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Research Article
Polymorphism Analysis of Heading Date Exon 2 Gene Region in *Japonica* Rice

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Abstract

Background and Objective: Temperate *japonica* rice varieties when grown in tropical climates have short daylight periods and high temperatures, so the heading date will be very early without enough panicles development, leading to low grain yields. This study aims to investigate the divergence of the *Hd1* exon 2 gene region related to the flowering time serving *japonica* rice breeding for the tropics. **Materials and Methods:** Forty-one *japonica* rice varieties (*Oryza sativa* L.) were used to evaluate flowering phenotypes and agronomic characteristics combined with sequencing of the heading date (*Hd1*) exon 2 gene region to determine the relationship between genotype and phenotype of the target trait. **Results:** The phenotypic assessment categorized 41 *japonica* rice varieties into four groups based on growth duration. Late varieties exhibited higher yields compared to early ones. Polymorphism analysis of *Hd1* exon 2 in 7 rice varieties revealed four Single Nucleotide Polymorphisms (SNPs) at positions 897, 1120, 1131 and 1159. The very early variety AKP4 showed no variation, while early varieties (Wc2811 and J16) had SNPs at positions A897C and G1131C, resulting in amino acid changes R299S and Q377H. Medium and long-duration varieties (A2 and B groups), in addition to the SNP at position A897C, might also have 1-2 SNPs at positions G1120A and G1159A that changed amino acids E374K and G387S, respectively. **Conclusion:** The SNP at G1120A and G1159A positions that changed amino acids E374K and G387S, respectively, may help to inactivate the *Hd1* gene function leading to the adaptation of temperate *japonica* rice to tropical regions.

Key words: Flowering time, *Hd1* gene, *japonica* rice, single nucleotide polymorphisms

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food crop of most Asian people, of which *japonica* rice varieties are very popular in Northeast Asian countries such as China, Japan and Korea, where are grown on large areas[1]. Vietnam is one of the world's leading rice exporters, but the amount of *japonica* rice exported is very limited, mainly because our country is located in the tropical monsoon climate zone, where is not suitable conditions for *japonica* rice production. The growth cycle of general rice and particularly *japonica* rice varieties is greatly influenced by environmental factors such as temperature, daylight, nutrients and water. Most rice varieties can only change to the reproductive stage when they meet one of two conditions: Achieving a total effective temperature accumulation (depending on the variety) or having a suitable day length[2,3].

Many genes regulate the flowering time of plants through their association with photoreceptors and circadian rhythms. Two key components, CONSTANS (CO) and Flowering locus T (FT), regulate the photoperiod pathway for flower development in Arabidopsis[4].Constans induces FT expression in inductive long-day (LD) conditions and regulates the circadian clock and photoperiod in determining flowering time. The *GIGANTEA* (GI) gene is a clock-associated gene regulating CO expression. The Arabidopsis GI-CO-FT module is a conserved mechanism found in various plant species, including the OsGI-Heading date 1 (*Hd1*)-Heading date 3a (*Hd3a*) module in rice[5]. This *Hd1* gene encodes zinc-finger-type transcriptional activators with CCT domains, promotes flowering under short-day (SD) conditions and represses flowering under LD conditions[6]. However, in another study, *Hd1* consistently promoted heading date in Zhenshan 97 background by upregulating *EHd1*, *Hd3a* and RFT1 expression under both SD and LD[7]. The full genomic *Hd1* gene length is 2,285 nucleotides, with a total coding region of 1,188 nucleotides divided into 2 exons. Exon 1 includes 828 nucleotides coding for 276 amino acids and exon 2 has 360 nucleotides coding for 120 amino acids. The sequence of this gene region varies greatly between *japonica* rice varieties[8,9]. Published studies have identified the *Hd1* gene related to the photoperiod sensitivity of temperate *japonica* rice varieties. If this gene is inactivated or inhibited by some other genes, the growth period of the rice will be prolonged enough to produce high yields even in tropical climate conditions[6,10-14]. The utilization of natural genetic diversity offers the chance to discover genetic variations affecting desired traits that can be selected to improve the agronomic characteristics of crops[15,16]. In order to explore the potential of using *Hd1* gene polymorphisms for MAS in *japonica* rice breeding for tropical regions, this study examined the association between flowering time, yield components and *Hd1* exon 2 variations.

MATERIALS AND METHODS

Study area: The study was carried out from March to September, 2023 at Net House and Molecular Biology Laboratory of Food and Biotechnology Institute, Can Tho University.

Materials: Forty-one *japonica* rice varieties imported from Dale Bumpers National Rice Research Center (USDA) and collected by Cuu Long delta Rice Research Institute (CLRRI) were used in this study including Quimimpol, Wc2811, AoChiu-2-hao, CriolloChivacoa, SecanoDoBrazil, R75, BritishHonduraCreole, C8429,Wc3532, PadiPohonBatu, Sipirasikkam, Morobereken, GPNO 1106, Grassy, Wc4443, Mitak, AKP4, Gallawa, DNJ121, Karayal, TiaBura, PadiTarabArab, Wir911, Coppocina, Pakkali, Wab462, Shinmei, Hananomai, J01, J03, J13, J16, J19, DS1, KRJ01, OM46, Shinmei01, Hatri200, *japonica*02, *japonica*01 and Amarose.

Methods

Evaluation of flowering phenotype and agronomic characteristics of *japonica* rice varieties: The experiment was carried out in a Completely Randomized Design (CRD), with three replications for 41 *japonica* rice varieties. Each replication was cultivated in a pot with a volume of 0.04 m^3 (40 cm in diameter ×32 cm depth). Each pot transplanted 2 rice plants when the seedlings were 12 days old (4-5 leaves). The amount of fertilizer for each pot was 0.8 g N+0.5 g P_2O_5 and 0.5 g K_2O.

Data observation according to Vietnamese National Standard TCVN 13381-1:2021 included flowering time (day to heading, abbreviated as DTH), growth duration (sowing to harvest, abbreviated as GD), plant height (cm), number of panicles/hill, number of filled grain/panicle, unfilled grain rate (%), dry grain yield/hill (g) and dry 1,000-grain weight (g). The growth duration of *japonica* rice varieties was divided into 4 groups according to TCVN 13381-1:2021 for the Mekong Delta Region including very early assigned as A0 (<90 days), early (A1 from 90 to 105 days), medium-duration (A2 from 106 to 120 days) and long-duration or late varieties (B above 120 days).

***Hd1* exon 2 gene region polymorphisms analysis:** Seven *japonica* rice varieties with different growth durations

according to TCVN 13381-1:2021[17] were chosen for *Hd1* exon 2 gene region analysis. Their young leaves were used to isolate DNA and perform PCR reactions using the P4 primer pair according to Kim *et al.*[8]. The expected product of P4 primer is 765 bp with the following sequence information:

Forward: 5'GAAAGACCTCATGAAAAGTAGG 3'
Reverse: 5'GCTATCAGGAAATTACAAAGCA 3'

The PCR reactions were performed in a total volume of 25 µL, the mixture included 2 µL of template DNA (~50 ng); 17.75 µL sterile double distilled water; 5 µL Buffer 5X; 1 µL forward primer (10 pM) and 1 µL reverse primer (10 pM); 0.25 µL Taq polymerase (5 U/µL). The reaction was amplified using a DNA Thermal Cycler machine-Model: GeneAmp PCR System 9700 (USA) with an initial thermal cycle of denaturation at 94°C for 5 min, 32 repeating cycles with 3 main steps: Denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec, extension at 72°C for 60 sec and termination at 72°C for 7 min and storing samples at 4°C. The PCR products were then tested by electrophoresis technique on a 2% agarose gel at a voltage of 50 V in 1X TBE buffer solution for 30-45 min. A 100 bp ladder was used to estimate the size of PCR product fragments. Electrophoresis results were recorded using a Biorad UV 2000 gel camera (USA). The PCR products of sufficient quality were sent for *Hd1* exon 2 gene sequencing by the Sanger method at Macrogen company (10F, 254, Beotkkot-ro, Geumcheon-gu, Seoul, Korea).

Data analysis: Phenotypic observation data were processed in Microsoft Excel, Variance analysis of phenotypic data, *post hoc* comparison and correlation were performed using SPSS software. The *Hd1* exon 2 gene sequence of *japonica* rice varieties was aligned by Bioedit software comparison with the reference *japonica* rice variety Nipponbare to find out Single Nucleotide Polymorphism (SNP). From the SNPs, the sequence of amino acids on the corresponding protein can be deduced. Compare genotypes and phenotypes to identify varieties with inactivated *Hd1* gene as breeding material and molecular markers linked to the late flowering gene to support selection.

RESULTS

Evaluation of flowering phenotype and agronomic characteristics of *japonica* rice varieties: The 41 *japonica* rice varieties were observed and recorded in Fig. 1. They were divided into 4 growth duration groups with 6 varieties belonging to the very early group (<90 days) accounting for 15% (group A0), seven varieties were observed in the early group (90-105 days) accounting for 17% (group A1). Medium-duration varieties (106-120 days) were observed with 8 varieties, accounting for 19.5% (group A2). There were 20 late rice varieties (group B) obtained with a growth period longer than 120 days, accounting for 48.5%.

Agronomic characteristics of 41 *japonica* rice varieties were recorded in Table 1 showing that, six very early rice

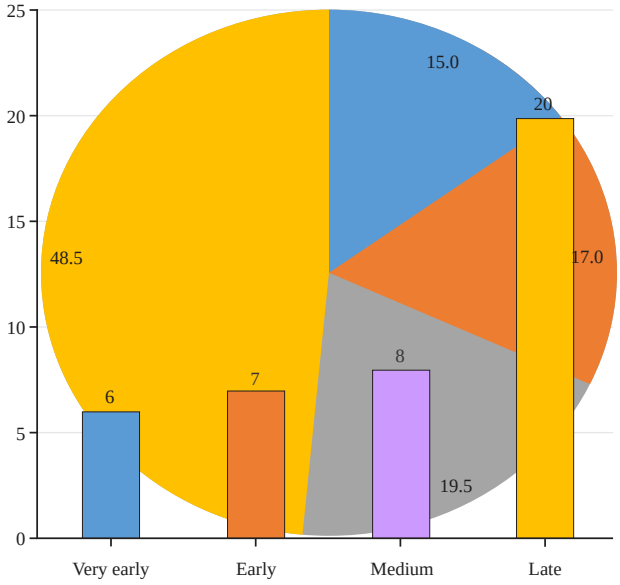

Fig. 1: Number of *japonica* rice varieties in each group of growth duration

Table 1: Day to heading and yield component of 41 *japonica* rice varieties

Characteristics	Very early	Early	Medium	Late
Count	6	7	8	20
DTH	39-59	64-75	77-90	92-148
Plant height (cm)	70.7-136.3	97.0-166.0	87.7-153.3	83.3-178.7
Panicles/hill	7.2-8.9	6.8-9.6	5.9-9.3	5.5-8.9
Filled grains/panicles	67.6-90.6	86.7-94.5	78.4-106.1	76-121.9
Unfilled grain rate (%)	8.5-30.1	19.6-34.4	19.4-32.2	10.9-35.7
1000 grains weight (g)	25.2-31.2	22.0-29.6	24.0-31.5	24.8-32.0
Grain yield (g/hill)	14.54-20.04	16.80-22.72	14.94-22.98	14.99-24.7

Table 2: Growth and yield components of 41 *japonica* rice varieties

Varieties	DTH	Plant height (cm)	Panicle/hill	Filled grain/panicle	Unfilled grain rate (%)	1000 grain weight (g)	Grain yield (g/hill)
Quimimpol	118^{c}	151.7^{de}	6.8^{d-h}	96.9^{b-h}	21.8^{j-m}	31.4^{ab}	20.44^{b-h}
Wc2811	75^{n}	166.0^{b}	9.6^{a}	91.4^{f-l}	31.4^{a-d}	25.9^{l-p}	22.72^{a-d}
AoChiu-2-hao	85^{kl}	142.3^{f}	7.2^{c-h}	96.1^{b-i}	32.2^{abc}	25.4^{n-q}	17.49^{f-i}
CriolloChivacoa2	139^{b}	107.7^{l}	6.8^{d-h}	105.3^{b-f}	24.1^{f-l}	26.1^{k-p}	18.63^{d-i}
SecanoDoBrazil	89^{ik}	116.3^{k}	9.3^{ab}	82.8^{i-n}	27.5^{c-j}	28.7^{d-j}	22.13^{a-e}
BritishHonduraCreole	59^{rs}	136.3^{g}	7.6^{b-f}	73.8^{no}	30.1^{a-f}	27.0^{h-o}	14.83^{i}
R75	92^{i}	125.3^{j}	7.1^{c-h}	105.0^{b-e}	17.1^{mno}	29.7^{b-r}	21.68^{a-f}
C8429	106^{fg}	147.7^{e}	8.5^{a-d}	83.5^{h-n}	28.3^{c-i}	30.8^{a-e}	21.65^{a-f}
PadiPohonBatu	83^{lm}	138.3^{fg}	8.9^{abc}	97.1^{b-g}	30.0^{a-g}	26.8^{i-o}	22.98^{abc}
Sipirasikkam	108^{efg}	128.3^{ij}	5.6^{gh}	121.9^{a}	24.9^{e-l}	25.3^{n-q}	17.24^{hi}
Morobereken	90^{i}	137.7^{fg}	8.5^{a-d}	78.4^{l-o}	30.4^{a-e}	26.0^{l-p}	17.33^{ghi}
Wc3532	92^{i}	135.0^{gh}	8.0^{a-e}	108.2^{b}	28.0^{c-j}	24.8^{o-q}	21.50^{a-g}
GPNO1106	100^{h}	157.3^{c}	7.8^{a-f}	97.3^{b-g}	24.0^{f-l}	29.6^{b-g}	22.24^{a-e}
Grassy	113^{cde}	175.0^{a}	8.0^{a-e}	89.7^{f-l}	20.7^{k-n}	30.9^{a-d}	22.35^{a-e}
Wc4443	93^{i}	131.3^{hi}	8.0^{a-e}	107.1^{bc}	10.9^{pq}	28.1^{f-l}	24.15^{ab}
Mitak	93^{i}	127.7^{ij}	8.0^{a-e}	76.0^{mno}	35.7^{a}	29.2^{b-g}	17.85^{f-i}
AKP4	39^{v}	101.3^{mno}	8.6^{a-d}	90.6^{f-l}	15.3^{nop}	25.2^{n-q}	19.68^{c-h}
Gallawa	69^{op}	124.7^{j}	7.7^{b-f}	87.4^{g-m}	34.4^{ab}	25.7^{m-p}	17.08^{hi}
DNJ121	64^{qr}	139.0^{fg}	8.9^{abc}	91.3^{f-l}	19.8^{k-n}	22.0^{r}	17.72^{f-i}
Karayal	73no	127.3^{ij}	7.6^{b-f}	94.5^{c-j}	22.7^{i-m}	23.4^{qr}	16.80^{hi}
TiaBura	92^{i}	156.7^{c}	7.4^{c-g}	80.7^{k-n}	25.8^{d-k}	28.9^{d-i}	17.14^{hi}
PadiTarabArab	112^{df}	178.7^{a}	8.9^{abc}	86.7^{g-m}	25.4^{d-l}	32.0^{a}	24.70^{a}
Wir911	54^{tu}	127.7^{ij}	8.0^{a-e}	90.3^{f-l}	12.3^{opq}	25.8^{m-p}	18.64^{d-i}
Coppocina	110^{def}	148.0^{e}	8.5^{a-d}	95.3^{b-j}	25.3^{d-l}	26.8^{i-o}	21.53^{a-g}
Pakkali	83^{ml}	153.3^{cd}	6.4^{e-h}	97.5^{b-g}	23.2^{h-m}	24.0^{pqr}	14.94^{i}
Wab462	65^{pq}	97.0^{opq}	6.8^{d-h}	93.5^{d-k}	19.6^{k-n}	29.6^{b-r}	18.46^{e-i}
Shinmei	94^{i}	93.3^{q}	7.4^{c-g}	92.4^{e-k}	21.1^{k-n}	29.1^{c-g}	19.73^{c-h}
Hananomai	137^{b}	95.7^{pq}	5.5^{h}	94.8^{c-j}	23.1^{h-m}	29.0^{d-i}	14.99^{i}
J01	78^{mn}	87.7^{rs}	8.1^{a-e}	94.4^{c-j}	27.6^{c-j}	25.4^{n-q}	19.35^{c-h}
J03	No flowering						
J13	75^{n}	103.3^{lmn}	8.4^{a-d}	86.7^{g-m}	29.2^{b-h}	27.4^{g-n}	19.87^{c-h}
J16	75^{n}	104.0^{lm}	8.4^{a-d}	92.7^{e-k}	28.8^{b-i}	28.4^{f-k}	22.17^{a-e}
J19	103^{gh}	83.3st	7.2^{c-h}	91.5^{f-l}	23.2^{h-m}	26.7^{i-o}	17.59^{f-i}
DS1	106^{fg}	93.7^{q}	8.0^{a-e}	90.5^{f-l}	28.2^{c-i}	28.5^{e-j}	20.77^{a-h}
KRJ01	52^{u}	88.7^{r}	7.2^{c-h}	82.1^{j-n}	8.5^{q}	30.0^{a-f}	17.62^{f-i}
OM46	84^{l}	108.0^{l}	6.8^{d-h}	95.5^{b-j}	23.8^{g-l}	26.6^{j-o}	17.14^{hi}
Shinmei01	58st	83.0^{t}	8.9^{abc}	72.0^{no}	17.3^{mno}	31.2^{abc}	20.04^{b-h}
Hatri200	77^{n}	99.0^{nop}	5.9^{fgh}	106.1^{bcd}	19.4^{lmn}	31.5^{ab}	19.76^{c-h}
*Japonica*02	148^{a}	88.7^{r}	7.2^{c-h}	101.6^{b-f}	23.7^{h-l}	27.9^{f-m}	20.35^{b-h}
*Japonica*01	114^{cd}	95.7^{pq}	7.6^{b-f}	96.7^{b-h}	24.3^{e-l}	28.1^{f-l}	20.56^{b-h}
Amarose	55^{stu}	70.7^{u}	8.3^{a-d}	67.6^{o}	12.8^{opq}	25.8^{m-p}	14.54^{i}
Mean	89	121.8	7.7	92.1	23.8	27.6	19.41
F	213**	319.11**	3.18**	7.71**	11.84**	12.58**	4.63**
CV (%)	28	22.72	15.83	12.96	28.40	9.30	15.98

**Significant at 1% and Numbers followed by the same character are not statistically different in the same column

varieties had a day to heading ranging from 39-59 days. There was not much difference in plant height, unfilled grain rate and 1000 grain weight of the very early group compared to other growth duration groups, but had the fewest filled grains/panicle and the lowest grain yield in comparison with other groups (Table 2).

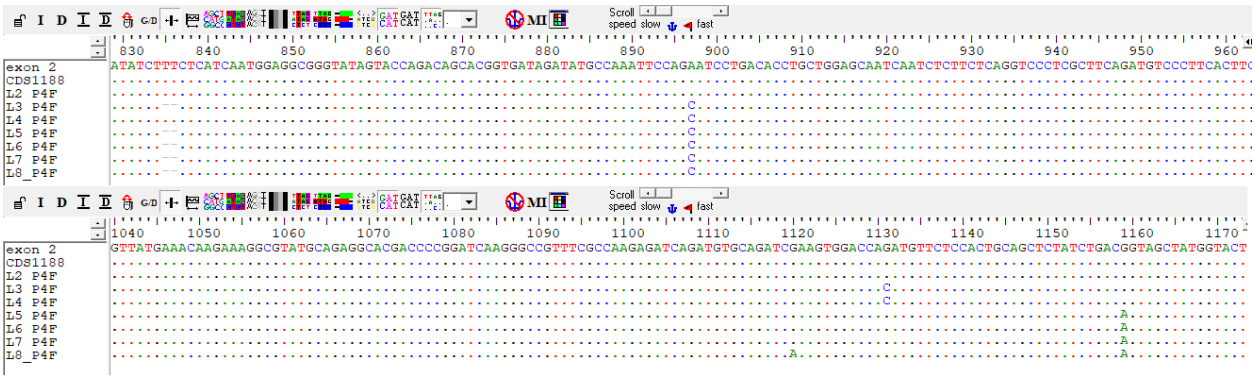

Fig. 2: Comparison results of *Hd1* exon 2 gene region sequences of seven rice varieties with Nipponbare variety
CDS1188 full coding DNA of *Hd1* gene, L2 P4-F AKP4; L3 P4-F Wc2811; L4 P4-F J16; L5 P4-F SecanoDoBrazil; L6 P4-F PadiPohonBatu; L7 P4-F Wc4443; L8 P4-F PadiTarabArab

Table 3: Pearson correlation coefficient of *japonica* rice varieties agronomic traits

Traits	DTH	PH	PPH	FGPP	UGR (%)	TGW	GY
DTH	1.000						
PH	0.160	1.000					
PPH	-0.315*	0.213	1.000				
FGPP	0.453**	0.074	-0.538**	1.000			
UGR (%)	0.258	0.308*	0.176	-0.067	1.000		
TGW	0.278	0.021	-0.072	-0.151	-0.134	1.000	
GY	0.314*	0.336*	0.428**	0.292	0.030	0.442**	1.000

DTH: Days to heading, pH: Plant height, PPH: Panicles per hill, FGPH: Filled grain per panicle, UGR: Unfilled grain rate, TGW: Thousand grain weight, Grain yield. *T-test significant at 5%, **t-test significant at 1%, Pearson correlation coefficient from 0.0 to ±0.2 no correlation, Pearson coefficient from ±0.2 to ±0.4 weak correlation when t-test significant, pearson coefficient from ±0.4 to 0.6 moderate correlation when t-test significant

Table 4: *Hd1* exon 2 gene region polymorphism

SNP positions	897	1120	1131	1159	Varieties (group)
Amino acid change	R299S	E374K	Q377H	G387S	
	A	G	G	G	Nipponbare (reference)
	-	-	-	-	AKP4 (A0)
	C	-	C	-	Wc2811 (A1), J16 (A1)
	C	-	-	A	SecanoDoBrazil (A2), PadiPohonBatu (A2), WC4443 (B)
	C	A	-	A	PadiTarabArab (B)

Analysis of the correlation between traits, Table 3 showed that the DTH of rice varieties had an average positive correlation (Pearson coefficient 0.453) with the number of filled grains/panicles at the 1% significance level, a weak negative correlation with the number of panicles/hill (-0.315) and positively correlation with grain yield (0.314) both at the 5% significance level. Grain yield of rice varieties had a moderate correlation with the number of panicles/hill (0.428) and 1,000 grains weight (0.442) at the 1% significance level, weakly correlation with plant height (0.336) and DTH (0.314) at the same 5% significance level. In addition, there were two other pairs of traits that were moderately and weakly correlated: Number of panicles/hill with the number of filled grains/panicles (-0.538) at the 1% significance level and plant height with the unfilled grain ratio (0.308) at the 5% significance level. Most of the traits were positively correlated with each other, only two pairs of traits, including the number of panicles/hill with number of filled grains/panicles and DTH with number of panicles/hill, were negatively correlated. Thus, although there was a weak to moderate correlation, early-flowering time varieties had more panicles/hill but fewer filled grains/panicles and had lower grain yields. Late rice varieties had fewer panicles/hills but higher grains/panicles and produced higher grain yields.

***Hd1* exon 2 gene region polymorphisms analysis:** From the results of morphological evaluation, 7 varieties represented for 4 growth duration groups were selected for *Hd1* exon 2 gene region polymorphism analysis, including AKP4 (A0), Wc2811 and J16 (A1), SecanoDoBrazil and PadiPohonBatu (A2) and WC4443 and PadiTarabArab (B). The *Hd1* exon 2 sequencing results of 7 *japonica* rice varieties showed a stable signal region of 765 bp length, extending from the 829th nucleotide position to the 1593rd nucleotide position. After aligning with

the referent variety Nipponbare, four SNPs were found at 897, 1120, 1131 and 1159 (Fig. 2 and Table 4). Among 7 varieties sequenced, there was one variety without any nucleotide substitutions (AKP4), one variety with 3 SNPs found (PadiTarabArab) and five varieties with 2 SNP markers recorded (Wc2811, J01, SecanoDoBrazil, PadiPohonBatu, WC4443).

All four SNP markers are missense mutations. Specifically, at the position 897th, nucleotide A changes to C, corresponding to the amino acid arginine changed to Serine at position 299th (R299S), this change was found in 6 rice varieties Wc2811, J16, SecanoDoBrazil, PadiPohonBatu, WC4443 and PadiTarabArab. The nucleotide G changed to A at position 1120th, leading to the amino acid Glutamate change to Lysine at position 374th (E374K), obtained in the PadiTarabArab variety. The nucleotide G changed to C at position 1131st, corresponding to the amino acid Glutamine changed to Histidine (Q377H), found in two varieties Wc2811 and J16. The nucleotide G changed to A at position 1159th, distorted the amino acid Glycine to Serine at position 387th (G387S), presented in 4 varieties SecanoDoBrazil, PadiPohonBatu, WC4443, PadiTarabArab (Table 4).

The *Hd1* exon 2 gene sequencing results were consistent with the phenotypic assessment of flowering traits. Specifically, the AKP4 variety belongs to the very early group, showing no SNP markers compared to the Nipponbare variety. The Wc2811 and J16 varieties were found in the early group with 2 SNP markers at positions 897th and 1131st. SecanoDoBrazil, PadiPohonBatu, WC4443 and PadiTarabArab varieties corresponding to the medium and long duration groups, two SNP markers were found in SecanoDoBrazil, PadiPohonBatu and WC4443 varieties at nucleotide positions 897th and 1159th; three SNP markers were detected for the PadiTarabArab variety at positions 897, 1120 and 1159th on exon 2 of the *Hd1* gene region.

DISCUSSION

The flowering time of 41 *japonica* rice varieties in this study ranged from 39 to 148 days with 6 varieties showing very early heading dates (<60 DTH). This divergent was similar to the study of Kim *et al.*[8] in which, 45 *japonica* rice varieties were evaluated and observed 8 very early rice varieties. These very early varieties showed an irregular flowering time among tillers in a single plant with the main tiller flowering very quickly (~45 days) and the following tillers flowering in order. In another research, Fujino *et al.*[6] reported that the non-function *Hd1* gene (homogenous *Hd1*) exhibited later flowering time in comparison with the function *Hd1* gene (homogenous *Hd1*) in short-day conditions. Subudhi *et al.*[11] suggested *Hd1* gene separated as a single gene with a ratio of 1:2:1 for the F2 population and promoted flowering in short-day conditions but delayed flowering in long-day conditions. Ye *et al.*[12] reported that Loss-of-function alleles of *Hd1*, *Ghd7* and *DTH7* genes contributed to early rice heading dates in the northern regions of Northeast China, while functional alleles promoted late rice heading dates in the southern regions of Northeast China.

The growth duration of 41 *japonica* rice varieties in the current study was more diverse than the report by Zhao *et al.*[17]. This study was conducted on 7 *japonica* rice varieties in China with growth duration from 126 to more than 144 days. In another research, Reig-Valiente *et al.*[18] conducted an experiment in Spain at a high-latitude location (39°28'N) and evaluated 217 rice varieties collected from different geographical regions. They reported that the growth duration of these varieties ranged from 48 to more than 107 days.

The grain yield of *japonica* rice was reported positive correlation with flowering time in various geographical locations (ranging from lower to higher latitudes) represented by grain number per main panicle. Analysis of the correlation between flowering time with other traits in the current study indicated that the early-flowering time varieties had more panicles/hill but fewer filled grains/panicles and had lower grain yields. This result was similar to the report by Gao *et al.*[19], in which the grain yield was lost because of less grain per panicle in the early varieties. In the other research, a positive correlation was observed between flowering time with spikelets per panicle under short-day conditions but changed to a negative correlation under long-day conditions[13]. Spikelet number per panicle and grain yield per plant were reported to correlate positively and extremely significantly with flowering time and flowering time influenced yield mainly through the spikelet number per panicle[12].

The *Hd1* exon 2 gene region polymorphism analysis of seven *japonica* rice varieties in various growth durations detected four SNPs at the positions of 897, 1120, 1131 and 1159th, changed the corresponding amino acid R299S, E374K, Q377H and G387S, respectively. This result is not exactly similar to research by Kim *et al.*[8] and Leng *et al.*[9]. It may be because this gene region is very divergent between rice varieties. Kim *et al.*[8] reported that four divergences were detected from the 829th to 1593rd nucleotide position including a frameshift insertion at the position of 897th and 4 bp frameshift deletion at the 1089th position, two SNP at A933C and G1195A nucleotide positions observed changing A311C and G399S in amino acid sequence, respectively. In the other research, Leng *et al.*[9] indicated that six divergences were

detected in the same gene region consisting of five SNPs at the nucleotide position of 953rd, 1031st, 1145th, 1169th and 1534th and a 2 bp frameshift deletion at 1472nd position.

In general, the results of *Hd1* exon 2 gene region polymorphism analysis in this study showed that early rice varieties (A1) Wc2811 and J16 had 2 SNPs that distort 2 amino acids R299S and Q377H; while the medium duration rice varieties (A2) SecanoDoBrazil, PadiPohonBatu also had 2 SNPs but change 2 amino acids R299S and G387S; particularly, the late varieties group (B) had 2-3 SNPs which changed amino acid the same as the medium-duration varieties group (A2), or had an additional SNP at the 1120th nucleotide position that changed the 374th amino acid, Glutamate to Lysine (E374K). No SNPs were found in the very early rice variety AKP4. This result contributes to strengthening the hypothesis of nucleotide changes in the genotype (SNP) compared to the original gene sequence, helping to select *japonica* rice varieties with late flowering genotypes as breeding materials for tropical *japonica* rice breeding.

CONCLUSION

Phenotypic evaluation of 41 *japonica* rice varieties showed that four different growth duration groups were observed including 6 very early varieties, 7 early varieties, 8 medium-duration varieties and 20 late varieties. Early-flowering rice varieties produced more panicles/hill but fewer filled grains/panicles and had lower grain yields than the late-flowering varieties. The *Hd1* exon 2 polymorphism analysis of 7 rice varieties in 4 different growth durations recorded 4 SNPs at nucleotide positions 897, 1120, 1131 and 1159, distorting 4 amino acids R299S, E374K, Q377H and G387S. The SNPs in the *Hd1* exon 2 gene region could distinguish between very early rice varieties (A0, no SNPs), early rice varieties (A1, with 2 SNPs), medium-duration rice varieties (A2, with 2 SNPs) or long-duration (B, had 2-3 SNPs) but could not distinguish between groups A2 and B. The SNP at G1120A and G1159A positions that changed amino acids E374K and G387S, respectively, may help to inactivate the *Hd1* gene function leading to the adaptation of temperate *japonica* rice to tropical regions.

SIGNIFICANCE STATEMENT

Temperate *japonica* rice varieties when grown in tropical climates will be very early heading without enough panicles development, leading to low grain yields. This study aims to investigate the divergence of the *Hd1* exon 2 gene region related to the flowering time serving *japonica* rice breeding for the tropics. The phenotypic assessment categorized 41 *japonica* rice varieties into four groups based on growth duration. Late varieties exhibited higher yields compared to early ones. Polymorphism analysis of *Hd1* exon 2 in 7 rice varieties belong to 4 groups of growth duration time revealed four Single Nucleotide Polymorphisms (SNPs) at positions 897, 1120, 1131 and 1159 in comparison with that of temperate *japonica* rice, Nipponbare. The SNP at G1120A and G1159A positions that changed amino acids E374K and G387S, respectively, may help to inactivate the *Hd1* gene function leading to the adaptation of temperate *japonica* rice to tropical regions.

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