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RESEARCH ARTICLE

Diversity of *Heading date 1 (Hd1)* gene that conditions flowering time in traditional tropical *japonica* and traditional *indica* rice from Thailand

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Abstract

The traditional rice cultivars are genetic resources in breeding programs that possess variable DNA sequence of genes, but variants of genes of these resources have been limited in Thailand. Therefore, DNA sequence variation of the *Heading date 1* (*Hd1*) gene was explored in the rice cultivars collected from north (*tropical japonica* rice) and northeastern (*indica* rice) regions of the country. Results from re-sequencing of the *Hd1* gene, identified 3 non-synonymous SNPs in the coding sequence (exon2) and there are four SNPs in the non-coding sequence of the gene. For coding sequences, the first non-synonymous SNP, AGT/GGT leading to amino acid sequence changes in *Hd1* protein at position 339 of 407 residues. The allele S (AGT:Serine) of *Hd1* gene were dominant in *tropical japonica* rice in the northern region, whereas the allele G (GGT:Glycine) was mostly found in *indica* rice from the northeastern region. In addition, in two traditional cultivars from the northern, a premature stop codon at exon2 was identified. Furthermore, 2 others non-synonymous SNPs was distributed in both populations, including allele S (AGC:Serine) was dominant in *tropical japonica* rice, while the allele A (AGA:Arginine) were mostly found in *indica* rice.

Keywords: Traditional rice cultivar, flowering time, heading date 1 gene, single nucleotide polymorphism, allele distribution

Introduction

The flowering time or heading date of cultivated rice (*Oryza sativa*) is an important agricultural trait for yield production. In general, rice is known as a short-day plant that induces a transition from the vegetative phase to the reproductive phase when it senses a decrease in day length (Takahashi et al. 2009). Initially, the molecular genetic pathway for short-day flowering in cultivated rice has been illustrated (Hayama et al. 2006; Takahashi et al. 2009; Purwestri et al. 2017), and several studies or articles have been provided on the regulation of rice flowering (Tsuji et al. 2013; Shrestha et al. 2014; Wu et al. 2020). According to these studies, three key genes, including *Early heading date 1* (*Ehd1*), *Hd3a* and grain number, plant height, and heading date 7 (*Gh7*) were affected by rice flowering time. In addition, results from studies of gene expression of Takahashi et al. (2009), found that heading date 1 (*Hd1*) is a major determinant of variation in flowering time of cultivated rice. In addition, results from experimental studies strongly suggest that under short-day length, three key genes, including *GIGANTEA* (*OsGI*), *Heading date 1* (*Hd1*) and *Heading date 3a* (*Hda3*), play an important role for the flowering promotion of rice (Kojima et al. 2002; Tamaki et al. 2007; Komiya et al. 2008).

To date, the mutations that caused morphological and physiological change, followed by human selection in rice, as well as the molecular mechanisms generating the diversity of flowering time in cultivated rice, are under investigation. For example, Wu et al. (2020) reported that the DNA sequence information of *Heading date1* gene is associated with flowering phenotypes of cultivated rice in different regions, and photoperiod sensitivity controlled by

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cooperation and competition among genes of *Hd1*, *Ghd7* and *DTH8* (Zong et al. 2021). *Hd1* gene of rice has two exons and was identified as *Arabidopsis* orthologue CONSTANS and encodes zinc-finger type transcriptional activators with the CO, CO-like, and TOC1 (CCT) domains (Yano et al. 2000). The DNA sequence of exon2 contains the CCT domain, with function as a nuclear localization signal. Furthermore, the mutant without the CCT domain in CO showed a defect in protein function (Robson et al. 2001). In addition, Hd1 protein functioned as suppressing flowering under long day (LD) conditions and promoting flowering under short day (SD) conditions. The genetic pathway suggested that *Hd1* upstream regulates the expression of *Hd3a* (Takahashi et al. 2009). A current study reported that *Hd1* has a much higher level of polymorphism than the other genes (*Hd3a, Ehd1, OsMADS51*), and it is a main source of flowering time diversity in cultivated rice and type of alleles associated with major agronomic traits (Takahashi et al. 2009; Mo et al. 2021).

In temperate regions, results from a physiological experiment by Izawa et al. (2002) indicated that the expression of *Hd3* is repressed, while *Ehd1* is a key regulator of floral transition in temperate regions. In addition, the areas south of 31N (31degreeN), flowering is regulated by both *Hd1* and *Ehd1* promotion and resulted in short-day flowering, while in areas north of 31N the expression of Hd3a is regulated by *Hd1* repression and *Ehd1* promotion (Izawa et al. 2002), which resulted in early flowering under long-day conditions (Doi et al. 2004; Izawa et al. 2002). Historically, some areas of Thailand have been archeological recorded of different cultivated rice varieties during the development of the country as early as the Metal/Iron Age (Castillo, 2011). There are more than 10,000 rice seed samples of Thai rice cultivars with 3,500 different cultivars named which were collected for germplasm conservation around the country by the Pathum Thani Rice Research Institute during 1982-1986. These cultivars are attributed to the dissimilar ecological environments and/or agroecological conditions found in the northern region (mountain areas) through the central (large central plain), northeastern and southern regions (lower areas) of the country. Each region has a number of minority tribes, such as *Khamu, Mein, Lisu, Hmong and Karen* in the northern region. Traditionally, these people have cultivated local upland rice cultivars. The upland rice cultivars are grown under rainfed conditions in the rainfall season (start on May) and these rice cultivars have a characteristic of early maturity (flowering time on September). For Thai lowland rice cultivars, the characteristics of early, middle, and late maturity depends on the genetic background of each cultivar. In addition, most of these upland rice cultivars have been classified as tropical *japonica* rice type by using DNA markers *i.e.,* ORF100, plastid subtype ID sequences (Kanno et al. 1993; Chen et al. 1994; Nakamura et al. 1997). There are several previous reports to characterize upland rice and lowland rice cultivars from Thailand (Prathepha 2008; Prathepha and Baimai 2004; Fongfon et al. 2021). Nowadays a very few rice traditional rice cultivars have grown in some areas of the northern and northeastern regions of the country, and some of them disappear from rice fields. Knowledge of the variation of genes that are associated with agronomic traits of local rice germplasm collections is important information for genetic improvement. Furthermore, the overall population structure of global *O. sativa* germplasm has been well characterized, more detailed analyses of rice germplasm on a regional or country-specific basis have only just begun (Thomson et al. 2007). For such example is Thailand, a country with a wealth of rice diversity that is largely untapped. A long history of traditional rice production across numerous environments in Thailand has led to a diverse array of traditional rice cultivars.

Existence of traditional rice cultivars reflex to food security and health benefits of local people. For example, the temperate regions in north India have been an abode of traditional rice varieties since prehistoric times and were recommended for medical uses by traditional healers and local farmers. These varieties thus fit into the description of healthy and functional foods. Furthermore, local varieties need to be conserved and promoted by commercialization and general public awareness about their medicinal benefits (Bhat and Riar 2015)

In Thailand, most of the rice type is *indica* type. Historically, *tropical japonica* rice has been explored and reported first in Chiang Rai province (Oka and Chang 1963). Some traditional rice cultivars, both tropical *japonica* and *indica* rice types have been made in breeding programs and recommended to farmers since 1963, such as KDML105 (non-glutinous and *indica* rice), Khao Pong Krai (glutinous and tropical *japonica* rice). To date, traditional rice cultivars have mostly disappeared from the paddy fields of local farmers. These traditional rice cultivars were replaced by modern or new-release varieties recommended by the Department of Rice. Moreover, it might be lost and scientists pay no attention to carrying out research as well. However, some traditional rice cultivars had been collected and genomic DNA was extracted and kept at long-term storage (-80°C) for research purposes (Prathepha 2008). It would be interesting to figure out whether this gene is conserved between different rice types (*indica* and *tropical japonica*). Future studies could use similar genetic tools in other local rice landraces to identify DNA sequence differences that respond to photoperiod sensitivity. Currently, the studies of *Hd1* gene of local rice landraces in Thailand have no reports. In addition, to study the plant molecular population genetics, additional sampling of local populations is required (Wright and Gaut 2005). The study of DNA sequence polymorphism in the *Hd1* gene in the two types of Asian cultivated rice, *i.e.,* tropical *japonica* type and *indica* type rice will help us gain

more insight into the genetic background of this gene. In addition, to address this paucity of information about this gene, re-sequencing of the *Hd1* gene of traditional rice cultivars were carried out. The objective of this study was to explore the DNA sequence variation and comparison of *Hd1* gene between *tropical japonica* type which is grown by ethnic groups in the northern region of Thailand and *indica* type of traditional lowland rice cultivars from the northeastern region of the country.

Materials and methods

Sample collection

For the northern region of Thailand, samples of traditional rice cultivars were collected from rice fields of local farmers in mountain regions of ethnic groups in Chiang Rai (CR) and Tak provinces since 2006-2008. Thirty traditional rice cultivars were selected for use in this experiment. Wild rice (*Oryza rufipogon)*, weedy rice (*Oryza sativa* f. *spontanea*), KDML 105, a photoperiod sensitivity cultivar, and Chainart 1, a non-photoperiod sensitivity cultivar were also used in this study. The local name and description of rice genotypes is shown in Table 1. For the northeastern region, traditional lowland rice cultivars and some upland rice cultivars were collected from local rice fields in five provinces: Mukdahan (MDH), Sakon Nakhon (SKN), Kalasin (KLS), Maha Sarakham (MSK), and Yasothon (YST) during 2004-2005.

An example of traditional rice cultivars, both tropical *japonica* and *indica* rice, is shown in Fig. 1. Genomic DNA of all accessions of the local rice landraces, weedy rice and wild rice were extracted from young leaves, given sample code and stored at -80° C. For genomic DNA extraction, approx. 100 mg of young leaves or flag leaves of each individual rice sample was isolated using the CTAB method following the procedures described by Doyle and Doyle (1987). DNA stock of traditional rice cultivars were used as samples for analyses of previous experiments and have been reported by Prathepha and Baimai (2004).

There are two categories of rice cultivation in Thailand: in-season and off-season rice cultivation. In-season rice cultivation refers to regular rice cultivation during the rainy season. It begins in May until October followed by a harvest period that ends in February. In-season cultivation produces rice only once a year. During the cultivation period, rice flowering can be expected precisely on the same month and day. Traditional rice cultivars had grown only in in-season cultivation in Thailand. Almost all rice cultivars had a characteristic of photoperiod sensitivity and were classified into three groups based on flowering time (month) that were observed, discussed and informed by local farmers, i.e., early- (flowering in 10-15 September), middle- (flowering in 10-12 October), and late- (flowering in 10-12 November) maturity (Table 1). This study, a collection of thirty traditional upland and lowland rice cultivars in a stock DNA sample was selected to analyze DNA variation of *Hd1* gene.

PCR amplification and DNA sequencing

Primer pairs used in the PCR and DNA sequencing experiment that cover entire the *Hd1* gene are as follows: Hd1F-6: 5′-ACACAGCAATCACCACACGA -3′/ Hd1R-6:5′-AGATAGGCCGTGCTGGAAA-3′; Hd1F-9: 5′-CGACAACCGCATCGAAAACA-3′/Hd1R-9:5′-CCCGCCTCCATTGATGAGAA-3′/Hd1F-10: 5′-CTCCATAGGACCCGCCAAAG -3′/Hd1R-10:5′-TTCGATGCGGTTGTCGTAGT-3′.

The PCR conditions for the three primer pairs were as follows. An initial denaturation step of 3 minutes at 94°C followed by 35 cycles as follows: 45 s at 94°C, 2 minutes at 55°C, then 1-minute 5 seconds at 72 °C. After 35 cycles, a final extension of 5 min at 72°C was performed. PCR products were then separated by 1.0% agarose gel electrophoresis and visualized with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA). The amplified bands were cut and purified using PureDireX PCR Clean-Up&Gel Extraction Kit (The BIO-HELIX Co./Ltd., Taiwan). PCR products were sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA) which was performed by the 1st BASE Laboratories, Selangor, Malaysia.

DNA sequence analysis

First, all DNA sequence data were manually edited using BioEdit version 7.0.9.0 (http://www.mbio.ncsu.edu/ BioEdit/ BioEdit.html), then the data set was aligned using Clustal W (Thompson et al. 199). Then, DNA sequences of the *Hd1*orthologues of Asian cultivated rice (GenBank acc. no., AB041840.1), wild rice (GenBank acc. no. JN594496) retrieved from the GenBank, NCBI (http://www.ncbi.nlm.nih.gov/) was used as references in the analysis. For DNA sequence variation, DNA sequences of the rice samples were calculated using the DnaSP software version 6.12.03. (Rozas et al. 2003). Statistical analysis included the number of polymorphic segregation sites (S), nucleotide diversity (π): the average number of nucleotide differences per site between two sequences (Nei 1987), number of haplotypes and haplotype diversity (Hd). The number of haplotypes was estimated by counting method. Haplotype frequency and diversity were calculated as following equations:

$$\text{Haplotype frequency} = \frac{\text{Number of observed haplotype}}{\text{Number of total haplotype}}$$

$$\text{Haplotypediversity} = n(1\text{-summation } Pi^2)/(n\text{-}1)$$

where n: number of populations, *Pi*: is haplotype frequency in a tested population, and the average number of pairwise nucleotide differences per site calculated on the silent sites (synonymous sites plus non-coding sites) or non-synonymous sites among sequences, (π_{sil}) (Tajima 1983), was used as a diversity measure.

Table 1. Local name of traditional rice cultivars collected from northern and northeastern regions of Thailand. Modern rice varieties (Chainart1 and KDML 105), wild, weedy rice used in this study

Cultivars'name/stock code/ subspecies*	Cultural type/ ethic group	Locality/ province	1st nonsyn. (AGC/AGA)	2nd nonsyn. (CGA/TGA)	3rd nonsyn. (AGT/GGT)	Flowering time (month)***
Bael Dir (N12)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Bael Jai (N13)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Bael Lue (N1)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Bael Kao Sue (N18)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Bael Ma Kael (N14)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Bael Plao Sang (N17)/J	Upland/Hmong	N/CR	AGA	CGA	GGT	early maturity
Bao Bud (N10)/J	Upland/Mien	N/CR	AGC	CGA	AGT	early maturity
Bao Ku (N2)/J	Upland/Mien	N/CR	AGC	CGA	AGT	early maturity
Be Dao Derk (32)/J	Upland/Hmong	N/Tak	AGC	CGA	AGT	early maturity
Be Dai Nang (N33)/J	Upland/Hmong	N/Tak	AGC	CGA	AGT	early maturity
Be Lao Da (N39)/J	Upland/Hmong	N/Tak	AGC	CGA	AGT	early maturity
Ja Lai (N8)/J	Upland/Khamu	N/CR	AGC	CGA	AGT	early maturity
Ja Ngai (N7)/J	Upland/Khamu	N/CR	AGC	CGA	AGT	early maturity
Ling Bao (N15)/J	Upland/Mien	N/CR	AGA	CGA	GGT	early maturity
Pern Nern Yim (N11)/J	Upland/Khamu	N/CR	AGC	TGA**	AGT	early maturity
Ram Tang (N9)/J	Upland/Khamu	N/CR	AGC	TGA**	AGT	early maturity
Sew (N16)/J	Upland/Hmong	N/CR	AGA	CGA	GGT	early maturity
Yim (N6)/J	Upland/Hmong	N/CR	AGC	CGA	AGT	early maturity
Ga Saen (NE29)/J	Upland/Thai	NE/MDH	AGA	CGA	GGT	early maturity
Hao' Kaen Du (NE31)/J	Upland/Thai	NE/MDH	AGA	CGA	GGT	early maturity
Khi Tom (NE30)/I	Lowland/Thai	NE/KLS	AGA	CGA	GGT	middle maturity
Lao Taek (NE24)/I	Lowland/Thai	NE/MDH	AGA	CGA	GGT	middle maturity
Mae Hahng (NE36)/I	Lowland/Thai	NE/SKN	AGA	CGA	GGT	middle maturity
Mahk Yom (NE45)/I	Lowland/Thai	NE/KLS	AGA	CGA	GGT	middle maturity
Lu Nee (NE25)/I	Lowland/Thai	NE/KLS	AGA	CGA	GGT	middle maturity
Pong Aew (NE26)/I	Lowland/Thai	NE/MSK	AGA	CGA	GGT	early maturity
U Kham (NE32)/I	Lowland/Thai	NE/MDH	AGA	CGA	GGT	middle maturity
Leung Bun Mah (NE49)/I	Lowland/Thai	NE/MSK	AGA	CGA	GGT	early maturity
Hohm Nang Nuan (NE23)/I	Lowland/Thai	NE/YST	AGA	CGA	GGT	early maturity
Perd Nam (NE55)/I	Lowland/Thai	NE/SKN	AGA	CGA	GGT	middle maturity
Oryza rufipogon (NE)	Wild rice	NE/SKN	AGC	CGA	GGT	late maturity
Oryza sativa f. spontanea	Weedy rice	NE/MSK	AGC	CGA	GGT	July to August
Oryza sativa (cv. Chai Nart1)	Lowland/Thai	NE/MSK	AGA	CGA	GGT	non-photoperiod sensitive
Oryza sativa (cv. KDML105)	Lowland/ Thai	NE/MSK	AGA	CGA	GGT	middle maturity

* I= indica; J=japonica; assessed by indels of ORF in cp-DNA (according to Prathepha and Baimai 2004); N= Northern, NE= Northeastern; nonsyn. = Nonsynonymous single nucleotide polymorphism; **a premature stop codon, *** early maturity (flowering time on 10-15 September), middle maturity (flowering time on 10-12 October), and late maturity (flowering on 10-12 November) as described in materials and methods.

Fig. 1. A representative of traditional rice cultivars from northern, cv. Pern Nern Yim (A) and northeastern Thailand, cv. Khi Tom (B)

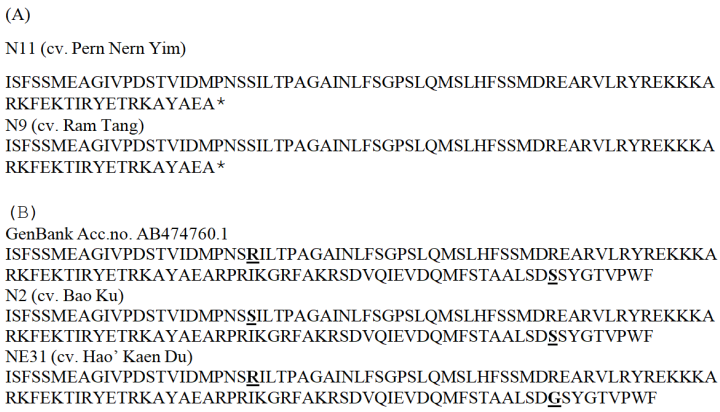

Fig. 2. Comparison of amino acid substitutions in exon2 of Hd1 protein among three non-synonymous single nucleotide polymorphism (SNP). (A) Two traditional cultivars (N11 and N9) show a premature stop codon. (B) Two non-synonymous SNP cause amino acid substitutions of Hd1 protein in representative traditional rice cultivars from Thailand

The genetic relationship was determined with Neighbor-Joining (NJ) analyses as implemented by using the program MEGA version 11.0 (Tamura et al. 2021). The DNA sequences were translated into amino acid sequences by using Open Reading Frame Finder software (ncbi.nlm.gov/orffinder). Amino acid sequences of Hd1 protein were identified and compared with the previously published (Fujino et al. 2010).

Results

DNA sequence comparison of the Hd1 gene

Hd1 gene structure contains of 2 coding sequences, exon1 and exon2 with lengths of 864 and 357 bp, respectively. The two exon encode Hd1 protein with a length of 407 amino acid sequence and intervened by intron with 624 bp length. The alignment of the DNA sequence of both coding sequence (exon1 and exon2) and non-coding sequence (intron) of traditional rice samples (n=30) with a reference accession number AB474759 from GenBank (https//www.ncbi.nlm.nih.gov). The DNA sequence of exon1 has no variation. These rice accessions give different results than previously reported of DNA sequence of exon1 of the *Hd1* gene in Korean rice (Mo et al. 2021). Four SNPs were found in the intron of the gene, including G/T, C/T, C/G and G/C/A SNPs at positions 1827, 1905, 2019 and 2044, respectively. Three non-synonymous SNPs was identified in exon2, resulting in encoding different amino acid. First, AGC: Serine/AGA: Arginine at position 311 of the total of 407 amino acid sequence of Hd1 protein. Wild rice and weedy rice exhibited AGC:Serine allele(S allele), which is the dominant allele of local traditional rice of the northern region. While cv. KDML105 and cv. Chainart 1 carried AGA:Arginine allele (A allele). Second, two cultivars (cv. Ram Tang, and cv. Pern Nern Yim) of the northern region identified a premature stop codon TGA at the 370th amino acid sequence, whereas the other cultivars had a non-synonymous SNP and produced a codon CGA that encodes an amino acid Arginine (Arg) (Fig. 2). The last non-synonymous SNP: AGT (Serine)/GGT (Glycine) was identified at position 399th of the amino acid sequence of Hd1 protein (Supplementary data 1-4, S1-S4).

DNA sequence variation of the aligned lengths of intron and exon2 (981 bp) of rice samples was considered in the study. Overall, the nucleotide diversity (π_{sil}) of rice samples was 0.00337. There are seven haplotypes (h = 7) with haplotype diversity (Hd) of 0.772. The nucleotide diversity of the northern population (0.00250) was lower than the northeastern population (0.00339). The northern population had four haplotypes, while the northeastern population showed six haplotypes, with haplotype diversity (Hd) of 0.619 and 1.00, respectively.

Phylogenetic analysis

DNA sequence of intron and exon2 of thirty rice samples were used to construct a phylogenetic tree using the MEGA program version 11. The phylogenetic tree constructed by NJ method shows the relationship among 30 DNA sequences of traditional rice cultivars of the *Hd1* gene (Fig. 3). There are six major clusters. Group 1 consists of 14 cultivars of tropical *japonica* from northern (N); group 2 consists of one upland rice cultivar (HKD-NE31, cv. Hao' Kaen Du), which is a traditional tropical *japonica* type collected from Mukdahan province; group 3 and 4, each consists of 1 *indica* rice, cv. Lu Nee (LN-NE25) and cv. Pong Aew (PAW-NE26), respectively; groups 5 and 6 consist of traditional rice cultivars from the northeastern.

Discussion

The present study was conducted to determine the variation of the *Hd1* gene of traditional rice cultivars from Thailand. The variation of non-synonymous mutation in the coding sequence caused amino acid changes in Hd1 protein were identified in the rice samples. In addition, some rice cultivars revealed the mutation causing a premature stop codon. These mutations probably alter the stability and function

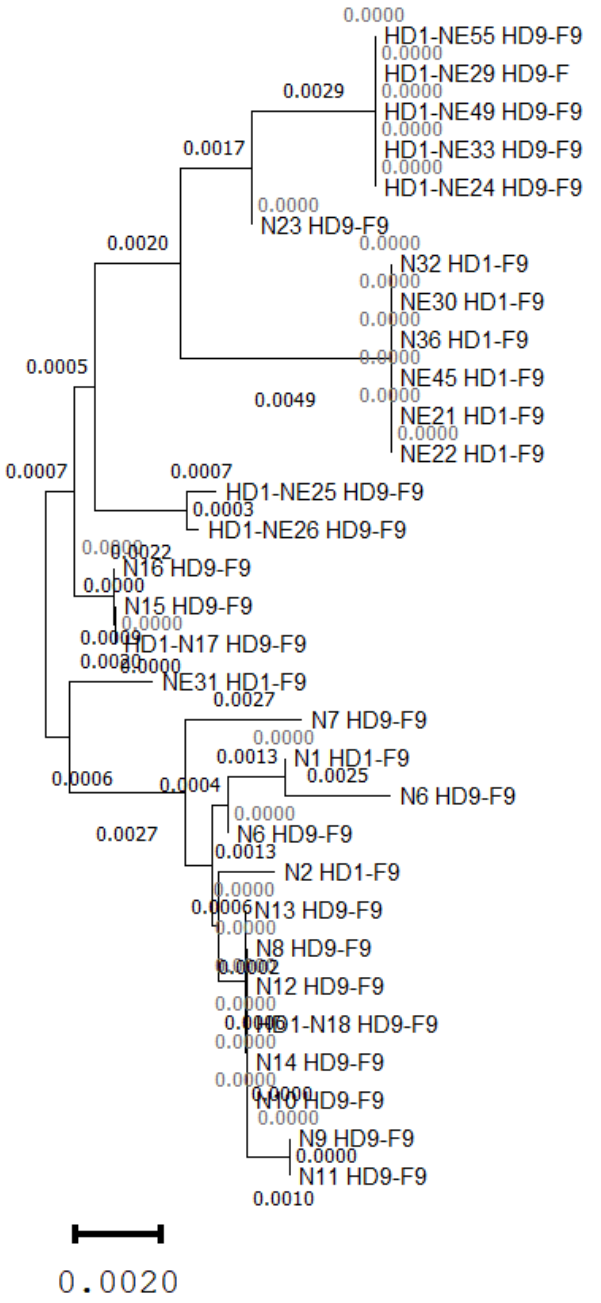

Fig. 3. The evolutionary relationship was inferred using the Neighbor-Joining method and conducted in MEGA11. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1000 positions in the final dataset

of transcripts and the protein function of *Hd1* gene. These data support the finding that the structural variation of proteins caused by amino acid change of PvHd1 gene in switchgrass lead to functional divergence (Choi et al. 2023). Molecular genetic studies of flowering time genes in rice have taken a very long time to address many questions of genes controlling flowering time and how expression and how many genes are involved. In addition, several studies provide several key genes involved in the field and under laboratory conditions. For example, Endo-Higashi and Izawa (2011) reported that *Hd1* and *Ehd1* genes together control the number of primary branches in the panicle. Furthermore, different levels of expression of *RFT1* and *Hd3* genes are associated with panicle size (Kojima et al. 2002; Tamaki et al. 2007). *Hd1* gene is one of the first key genes of flowering time that was cloned and identified from rice (Yano et al. 2000). There is no doubt that the study of *Hd1* gene in rice will provide new insights into the adaptation of rice in different conditional environments. Previously, nucleotide polymorphism of this gene has been studied in wild, weedy and cultivated rice. Some reports provided several significant findings, such as some alleles of the *Hd1* gene present in weedy rice and introgression to cultivated rice (Wu et al. 2020).

Takahashi et al. (2009) reported that DNA sequencing analysis of *Hd1* gene from a core collection of rice varieties shows 17 types of alleles and 15 types of proteins. Overall, identified mutations consist of deletion, insertion and substitution. These mutations lead to altered amino acid sequences caused by frame shifts and premature stop codons. Among unknown factors, the authors suggested that variation in the activity of *Hd1* alleles might be a factor of that influences flowering time variation in cultivated rice. In the present study was focused on the genetic diversity of traditional rice cultivars grown in different natural agroecosystems between northern mountain areas (upland rice) and northeastern lowland areas (lowland rice). The distribution of the allele S (AGT:Serine) of *Hd1* gene which were dominant in tropical *japonica* rice from northern region, while the allele G (GGT:Glycine) was mostly found in *indica rice* from northeastern region. In addition, two traditional rice cultivars from the northern, a premature stop codon at coding sequences (exon2) was identified, but not for lowland rice. Furthermore, 2 others non-synonymous SNPs(*i.e.*, AGC (Serine), AGA:Arginine) was distributed in both populations. The *Hd1* gene of these traditional rice cultivars shows no insertion/deletion (InDel) mutations occurring in coding sequences. The results of this study suggest that the genetic variation in terms of allele distributions between two locations may reflect to variation of mode of natural adaptations and/or result from the artificial selection made by local farmers. In mountain areas, local farmers have grown local traditional rice in their shifted fields. These traditional rice cultivars are photoperiod sensitive, growing in in-season rice production. The findings from this study also support the suggestion that is natural genetic variation in plant response to photoperiod contributed to the expansion of domesticated rice cultivation to a wider geographic range (Doebley et al. 2006), together with genes that control flowering time should show allelic differentiation across environmental gradients (Huang et al. 2012; Itoh et al. 2018).

In addition, the adaptation of plants to natural environments depends on the adaptation of flowering-time control at the appropriate season to set seeds (Izawa 2007). However, the non-synonymous SNPs in these rice cultivars is probably affect to gene or protein function that should be further investigation. An important regulator of photoperiod-sensitive flowering is the *Hd1* gene (Yano et al. 2000).

Asian cultivated rice (*Oryza sativa*) were classified as a short-day species that was domesticated from wild rice (*Oryza rufipogon*) in tropical and subtropical regions. Zong et al. (2021) showed the results from an experiment showed that is many landrace rice in tropical and subtropical regions, such as wild rice, *Oryza rufipogon*, contain *Hd1* allele and have strong or very strong photoperiod sensitivity for flowering. In addition, landrace rice could only be cultivated in natural short-day conditions in tropic and subtropic regions. One of the most important findings that is the evolution of rice with photoperiod sensitivity from very strong in the wild rice and landrace rice to various reduced degrees in the modern varieties during rice domestication and breeding, mainly involving natural allelic variations. Therefore, reliable information about the flowering gene network of cultivated rice is needed to investigate for a better understanding of different flowering times in photoperiod sensitivity rice cultivars.

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