at the photoperiod of 11 hours and 45 minutes at both locations. According to the postulated SP for the range of tested photoperiods, it is suggested that the photoperiod under 11 hours and 45 minutes giving the lowest DF is not the the optimum photoperiod for SP for accession 4237, reconfirming the non-correlation of DF and SP in accession 4237 (Table 2).

Gene network model analysis for identification of key flowering time genes involved in vegetative growth and yield

Different gene models were generated to predict the possible gene interactions in the three tested rice accessions for the DF and yield under three photoperiods (Figure 6). The model explains the behaviour of accession 5530 as follows: under short-day conditions, *OSG1* promotes the *Hd*1, which promotes the *Ehd1* and *Hd3a* expression for flowering initiation (Endo-Higashi & Izawa, 2011). In addition to the effect of *Hd1* on flowering, there are reports on its pleiotropic effects on plant height and yield traits of SP and number of grains per panicle (NGP), in the genetic background of the *indica* rice variety ZS97 (Zhang *et al.*, 2012; Yan *et al.,* 2011).

Therefore, the significant increase in SP under the short-day photoperiod of 11 hours and 45 minutes in accession 5530 could be due to the direct influence of *Hd1* as reported by Zhang *et al.* (2012).

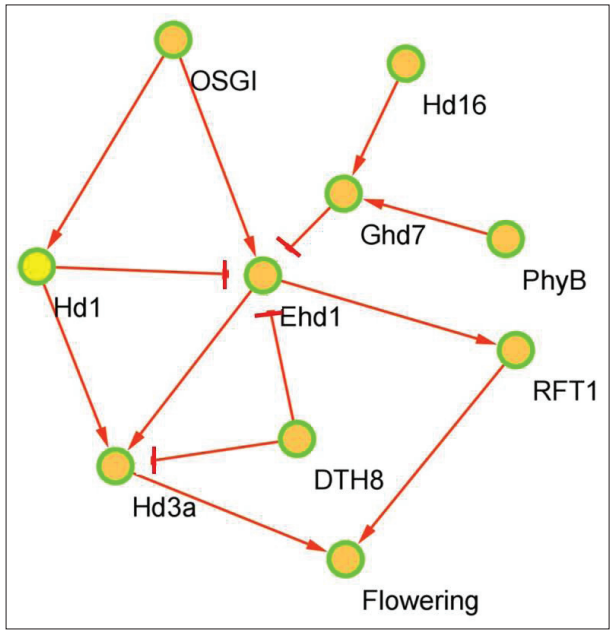

Figure 6: Proposed generalized gene network models for rice accessions 4237, 5530, and 6412 on flowering initiation under short-day and long-day photoperiods.

In the gene pathway model for accession 5530 under long-day condition, the presence of functional *Ghd7* is suggested for the interaction with *Hd1* to delay flowering (Figure 6). A night break of even 10 minutes results in delayed flowering through adversely affecting *Hd3a* expression (Ishikawa *et al.*, 2005). Therefore, the model indicates the unavailability of *Hd3a* under 12 hours, and 12 hours and 05 minutes in accession 5530. According to the model, *DTH8* is not present in the accession 5530. As a result, the delayed number of DF under long-day conditions negatively influenced the SP. The red-light photoreceptor, *Phytochrome B* and *Hd1*6 induce *Ghd7,* leading to delayed flowering under long-day conditions. It is postulated that *Phytochrome B* may affect the *Hd1* function adversely at the post-transcription level (Zhou *et al.*, 2021).

The accession 6412 exhibited the shortest DF (59.0 ± 1.8 days) among the three tested accessions under short-day conditions. Accordingly, accession 6412 is not supposed to have functional *Ghd7*, expression of which delays flowering under short-day conditions also (Zhang *et al.,* 2019). Two *Hd1*-induced pathways were proposed for accession 6412 with the presence of *Hd3a, Hd1, Ehd1* and *RFT1* (Figure 6). Among the previously derived pathways, those involving *Ghd7* were presumed to be absent in this accession, allowing *Hd1* to initiate

early flowering in 6412 under short-day conditions. *Hd16* was reported to shorten flowering time by 20 days under long days and to extend DF under short days in the non functional *Ghd7* background (Hori *et al.*, 2013; Kwon *et al.*, 2014). *Hd1* promoted early heading under two pathways, where one promoted *Ehd1* and then *Hd3a,* as observed in the 5530 accession. In the second pathway, *Ehd1* expression under short-day conditions promotes *RFT1* expression (Zhu *et al*., 2017). Simultaneously, *Hd1* induces higher SP under short-day conditions as well.

A genetic model with two pathways was proposed for 4237 with *DTH8, Ehd1,* and *RFT1* (Figure 6). In the absence of *Hd1, Ehd1* may have induced the expression of *RFT1*, leading to early flowering in accession 4237 under short-day conditions. The presence of *DTH8* in the model explains the increase of SP under long-day conditions of 12 hours and 5 minutes with delayed flowering; *DTH8* down-regulates the transcriptions of *Ehd1* and *Hd3a* under long-day conditions (Wei *et al*., 2010). Our results can be supported by the previous work reporting the findings that *DTH8* plays an important role in the signal network of photoperiodic flowering as a suppressor, as well as in the regulation of yield (Wei *et al.*, 2016).

The *Hd1* floral repressor activity basically requires *Ghd7* in long-day conditions in functional *DTH8* background (Nemoto *et al*., 2016). The heading repression in long-day conditions by *Hd1* also depends on *DTH8* in functional *Ghd7* background (Du *et al*., 2017). Post-transcriptional interactions of *Hd1*-*Ghd7* negatively influences flowering initiation by binding to the *Ehd1* promoter and suppressing the expression of *Ehd1* under long-day conditions (Zhou *et al.*, 2021).

The comprehensive mechanism by which these genes interactively control the various extents of photoperiod sensitivity in the same genetic background needs to be further addressed. Our results sheds light on gene network manipulations in the future for breeding Sri Lankan traditional rice to contribute to food security under climate change.

CONCLUSION

The short-day photoperiod of 11 hours and 45 minutes, which is close to the prevailing shortest photoperiod under tropical Sri Lankan conditions during the vegetative phase of accessions 4237, 5530 and 6412 is the optimum photoperiod for significant early flowering.

Early flowering under the photoperiod of 11 hours and 45 minutes is correlated with the highest number of spikelets per first panicle (214.6 ± 0.9 , 145 ± 3 at Ibbagamuwa) and (220 ± 4, 150.6 ± 1.4 at Vantharumoolai) in accessions 5530 and 6412 respectively ($p < 0.05$). The number of spikelets per first panicle is in a regression relationship with photoperiod exposure. According to the regression analysis, the optimum photoperiod of 11 hours and 45 minutes produces the highest SP in accessions 5530 and 6412 under tested conditions. Gene network model analysis revealed the pleiotropic genetic factors leading to higher yield and early flowering under inductive photoperiod. The gene network model suggests that specific genes, *Hd1*, *Ehd1*, and *Ghd7*, are involved in the photoperiodic response of the three rice accessions. In accession 5530, the significant increase in SP under the short-day photoperiod of 11 hours and 45 minutes aligns with the role of *Hd1* in promoting flowering and yield traits under these conditions. The varied responses of the three accessions to photoperiods, including differences in days to flowering and number of spikelets per first panicle, are strongly influenced by the functional presence or absence of *Ghd7* and *DTH8* under short-day and long-day conditions.

Acknowledgements

The authors acknowledge the Plant Genetic Resources Centre (PGRC), Gannoruwa, Sri Lanka for provision of the traditional rice seeds.

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