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Photoperiodic stress induces genotype-specific shift in DNA methylation in Tartary buckwheat

Muhammad Saad[1], Helen Mary[1], Umar Amjid[1], Ghulam Shabir[2], Kashif Aslam[2], Shahid Masood Shah[1] and Abdul Rehman Khan[1]

[1]Department of Biotechnology, COMSATS University Islamabad, Abbottabad, Pakistan
[2]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

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Tartary buckwheat, known for its rich source of health beneficial secondary metabolites, is cultivated in many areas of the world. Among different environmental factors, photoperiod strongly influence its growth, flowering time, and ultimately the yield. In this context, epigenetics could contribute significantly in the regulation of plant response against changing environment. Therefore, with the aim to study the involvement of DNA methylation in photoperiod mediated plant response, genome-wide DNA methylation analysis was performed in two accessions (A1 and A2) of Tartary buckwheat using three photoperiodic treatments, i.e., 10-hr light/day (T1), 12-hr light/day (T2), and 14-hr light/day (T3). Flowering time and plant fresh weight data revealed that accessions A1 and A2 prefer T1 and T2 treatments, respectively. Total DNA methylation ratio increased with the increase in photoperiod in accession A1 but decreased under same conditions in accession A2. Full methylation increased significantly while intensive decrease in hemimethylation was noted from T2 to T3 in A1, whereas full methylation strongly increased and hemimethylation strongly decreased from T1 to T2 in A2. Overall, the DNA methylation events appeared more frequently than demethylation events. This study reports for the first time an accession-/genotype specific pattern of shift in the DNA methylation under different photoperiodic treatments that will pave the way toward identification of specific genes involved in the regulation of plant response against photoperiodic stress.

Original Article

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Author for correspondence:
Abdul Rehman Khan
e-mail: arehman@cuiatd.edu.pk

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INTRODUCTION

Buckwheat, a plant cultivated for its grain-like seeds, is used for human and animal consumption. Two commonly used species of buckwheat are common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn]. Tartary buckwheat is proving to have more health benefits. It contains different beneficial compounds like phenylpropanoid glycosides, flavonoids, organic acids, *trans*-resveratrol, and d-chiro-inositol (Kim et al., 2009; Matsui et al., 2010; N mcová et al., 2011; Ren et al., 2013). Its activity as antioxidant, hypocholesterolemia, anti-tumor, and antidiabetic has been reported (Guo et al., 2007; Wang et al., 2009; Yao et al., 2008; Zhao et al., 2018). In addition, it improves cognition and memory function (Abbasi et al., 2013; Choi et al., 2013). Despite all these benefits, the cultivation of this crop is limited and reducing. Different environmental conditions especially temperature and photoperiod strongly influence the growth and yield of this plant. These factors may limit the areas of cultivation in the countries where it is grown. Therefore, understanding the plant response mechanisms under such abiotic stress conditions is very important. Different plants respond differently to the change in day length. Broadly and roughly, plants can be divided into three classes according to their responses to photoperiod, i.e., day neutral, short-day, and long-day plants (Roden et al., 2002). In photoperiod responsive plants, proper day length condition is a significant factor that effects timing of different growth stages like flowering to be synchronized with the external environmental conditions to ensure the maximum productivity (Endo et al., 2016). Buckwheat is considered as short-day plant. In Tartary buckwheat, the vegetative growth regulation, floral development, and fruiting of the plant are strongly

influenced by the fluctuation of day length (Skok & Scully, 1955). Investigations in various species report the presence of a complicated network of photoresponsive and other related genes, regulation of which mediates the changes in the plant response (Song et al., 2015). Different genes showing shift in the gene expression due to day and night length have been reported in different plant species (Beales et al., 2007; Bentley et al., 2011). Understanding the underlying mechanism of these gene regulations is vital. Epigenetics is a useful tool to understand this regulation process.

Different epigenetic marks like DNA methylation and histone modification play their role in modulating the plant stress response. Among these, DNA methylation is most stable epigenetic mechanism that can have implications in crop improvement (Kapazoglou et al., 2018; Shafiq & Khan, 2015). Different studies have reported the involvement of genome-wide as well as locus-specific shift in the DNA methylation profile under stress conditions in modulating the plant response through gene regulation (Alakärppä et al., 2018; González et al., 2013; Kaleem et al., 2018; Sun et al., 2018) and transposable element regulation. (Ikeda & Nishimura, 2015). Therefore, understanding the shift in the DNA methylation landscape of Tartary buckwheat under photoperiodic variation can provide vital information about the plant adaptative response during unfavorable photoperiodic conditions that may help in breeding tolerant varieties. Therefore, this study was carried out with the aim to evaluate the involvement of DNA methylation in photoperiod-mediated plant response in Tartary buckwheat.

MATERIALS AND METHODS

Plant material development

The seeds of *F. tataricum* (L.) Gaertn were collected directly from the farmer fields present at two different sites (Shighar and Skardu; 35.42°N, 75.73°E, and 35.3°N, 75.61°E) in Gilgit Baltistan. As these accessions have never been conserved *ex situ*; therefore, they did not have any official cultivar IDs and they will be denoted as accessions A1 and A2 hereafter in the manuscript. These seeds were grown in semi-controlled condition in a tunnel with silty loam soil and average temperature between 18 and 25 °C in 3 months of cultivation. The plants were irrigated twice per day to maintain the soil field capacity. Three photoperiodic regimes: T1 (control) = 10-hr light/day, T2 = 12-hr light/day, and T3 = 14-hr light/day were used to study the effect of different photoperiods. Plants were provided with natural sunlight at daytime (9 hr 54 min to 12 hr 03 min; minimum to maximum daytime during the course of experiment) and artificial light through incandescent bulbs of 200 Watts (intensity around 78.34 Wm^{-2}) where required. The experiment was conducted in biological triplicate (three plants per treatment). The plants were grown till the seed set. Different phenotypic parameters like fresh plant weight (whole plant including root and shoot) and flowering time were evaluated. The youngest/upper three leaves (fully developed) from each plant of each treatment were sampled (pooled) for molecular analysis. Two replicates for each treatment was used for molecular analysis.

DNA extraction

Genomic DNA was extracted from 200 mg of leaves from each plant (control and treated plants) using modified CTAB protocol of extraction (Murray & Thompson, 1980). Each of the leaf samples was separately ground to powdered form in mortal and pestle. The powdered sample was transferred to 1.5 ml tube and 800 µl of prewarmed CTAB buffer was added, vortexed, and incubated at 65 °C for 45 min. Then, an equal volume of 24:1 chloroform:iso-amyl alcohol was added and mixed followed by centrifugation at 11,000 × *g* for 15 min at 4 °C. The supernatant was then transferred to a new 1.5-ml tube and an equal amount of chloroform: isoamyl alcohol was added to this supernatant and the centrifuged again with previously described conditions. The supernatant was again transferred in another 1.5-ml tube and an equal volume of chilled isopropanol was added followed by an overnight incubation at −20 °C. Next day, another round of centrifugation was performed for 15 min. After the removal of supernatant, two rounds of washing with 70% ethanol by adding 500 µl of ethanol followed by centrifugation for 5 min at 4 °C and removal of supernatant were performed. The DNA pallets were then dried for 2 hr and then dissolved in 50-µl TE buffer. The DNA samples were stored at −20 °C for further analysis.

Methyl sensitive amplification polymorphism (MSAP) analysis

MSAP, using a protocol described by Reyna-López et al. (1997) with slight changes, was employed to study genome-wide DNA methylation shift. In this method, 100 ng of genomic DNA was digested with *Eco*RI by incubation at 37 °C for 2 hr followed by enzyme inactivated at 65 °C for 20 min. This digested sample was then equally distributed to two new 1.5 ml tubes. One subsample was digested with *Msp*I restriction enzyme and the other subsample was digested with *Hpa*II by incubation at 37 °C overnight and enzymes inactivation at 80 °C for 20 min. The restricted samples were then ligated with *Eco*RI and *Hpa*II linkers (Table 1) using T4 DNA ligase enzyme (BioBasic®, Toronto, Canada) by incubating overnight at 4 °C. The linkers and primer sequences were taken from (Baurens et al., 2003) as shown in Table 1. Aliquot was then diluted to 1/5th in water and polymerase chain reaction (PCR) was performed with seven primer combinations (Table 1).

Two independent PCRs for each sample were carried out in PTC-100® Thermal Cycler (Bio-Rad, California, USA) using each of the primer combinations as described in Table 1. The PCR mixtures with the final volume of 22 µl consisted of 5 µl of master mix (Solis biodyne®), 1.5 µl of dNTP's (10 mM), 3 µl of $MgCl_2$ (50 mM), 2.5 µl of each primer (forward and reverse; 2 µM), 5 µl DNA, and 2 µl of water. Following PCR program was used: initial denaturation at 94 °C for 5 min followed by 12 cycles (touchdown program) of 94 °C for 30 s, 65 °C – 56.6 °C for 1 min (decreasing 0.7 °C per cycle), 72 °C for 1 min, then 23 cycles of 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The

Table 1. Sequence of adapters and primers used for amplification of DNA

Type	Name	Sequence (5′–3′)
Adopters	EcoR1 linker (F)	5′-CTCGTAGACTGCGTACC-3
	EcoR1 linker (R)	5′-AATTGGTACGCAGTCTAC-3′
	HLINK1 (F)	5′-GATCATGAGTCCTGCT-3′
	HLINK1 (F)	5′-CGAGCAGGACTCATGA-3′
Selective EcoRI primers	Eco-AC	5′-GACTGCGTACCAATTCAC-3′
	Eco-AG	5′-GACTGCGTACCAATTCAG-3′
Selective MspI/HpaII primers	HM-ACG[a]	5′-ATCATGAGTCCTGCTCGGACG-3′
	HM-ATT[a]	5′-ATCATGAGTCCTGCTCGGATT-3′
	HM-AAG[a,b]	5′-ATCATGAGTCCTGCTCGGAAG-3′
	HM-AAC[b]	5′-ATCATGAGTCCTGCTCGGAAC-3′
	HM-ACA[b]	5′-ATCATGAGTCCTGCTCGGACA-3′
	HM-ATG[b]	5′-ATCATGAGTCCTGCTCGGATG-3′

Note. [a]This primer was combined with Eco-AC primer for amplification.
[b]This primer was combined with Eco-AG primer for amplification.

PCR products along with a 100-bp ladder (Thermo Fisher, Vilnius, Lithuania) were then visualized on 8% denaturing polyacrylamide gel through ethidium bromide solution staining and the results were recorded.

Data recording and analysis

Fragments visualized on 8% PAGE were recorded using PyElph software (Pavel & Vasile, 2012) and only the reproducible fragments were selected for further analysis. The presence or absence of each single fragment was coded by 1 or 0, respectively. This data was then used for the identification of monomorphic and polymorphic fragments and for the comparative DNA methylation analysis.

Statistical analysis

The data for phenotypic parameters were measured in three replicates (i.e., each treatment contained three plants) and for molecular analysis was measured in duplicate (i.e., each treatment contained two plants). Statistical analyses were performed using "agricolae" package of R software, and graphs and tables were produced by MS Excel 2019 software. Analyses of variance were performed using the model $Y_{ij} = \mu + T_i + \epsilon_{ij}$ where T represents treatment effect (photoperiodic treatments) and ϵ_{ij} the residual. Least significant difference tests were performed with statistical significance thresholds of 0.05 and 0.01, respectively. In the figures, small alphabets were used to indicate the levels of significance in the differences in mean values between treatment and control plants.

RESULTS

Phenotypic analysis

Plant growth and development were significantly affected by different photoperiodic treatments used in this study. Plant fresh weight at harvest was measured for both accessions A1 and A2. Interestingly both the accessions showed different pattern in response to different photoperiodic treatments (Fig. 1a). In A1, highest fresh weight (30.4 g)

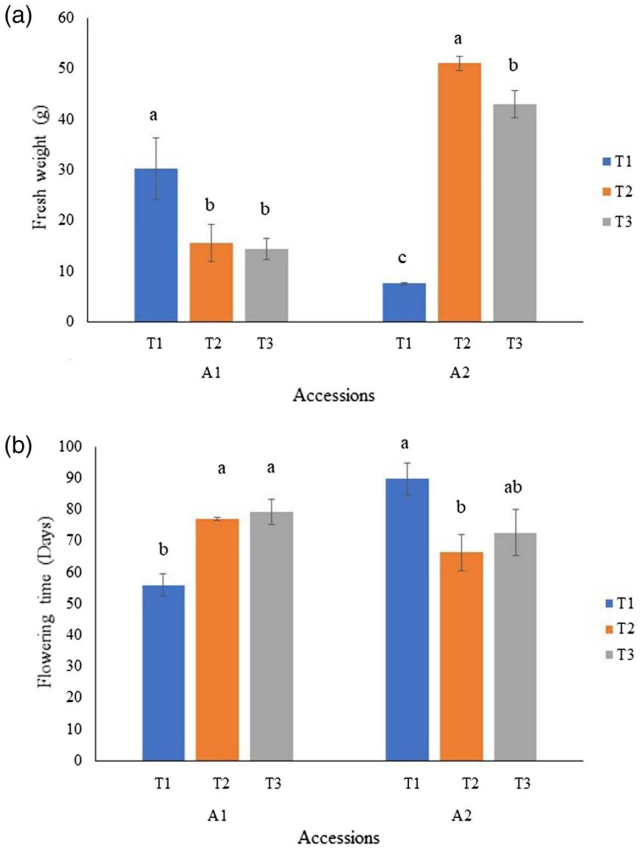

Fig. 1. Effect of photoperiodic treatments on growth parameters between the accessions. (a) Comparison of plant fresh weight at harvest between the accessions under photoperiodic treatments. (b) Comparison of flowering time between the accessions under photoperiodic treatments. The alphabet on top of each column indicates the levels of significance in the differences in mean values between the treatments. The error bars represent the standard deviation in the mean values of three plants per treatment. T1 represents 10-hr light/14-hr dark, T2 represents 12-hr light/12-hr dark, and T3 represents 14-hr light/10-hr dark

was observed in T1 (10-hr day light), which was significantly decreased in T2 (15.7 g). The plants treated with T3 showed 14.3 g of fresh weight, which was significantly

different from T1 but non-significant with T2. In A2, the highest plant fresh weight (51 g) was observed in T2, which significantly decreased in T3 (43 g) and T1 (7.5 g). T1 also showed significant decrease in plant fresh weight from T3. Flowering time, being an important trait effecting overall productivity of the crop, was also studied (Fig. 1b). In A1, the plants given the treatment T1 were the earliest to initiate flowering (56 days) compared to T2 (77 days) and T3 (79 days), which required significantly greater number of days to flower. Treatments T2 and T3 were statistically non-significant. In A2, plants given treatment T2 flowered earliest (66 days), which was statistically non-significant from the plants given treatment T3 (72 days). The plants, which were given the treatment T1, required significantly more days (89 days) to flower compared to T2 but was statistically non-significant from plants of T3. These results indicate that the two accessions have different preferences toward photoperiods. The plants of A1 appear to prefer shorter photoperiod (T1: 10-hr light/14-hr dark), whereas the plants of A2 prefer longer photoperiod.

Methylation profiling of genomic DNA of buckwheat after exposure to different photoperiodic treatments

To study the DNA methylation at 5′-CCGG-3′ sequence all over the genome of Tartary buckwheat under different photoperiodic treatments (10-, 12-, and 14-hr day light), seven primer combinations were used. The scoring of amplified bands revealed 176 and 155 bands in accessions A1 and A2, respectively (Fig. 2; Table 2). Under T1 (10-hr light/day), 94 and 80 non-methylated bands were detected

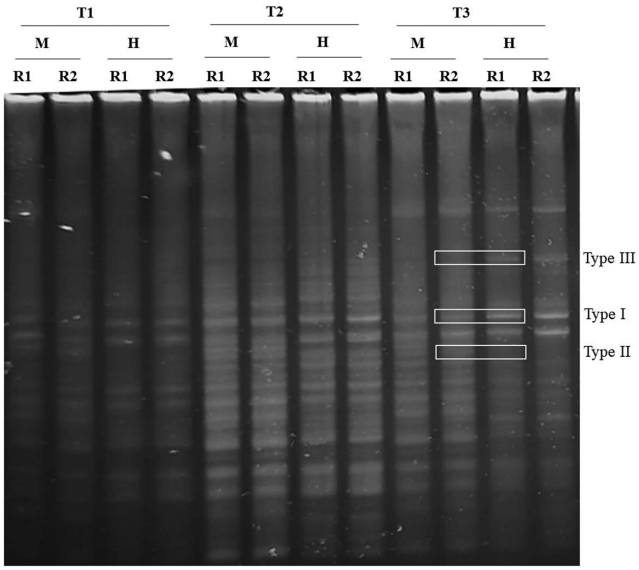

Fig. 2. A representative figure of selective amplification results by MSAP in accession A1of Tartary buckwheat using a primer combination. M represents *Msp*I digested sample, R1 and R2 represent two replicate H represents *Hpa*II digested sample, T1 represents 10-hr light/14-hr dark, T2 represents 12-hr light/12-hr dark, and T3 represents 14-hr light/10-hr dark. Type I represents unmethylated bands (bands present in both M and H), Type II represents fully methylated bands (bands present in M but absent in H), and Type III represents hemimethylated bands (bands present in H but absent in M)

(bands present in both *Eco*RI/*Hpa*II and *Eco*RI/*Msp*I lanes) and were called type I, 12 and 11 methylated bands were not observed in both *Eco*RI/*Msp*I and *Eco*RI/ *Hpa*II lanes (type II), 43 and 26 fully methylated bands were present only in the *Eco*RI/*Msp*I lane (type III), and 27 and 38 hemimethylated bands were observed only in the *Eco*RI/*Hpa*II lane (type IV) in A1 and A2, respectively. Under T2 (12-hr day/12-hr night), 88 and 83 non-methylated bands, 33 and 37 methylated bands, 28 and 14 fully methylated bands, and 27 and 21 hemimethylated bands were identified in A1 and A2, respectively. Under T3 (10-hr light/14-hr night), 85 and 84 non-methylated bands, 64 and 48 methylated bands, 17 and 8 fully methylated bands, and 10 and 15 hemimethylated bands were identified A1 and A2, respectively (Table 2).

As the DNA methylation pattern of the two accessions is different from each other, the results will be described separately. These results showed that the total methylation ratio in accession A1 of Tartary buckwheat subjected to photoperiodic treatments (10, 12, and 14 hr) slightly increased from 46.6% to 50% in T2 (12 hr) to 51.7% in T3 (14 hr). Interestingly, the pattern is somewhat different when this total methylation is fragmented in full and hemimethylation ratios. The full methylation ratio increased from 31.3% in T1 (10 hr) to 34.7% in T2 (12 hr) to 46% in T3 (14 hr), and the hemimethylation ratio remained constant at 15.3% in T1 and T2 and then strongly decreased to 5.7% in T3 (14 hr; Table 2), showing that a relatively more visible difference appeared at T3 in both full and hemimethylation ratios.

In A2, the total methylation ratio showed a slightly decrease with increase in day light duration. It decreased from 48.4% in T1 to 46.5% in T2 (12 hr) to 45.8% in T3 (14 hr). Similar to A1, a different pattern in the shift was observed when this total methylation was fragmented in full and hemimethylation ratios. The full methylation ratio increased from 23.9% in T1 (10 hr) to 33% in T2 (12 hr) to 36% in T3 (14 hr), and the hemimethylation ratio decreased from 24.5% in T1 to 13.6% in T2 to 9.7% in T3 (14 hr; Table 2), showing that a relatively more visible difference appeared at T2 in both full and hemimethylation ratios compared to T1.

Comparison between the two accessions revealed full and hemimethylation ratios in A1 showed strong shifts at T3 from T1 and T2, whereas, A2 showed strong shifts in full and hemimethylation ratios at T2. This indicated the different preference of the two accessions for the different photoperiodic treatments used. All these results confirmed the changes in photoperiod causes genotype- and type-specific shift in the genome-wide DNA methylation profile of buckwheat.

Dynamics of methylation/demethylation events in relation to photoperiodic treatments

To identify the change in the methylation and demethylation events, comparative MSAP profiling was performed by scoring all possible banding patterns between the three treatments used in this study (Table 3). In A1, out of the 176 bands, 38.6%, 35.8%, and 46% of the CCGG sites remained unchanged in T2 vs. T1, T3 vs. T1, and T3 vs. T2, respectively. Comparison of the demethylation events

Table 2. The MSAP analysis of two accessions of buckwheat subjected to different photoperiods

	A1			A2		
MSAP band types	T1 (10 hr)	T2 (12 hr)	T3 (14 hr)	T1 (10 hr)	T2 (12 hr)	T3 (14 hr)
Total amplified bands	176	176	176	155	155	155
Type I	94	88	85	80	83	84
Type II	12	33	64	11	37	48
Type III	43	28	17	26	14	8
Type IV	27	27	10	38	21	15
Total methylation ratio (%)	46.6	50	51.7	48.4	46.5	45.8
Full methylation ratio (%)	31.3	34.7	46.0	23.9	33.0	36.1
Hemimethylation ratio (%)	15.3	15.3	5.7	24.5	13.6	9.7

Note. Type I fragments refer to unmethylated loci and were present in both the EcoRI/MspI and EcoRI/HpaII lanes; Type II fragments display the methylated/demethylated loci and were absent in either enzyme combination in one of the samples; Type III fragments show fully methylated loci and were present only in the EcoRI/MspI lane; and type IV fragments demonstrate hemimethylated loci and were present only in the EcoRI/HpaII lane. Fully methylated ratio (%) = [(II + III)/(I + II + III + IV)] × 100%, hemimethylated ratio (%) = [(IV))/(I + II + III + IV)] × 100, total methylation ratio (%) = [(II + III + IV)/(I + II + III + IV)] × 100.

Table 3. DNA methylation patterns in two accessions of buckwheat subjected to different photoperiods

		Banding pattern				A1			A2		
		Control		Treatment							
Type of banding pattern	Class of banding pattern	M	H	M	H	T2 vs. T1	T3 vs. T1	T3 vs. T2	T2 vs. T1	T3 vs. T1	T3 vs. T2
No change	A	1	1	1	1	50	47	50	46	51	65
	B	0	1	0	1	2	1	0	7	5	5
	C	1	0	1	0	11	9	3	3	1	1
	D	0	0	0	0	5	6	28	4	5	30
	Total					68	63	81	60	62	101
	Percentage					38.6	35.8	46.0	38.7	40.0	65.2
Demethylation	E	0	1	1	1	18	19	19	21	18	9
	F	1	0	1	1	17	18	15	15	14	8
	G	0	0	1	1	3	1	1	1	1	2
	H	1	0	0	1	9	0	1	1	2	0
	I	0	0	0	1	2	4	3	5	5	4
	J	0	0	1	0	2	1	1	1	0	1
	Total					51	43	40	44	40	24
	Percentage					29.0	24.4	22.7	28.4	25.8	15.5
Methylation and hypermethylation	K	1	1	0	1	14	5	6	8	3	6
	L	1	1	1	0	10	5	12	7	4	4
	M	0	1	1	0	5	2	1	3	3	2
	N	1	1	0	0	20	37	20	19	22	8
	O	0	1	0	0	2	5	7	7	12	5
	P	1	0	0	0	6	16	9	7	9	5
	Total					57	70	55	51	53	30
	Percentage					32.4	39.8	31.3	32.9	34.2	19.4

revealed 29%, 24.4%, and 22.7% in T2 vs. T1, T3 vs. T1, and T3 vs. T2, respectively. The methylation events were found to be 32%, 39.8%, and 31.3% in T2 vs. T1, T3 vs. T1, and T3 vs. T2, respectively. The comparison between T2 and T1 showed 3.4% more methylation events than demethylation event, whereas comparison between T3 and T1 showed 15.4% more methylation events than demethylation events and comparison between T3 and T2 revealed 8.6% more methylation events than demethylation events. These results confirm that although more methylation events were found in all the three comparisons, the highest increase in methylation events was observed in the T3 vs. T1.

In A2, out of the 155 bands, 38.7%, 40%, and 65.2% of the CCGG sites remained unchanged in T2 vs. T1, T3 vs. T1, and T3 vs. T2, respectively. Comparison between T2 vs. T1, T3 vs. T1, and T3 vs. T2 revealed 28.4%, 25.8%, and 15.5% demethylation events, respectively, and 32.9%, 34.2%, and 19.4% methylation events, respectively. The comparison between T2 and T1 showed 4.5% more methylation events than demethylation events, whereas comparison between T3 and T1 showed 8.4% more methylation events than demethylation events and comparison between T3 and T1 showed 3.9% more methylation events than demethylation events. These results confirm that although

more methylation events were found in all the three comparisons, the highest increase in methylation events was observed in the T3 vs. T1. Comparison between the two accessions revealed more methylation events in A1.

DISCUSSION

Photoperiod, in addition to other environmental factors, has an effect on the plants to fine tune the timing of developmental stages especially flowering time to avoid adverse external condition and to maximize the benefits of favorable conditions. Various reports have confirmed its influence on different features of the plant growth including flowering time, photomorphogenesis, number of flowers, seed number, height of plant, and even plays its part in regulating circadian rhythms and stress tolerance (Duan et al., 2017; Serrano-Bueno et al., 2017). Similar to other photosensitive plant species, an alteration in the photoperiod effects plant growth, flowering time, and other related features in buckwheat (Hara et al., 2011; Hara & Ohsawa, 2013; Romanova et al., 2018). With the aim to study the effect of various photoperiodic treatments on different growth stages of buckwheat and to classify the two accessions, used in this investigation, on the basis of photosensitivity, different phenotypic traits were studied.

Both the accessions showed their specific pattern of photoperiodic sensitivities. Accession A1 appeared to be short day as it performed best under T1 (10-hr light/14-hr dark) conditions both in terms of plant weight and flowering time. Interestingly, A2 showed relatively different trend. The phenotypic data confirmed that A2 prefers longer day conditions compared to A1 as it gave best results at T2 (12-hr light/12-hr dark) conditions, which were statistically similar to T3 (14-hr light/10-hr dark). Similar pattern of variation in the photoperiodic requirements has been reported in many plant species (Bentley et al., 2013; Kondhare et al., 2018; Serrano-Bueno et al., 2017). In buckwheat, especially in common buckwheat, different investigations have also revealed similar kind of genotype-specific sensitivity toward photoperiod (Hara et al., 2011; Hara & Ohsawa, 2013; Romanova et al., 2018). Our phenotypic results confirm the influence of photoperiod in modulating the plant response in the studied accessions. This modulation is controlled by regulation of genes responsive of photoperiodic variation. DNA methylation is an important epigenetic mark that is known to regulate the gene expression (Shafiq & Khan, 2015). Therefore, genome-wide DNA methylation analysis was performed with the aim to investigate the DNA methylation change due to photoperiodic shift, the molecular study was conducted.

The genome-wide DNA methylation analysis revealed an accession-/genotype-specific shift in DNA methylation pattern in response to change in photoperiod. Total DNA methylation ratio increased with the increase in photoperiod in accession A1, whereas a decrease in the DNA methylation profile with the increase in photoperiod was observed in accession A2. The data indicate that DNA methylation events were more evident in A1 as compared to A2. This genotype-specific pattern of variability observed in this study is expected as both the accessions prefer different photoperiods. To our knowledge, this study first reports the involvement of genome-wide DNA methylation remodeling in the regulation of plant response due to photoperiodic variation in buckwheat. These results are in accordance with various studies in different species, where the genotype-specific shift in DNA methylation pattern under different photoperiodic conditions was reported (Brutch et al., 2019; Guzy-Wrobelska et al., 2013; Takeno, 2010). Takeno (2010) reported that DNA methylation pattern in *Perilla frutescens* and *Pharbitis nil* altered with the change in photoperiodic treatment. In rape seed, the genotype specific shift in global DNA methylation was reported under different photoperiodic conditions where spring rape seed showed strong differences in DNA methylation profile under different photoperiodic treatments compared to winter rape seed (Guzy-Wrobelska et al., 2013).

Upon discussing further dissection of this total DNA-methylation into full methylation $\begin{pmatrix} C\underline{C}GG \\ GG\underline{C}C \end{pmatrix}$ and hemi-methylation $\begin{pmatrix} \underline{C}CGG \\ GGCC \end{pmatrix}$, the shift in DNA methylation profile among different photoperiodic treatments became more pronounced in both the accessions. The level of full methylation in A1 is relatively higher compared to A2 (Supplementary Fig. S1a). Interestingly, our results revealed that in A1, the full methylation strongly increased from T2 to T3 whereas in A2, the full methylation strongly increased from T1 to T2 (Supplementary Fig. S1a). Inverse to full methylation percentage, the hemimethylation percentage decreased in T3 compared T1 in both accessions but in accordance to the previous results the pattern is highly accession/genotype specific. A1 showed strong decrease in hemimethylation from T2 to T3 and A2 showed strong decrease in hemimethylation from T1 to T2. Interestingly, the two components showed opposite pattern, i.e., full methylation, which is CpG methylation, increased with the increase in day length whereas hemimethylation, which is CpCpG methylation, decreased with increasing day length. This indicate that photoperiod influences the CpG and CpCpG sites differently. This specificity of methylation in different cytosine contexts indicates toward their distinct roles in plant response toward photoperiodic changes. Similar pattern of specificity in cytosine context (in both genome-wide as well as locus-specific) has been reported in different species under different environmental stresses (Ding et al., 2019; Khan et al., 2013; Mousavi et al., 2019). In *Isoetes sinensis*, full-methylation level or CG methylation decreased in Pb-treated and Cd-treated plants as compared to control, whereas hemimethylation level or CpCpG methylation increased in Pb-treated and Cd-treated plants as compared to control (Ding et al., 2019). Khan et al. (2013) reported that vernalization caused an increase in CpHpG to influence the gene expression of *VRN-A1* gene in wheat, whereas the CpG methylation remained constant.

It is important to understand at this point that both methylation and demethylation event simultaneously occur in an event of change of environmental conditions, which constitute the overall shift in the pattern of DNA methylation therefore to have a better understanding the comparative analysis to study the individual events was conducted.

De novo or hypermethylation events were observed in higher proportion compared to demethylation events in all the comparison in both the accessions (Table 3). In *de novo* or hypermethylation type, the class "N" (completely non-methylated in control and fully methylated in treatment) appeared most frequently in all the comparisons in both accessions. Similar pattern in *I. sinensis* was also observed against heavy metal (Pb and Cd) treatments where the N class was contributed the most among different methylation events (Ding et al., 2019). In demethylation type, the class E appeared to be most frequently in all the comparisons in both accessions. T3 showed highest level of methylation when compared with T1 in both the accessions. For these, it can be deduced that classes E and N play most important roles in demethylation and methylation events, respectively, against different photoperiodic treatments in Tartary buckwheat.

CONCLUSION FOR FUTURE BIOLOGY

In this study, we report, for the first time, genome-wide remodeling of DNA methylation in response to changes in photoperiodic conditions in Tartary buckwheat. It indicates toward the involvement of DNA methylation as an important mechanism in regulating plant response against variation in photoperiodic requirement in Tartary buckwheat. It can be inferred that DNA methylation shift influences the photo responsive genes, which in turn play their role in modulating the plant response against variation in photoperiodic conditions. Isolation of differential bands followed by sequencing and blasting will lead toward the identification of particular photo-responsive genes that will help in broadening our understanding toward the molecular mechanism regulating the plant response to a particular photoperiod.

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