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PHENOTYPIC, MARKER ASSISTED SELECTION AND IDENTIFICATION OF QTLS FOR HEADING DATE IN F_2 POPULATIONS (PSM X IR24) RICE USING SNP MARKER

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ABSTRACT

Paw San Hmwe rice is aromatic, good taste, short grain elongation, has low amylose content and premium eating quality. However, it can flower under short day length conditions and yield per ha is very relatively low. Paw San Hmwe rice variety needs to be developed as a photoperiod insensitive rice variety for year-round production. F_2 populations, (94 plants) derived from Paw San Hmwe (PSM) and IR24, were grown both in short day length and natural day length conditions in Tokyo University of Agriculture from April to October 2022. The F_2 plants from natural day-length condition were used for molecular marker analysis. These F_2 plants were headed under short day-length conditions and earlier than their parent plant, PSM. In addition, it was found that of all the F_2 plants, 56 plants were headed less than 130 days under natural day-length conditions. It might have photoperiod insensitive gene like *Hd1* in these 56 plants for early heading. Among 94 F_2 populations, 52 F_2 plants had the same *Hd1*(+), *DTH8*(-) and *ehd1* (+). PSM expressed two QTLs, *DTH*8 (+) and *ehd1*(+) and 4 plants had these two QTLs like PSM but IR24 expressed two QTLs, *Hd1*(+) and *ehd1*(+) and not expressed *DTH8*. This result revealed that phenotypic variation in the heading date of 52 F_2 populations was closely identical with IR24 and 4 F_2 plants were closely identical with PSM. 5 plants had *Hd1*(+), *DTH8*(+), and *ehd1* (+). Therefore, based on genotypic selection, 57 F_2 populations which contained *Hd1* gene combined with the plants that contained *ehd1* can be used for the next generations for future breeding programs. In the QTLs analysis, a total of three QTLs were mined on chromosomes 6, 8, and 10. Three QTLs were observed on the heading date (*Hd1*, *DTH8*, *and ehd1*). It also indicated that there were common QTLs that were found across the genetic background and specific QTLs that were found within the specific genetic background.

Key words: genotyping, heading date gene, phenotyping,

INTRODUCTION

Rice is a major food crop for more than fifty percent of the world's population and one of the major economic crops around the world. The population of Myanmar was 49.39 million in 2010 and is increasing at the rate of 1.15% per year (MOAI 2014). There are 5 groups of rice in Myanmar: Emata, Let Ywe Zin, Nga Sein, Byat and Meedon. Among Myanmar rice varieties, Paw San rice is one of the world's most recognized high-quality rice. Paw San rice is in the Meedon group and is the most popular and widely cultivated quality rice due to its superior quality and the strong demand for it, which enable it to command a high price in the local market (Win 1991). The names of Paw San rice vary by location

and official name. But Paw San has a similar aroma, grain quality, and eating quality to the reputable aromatic rice varieties of the world, namely Basmati of India and Pakistan and Jasmine of Thailand. Although it has a strong aroma similar to Jasmine rice, Paw San has not made it to the export market due to its low yield and a high domestic demand leaves little to export as well. It is famous for its elongation characteristics (up to three times longer than the original size after cooking) and its great taste. Among Paw San rice varieties, Paw San Hmwe (PSM), Paw San Bay Kyar (PSBK), Paw San Yin (PSY) and Bay Kyar Lay (BKL) are very popular in Myanmar. Paw San Hmwe was awarded world's best rice at the World Rice Conference 2011 held in Ho Chi Minh, Vietnam. A good quality Paw San Hmwe costs often more than 1,000 USD per MT (metric ton). It has the potential to become famous in the international market soon; it is challenging to develop superior quality rice in the international rice market (Thein 2011). Although Paw San Hmwe is famous for its superior quality, it is photosensitive and can only head under short day conditions.

Heading date is very important in rice cultivation. Rice is short day plant so flowering is promoted under short day- length conditions and inhibited by long day- length conditions (Nishida et al. 2001). Short day conditions accelerated inflorescence initiation, heading or flowering (Morinaga et al. 1955). Short days promoted reproductive development in rice (Asakuma 1958). Short photoperiods of less than 9 or 11 hours were less effective in shortening the vegetative period than longer photoperiods (Velasco and Dela Fuente 1958). The short-day conditions allow the production of plants in different flowering stages which greatly facilitates synchronous flowering for varieties that differ in their flowering time (Song et al. 2012). The heads rate of super hybrid rice Liang-you-pei 9 (LYP9) is 21% in the short day- length and high temperature conditions and 13.6% in natural day length and high temperature conditions (Zhou et al. 2011).

The wide range of flowering times in rice is controlled by many quantitative trait loci (QTL) that influence photoperiod sensitivity (Hori et al. 2016). An important regulator of photosensitive heading is *Heading date 1* (*Hd1*), a homolog of *Arabidopsis thaliana CONSTANS* (*CO*) (Putterill et al. 1995; Yano et al .2000). The first heading date QTL cloned based on natural variation in rice accession, *Hd1* is a homolog of Arabidopsis CO and promotes heading under short day conditions while represses heading under natural day. Moreover, under short day conditions *Hd1* promotes the expression of *Hd3*a but represses *Hd3a* expression under natural day conditions (Kojima et al. 2002). *Hd1*, *Hd3a*, and *Hd 6* have been cloned and are involved in the photoperiodic flowering pathway (Kojima et al. 2002; Takahashi et al. 2011; Yano et al. 2001). *DTH8* gene on chromosome 8 for days to heading is the core gene in the regulation of the photoperiod sensitivity of rice (Brambilla and Fornara 2017; Chen et al. 2021). *DTH8* is another major heading date gene consisting of a strong allele and a nonfunctional allele of *DTH8* (*DTH8*/*dth8*), is present in many Chinese hybrids, particularly in the two-line hybrids (40.8%), including the well known super hybrid rice Liang-you-pei 9 (LYP9) (Liu et al. 2016). The *Ehd1* pathway is a specific one pathway that plays a more dominant role in the photoperiod flowering pathway, which is independent of *Hd1*. The *Ehd1* is the central integrator of the non-*Hd1*-dependent flowering pathway in rice, a pathway that has no parallel in *Arabidopsis*. It is capable of inducing *Hd3a and* RFT1independently of *Hd1* and is induced or repressed by several other flowering time genes including *Hd9* and *Ghd7*. Mutants lacking both *ehd1* and *hd1* have extremely late flowering phenotypes (Xue et al. 2008).

Hence, the breeding strategy has been laid down to have photoperiod insensitive traits for early heading of PSM rice variety for year-round production. The development of new rice cultivars with photoperiod-insensitive trait become the major priority for rice breeding programs in Myanmar because of the high demand for quality rice in local and international markets. Therefore, this study was conducted to evaluate phenotypic and genotypic analysis for heading of F_2 populations and to identify QTLs associated with heading traits in rice using F_2 population derived from a cross between PSM and IR24 using SNP marker.

MATERIALS AND METHODS

Population development. PSM (female) was crossed with IR24 (male) in the greenhouse for generation advanced at the Tokyo University of Agriculture from April to October 2022. The hot water emasculation method was used for hybridization as described by Matsubayashi and co-workers (1965). F_1 and F_2 populations (PSM x IR24) were advanced by self-fertilization and in total, 100 F_2 plants were grown by giving short day- length and natural day length conditions from December 2022 to December 2023. The short day- length condition was applied to the plants after transplanting for one month in a greenhouse with a controlled temperature at 31°C and the photoperiod was 9 hours light and 15 hours dark. The natural day-length condition was as in greenhouse conditions. Phenotypic trait data for heading date was measured for each plant in the F_2 populations. The heading date for each plant was recorded as the date on which the first panicle emerged from the flag leaf sheath. F_2 plants (94 plants) from natural day-length condition were used for molecular marker analysis.

Genomic DNA extraction and DNA quality estimation. Total rice genomic DNA was extracted from young leaves of parents and F_2 populations by Rnase treated method using Nucleo Spin PlantII protocols. Fresh leaves (0.02 g) were added into 2 ml tubes and ground into powder using a grinding machine. 6 ml pre-heated DNA extraction buffer PL 1 was added into each tube and vortexed. Each tube was incubated at 65◦C for 5 min, vortexed and then incubated again at 65°C for 45 min. This was transferred into new tubes contained filter and centrifuged for 1 min.675 μl of binding buffer PC was added for precipitation. DNA pellets were washed with 400μl of wash buffer PW1, 700 μl of wash buffer PW 2 and 200μl of wash buffer PW2. Elution buffer PE (75 μl) was added and incubated at 65◦C for 5 min.

The genomic DNA was quantified both at 260 nm and 280 nm wavelengths spectrophotometrically. An optical density of 1 at 260 nm corresponds to 50μg of double stranded 91 DNA. Normalization of the DNA concentration was done to bring all the DNA concentrations to a relatively equal level (25ng/μl) by appropriate dilutions for PCR reaction. Dilution was done with double sterile water.

1k-RiCA SNP assay. The 1 k Rice Custom Amplicon assay or 1k-RiCA was designed on Illumina's TruSeq Custom Amplicon (TSCA) 384 index Kit technology using Illumina's proprietary workflow. The ability of the 1 k (1000-SNP) RiCA trait markers to identify the samples with the desired and undesired alleles was determined using SNP Quality Control methods (Platten et al. 2019). In this study, 94 F_2 plants were checked for the trait-related heading date with 1k-Rica SNP markers.

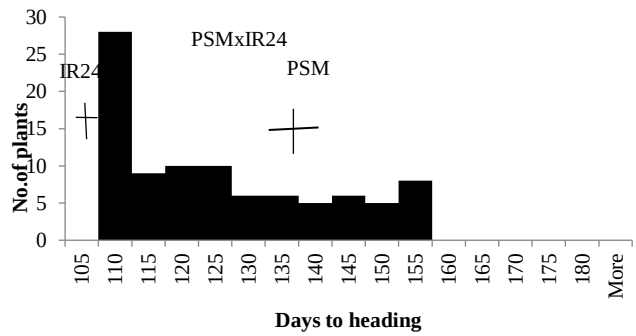
Statistical analysis and QTLs analysis. The visualization of days to the heading of 94 F_2 populations was performed using Microsoft Excel 2011. Analysis of QTLs was performed by using Windows QTL Cartographer version 2.5 (Basten, Weir, and Zeng 2005). The QTL parameters were estimated with composite internal mapping (CIM). One thousand permutations were run to obtain the empirical threshold of the experiment by randomly shuffling the trait values (Churchill and Doerge 1994). A probability level of 0.05 was used as the threshold for the detection of a putative QTL.

RESULTS AND DISCUSSION

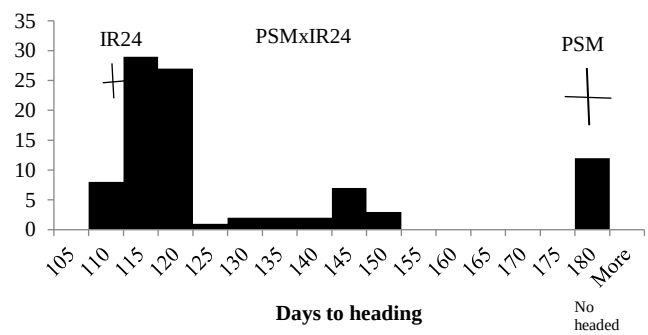
Phenotypic variation of days to heading in the F_2 population of PSM x IR 24. Days to heading of F_2 population under short day- length and natural day- length are shown in Figure 1. The parent genotype under short day length conditions had 152 days in PSM and 105 days in IR24 for days to heading. Among of F_2 populations (94 plants), we found 28 plants had days to heading 110 days, 9 plants at 115 days, 10 plants at 120 days, 10 plants at 125 days, 6 plants at 130 days, 6 plants at 135 days, 5 plants at 140 days, 6 plant at 145 days, 5 plants at 150 days, and 8 plants at 155 days. In this study, although PSM was late heading variety and photoperiod sensitive variety, it could response 9

hours light and 15 hours dark and headed in 152 days. 94 F_2 plants were also headed under short day-length condition and earlier than their parent plant, PSM. All F_2 plants were headed like PSM under short day- length condition because of giving 9 hours light and 15 darks. If short day- length condition is given to the F_2 plants derived from the crossing of PSM and IR 24, it was suitable condition for heading for F_2 plants. Zhou et al. 2011 reported that days to heading of some rice varieties were strongly influenced by their photoperiod-sensitive genetic in short day- length.

In natural day length conditions, the parental plants headed 110 days in IR 24 whereas PSM did not head until 31st of December, 2023. Based on the time of the flowering day in photoperiod insensitivity (IR24) and photoperiod sensitivity (PSM), the variation in the days to heading under natural day length conditions in the F_2 population of PSM x IR 24 showed 56 plants headed at 115-120 days, 17 plants headed at 125-150 and 12 plants headed at 175-180 while 9 plants did not head.PSM rice variety could not head when grown under natural day-length condition of Japan. This result confirmed that PSM is highly sensitive to photoperiod for heading. Although PSM did not head in natural day-length condition, 56 plants were headed without over 130 days. It might have photoperiod insensitive gene like *Hd1* in these 56 plants for early heading. Vergara et al. 1966 reported that photoperiod insensitive cultivars do not have duration of flowering longer than 130 days in the tropics. In addition, days to heading of F_2 population (PSM x IR24) under short day- length was earlier than those with natural day- length condition and all of F_2 populations were headed in short day length condition.



Short day length conditions



Natural day length condition

Figure 1. Phenotypic variation of days to heading in the F_2 populations of PSM x IR 24 under short day- length and natural day- length conditions

Marker assisted selection of F_2 population compared with parent genotypes by using SNP marker. Among the 94 F_2 populations derived from the cross of PSM and IR24, 52 F_2 plants were found to have the same *Hd1* (+), *DTH8* (-) and *ehd1* (+) (Table 1). PSM expressed two QTLs, *DTH8* (+) and *ehd1* (+) and 4 plants had these two QTLs like PSM but IR24 expressed two QTLs *Hd1* (+) and *ehd1* (+) and not expressed *DTH8* i.e PSM had *Hd1* (-), *DTH8* (+), *ehd1* (+) combination and IR 24 had *Hd1* (+), *DTH8* (-), *ehd1* (+) combination.

Table 1. Marker assisted selection of F_2 populations compared with parent genotypes by using SNP marker

Detected QTLs	**Plant No.**	**Total plant**
Hd1(+), *DTH8*(+), *ehd1* (+)	22,24,51,53,79	5
Hd1(-), *DTH8*(+), *ehd1* (+)	PSM, 71, 87, 90, 94	4
Hd1(+), *DTH8* (-), *ehd1*(+)	IR24, 2,3,4,5,6,7,8,11,12,13,14,15,20,26,27,28,31,32,34,35, 37,38,39,40,41,42,45,47,48,49,50,54,55,58,59,60,61,62, 66,67,68,70,72,75,76,77,81,82,83,84,85,93	52
Hd1(-), *DTH8(-)*, *ehd1*(+)	9,16, 19,21, 25,43, 57,63,64,65, 73,74,86	13
Hd1(+), *DTH8(+)*, *ehd1*(-)	N/A	0
Hd1(-), *DTH8*(+), *ehd1*(-)	1,10,56,91	4
Hd1(+), *DTH8*(-), *ehd1*(-)	N/A	0
Hd1(-), *DTH8*(-), *ehd1*(-)	N/A	0

(+) : presence of these QTLs; (-) : absence of these QTLs; N/A: QTLs not detected

These results revealed that phenotypic variation in heading date of 52 F_2 populations was closely identical with IR24 and 4 plants were closely identical with PSM. *Hd1* is dominant and confers early flowering (Doi and Yoshimura 1998). In this study, IR 24 carries a function allele *Hd1* whereas PSM did not carry *Hd1* because it did not contain *Hd1* alleles, and this genetic evidence indicated that *Hd1* can function to promote early flowering.

On the other hand, PSM did not express *Hd1*and this result can be confirmed that PSM rice did not head when exposed to natural day length condition. *Hd1* promotes the expression of *Hd3a* under short day length but represses under long day length and *Hd1* genes were expressed in non-photosensitivity (Kojima et al. 2002). IR 24 has a functional allele of *Hd1* and non-functional alleles of other heading date QTLs (*DTH 8, Ghd7, Hd3a, RFT1, Hd6 and Hd18*) (Itoh et al. 20018, Shibaya et al. 2016, Wei et al. 2016).

In the present experiment, 13 plants had *Hd1* (-), *DTH8* (-) and *ehd1* (+) combination; 5 plants had *Hd1* (+), *DTH8* (+) and *ehd1* (+) combination and 4 plants had *Hd1* (-), *DTH8* (+) and *ehd1* (-) combination. QTL (*DTH8*) was expressed in PSM. According to this result, *DTH8* might delay flowering time because of present in PSM which is photoperiod sensitive variety and this gene might act as a suppressor because of preventing early flowering. Therefore, *DTH8* might have a negative flowering effect.

When plants were grown in natural day length condition, it suppressed flowering by down regulating the expressions of *ehd1* and *Hd3a* (Gao et al. 2014). The lack of *ehd1* flowered extremely

late compared with wild-type plants under both conditions (Matsubara et al. 2008). The heading date genes *Hd1* and *ehd1* are effective for early heading and 57 F_2 plants contained the *Hd1* gene for early heading. In the present study, 94 F_2 populations derived from PSM and IR24 were used for genotyping with 1k-RiCA (1K- Rice Custom Amplicon) assay which is a novel genotyping amplicon based SNP assay.

QTLs analysis for heading date. Three QTLs were detected for heading on chromosome no. 6, 8, and 10 designated as *Hd1*, *DTH8*, and *ehd1*. The *Hd1*was located in the marker interval SNP1050-SNP 1051 on chromosome 6 with the LOD score of 20.27 and explained 22.60 % of the phenotypic variation for heading. The *DTH8* was located in the marker interval SNP1060-SNP 1061 on chromosome 8 with the LOD score of 9.42 and explained 24.55 % of the phenotypic variation for heading. The *ehd1* was located in the marker interval SNP1099-SNP 3100 on chromosome 10 with the LOD score of 3.71 and explained 21.65% of the phenotypic variation for heading (Table 2).

Table 2. QTLs for heading date on F2 populations

QTLs for heading date	Chromosome	Marker Interval	Position	LOD	Additive effect	PVE%
Hd1	6	SNP1050-SNP1051	9325033	20.27	0.2	22.60
DTH8	8	SNP1060-SNP1061	4334417	9.42	0.1	24.55
ehd1	10	SNP1099-SNP3100	17077589	3.71	0.12	21.53

QTLs associated with heading date were located on chromosome on 3, 5-8, 10 and 12 and QTLs on the chromosome 6 would be the same as QTLs reported in previous studies using doubled haploid and backcrossed populations of IR 64 (Guo et al. 2013). QTLs on Chromosome 3 were reported by Hittalmani et al. (2002; 2003) and Liu et al. (2007) and QTLs on Chromosome 8 were reported by Hittalmani et al. (2002; 2003), Liu et al. (2007) and Septiningsih et al. (2003). Heading date QTLs on Chromosome 7, 10 and 12 were found by Li et al. (2003), Liu et al. (2007) and Septiningsih et al. (2003).

CONCLUSION

Based on the phenotypic analysis of F_2 populations derived from PSM and IR24 (94 plants) found that 56 F_2 plants were headed without over 130 days and may have photoperiod insensitive genes such as *Hd1* in these 56 plants for early heading under natural day length condition.

Based on the genotypic analysis, 52 F_2 plants had the same *Hd1* (+), *DTH8* (-), and *ehd1* (+) and 57 plants had *Hd1* and *ehd1* gene together like IR24,13 plants had *DTH8* gene like PSM. Therefore, these 57 F_2 populations containing the *Hd1* gene combined with the plants containing *ehd1* can be used for the next generations for future breeding programs.

The F_2 population derived from PSM and IR24 was used to map the QTLs associated with heading traits. A total of three QTLs were detected in the QTLs analysis on chromosomes 6, 8, and 10. Three QTLs were observed for heading date (*Hd1, DTH8, and ehd1*).Some QTLs were located on the same chromosome and even at the same location, indicating the close association of the traits. It also showed that there were common QTLs that were found across genetic backgrounds and specific QTLs

found in specific genetic backgrounds. Molecular markers linked to the major QTLs for yield-related traits can potentially be used for the marker-assisted selection of the corresponding traits in the rice breeding programs.

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REFERENCES CITED

Asakuma, S. 1958. Some aspects of photoperiodisms in rice. Proc. Crop. Sci. Soc. Japan, 27: 61-66.

Basten, C., B. S. Weirand, Z.B. Zeng. 2005. QTL cartographer. A reference manual and tutorial for

Brambilla, V. and F. Fornara. 2017. Y flowering? Regulation and acitivity of CONSTANS and CCT-

Chen, R., Y. Deng, Y. Ding, J. Guo, J. Qiu, B. Wang, C. Wang, Y. Xie, Z. Zhang, J. Chen, L. Chen, C. Chu, G. He, Z. He, X. Huang, Y. Xing, S. Yang, D. Xie, Y. Liu and J. Li. 2021. Rice functional genomics: Decades' efforts and roads ahead. Scci. China Life Sci 65:33-92.

Churchill, G. A. and R. W. Doerge.1994. Empirical threshold values for quantitative trait mapping. Genetics, 138(3): 963-971.

Doi, K., A. Yoshimura, M. Okamoto, T. Nagamine and H. Tsunematsu. 1998. Rice QTL analysis for days to heading under the cultivation condition of Ishigaki island. Breed. Sci. 48 (Suppl.1):73.

domain proteins in *Arabidopsis* and crop species. Biochim. Biophys. Acta. Gene Regul. 1860:655-660.

Gao, H., M. Jin, X. Xheng, J. Chen, D. Yuan, Y.Xin, M. Wang, D. Huang, Z. Zhang, K. Zhou, P. Sheng, J. Ma, W. Ma, H. Deng, L. Jiang, S. Liu, H. Wang, C. Wu, L. Yuan and J. Wan. 2014. Days to heading 7, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. Proc. Nati. Acad. Sci. 111 (46): 16337-16342.

GROUP 708, a histone H3 lysine 36-specific methyltransferase, controls flowering time in rice (*Oryza sativa*). New Phytologist. 210: 577–588.

Guo,L., Z. Zhang and J. Zhuang. 2013. Quantitative trait loci for heading date and their relationship with genetic control of yield traits in rice (*Oryza sativa*). Rice Sci. 20:1-12.

heading date of rice (*Oryza sativa* L.). J. Genet. Genomics 34: 608–615.

Hittalmani, S., H.E. Shashidhar, P.G. Bagali, N. Huang, J.S. Sidhu, V.P. Singh and G.S. Khush. 2002.

Hittalmani, S., N. Huang, B. Courtois, R. Venuprasad, H.E. Shashidhar, J.Y. Zhuang, K.L. Zheng, G.F. Liu, G.C. Wang and J.S. Sidhu. 2003. Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. Theor. Appl. Genet. 107: 679–690.

Hori, K., K. Matsubara and J.C.M Dekkers. 2016. Genetic control of flowering time in rice: integration of Mendelian genetics and genomics. Theor. Appl. Genet. 129:2241-2252.

Itoh, H., K.C. Wada, H. Sakai, K. Shibasaki, S. Fukuoka, J. Wu, J. Yonemaru, M. Yano and T. Izawa. 2018. Genomic adaptation of flowering-time genes during the expansion of rice cultivation area. Plant J. 94: 895–909.

Kojima, S., Y. Takahashi, Y. Kobayashi, L. Nonna, T. Sasaki, T. Araki and M. Yano. 2002. *Hd3a*, rice ortholog of the Arabidopsis FT gene, promotes transition to flowering down stream of *Hd1* under short day conditions. Plant Cell Physiol. 43(10):1096-1105.

Li, Z.K., S.B. Yu, H.R, Lafitte, N. Huang, B. Courtois, S. Hittalmani, C.H.M. Vijayakumar G.F. Liu, G.C. Wang and H.E. Shashidhar. 2003. QTL × environment interactions in rice. I. Heading date and plant height. Theor. Appl. Genet. 108: 141–153.

Liu, B., G. Wei, J.L. Shi, J. Jin, T .Shen, T. Ni, W.H. Shen, Y. Yu, A.W. Dong. 2016. SET DOMAIN

Liu, G., J. Yang, H. Xu and J. Zhu.200.) Influence of epistasis and QTL × environment interaction on

Matsubara. K., U. Yamanouchi, Z.X. Wang, Y. Minobe, T. Izawa and M. Yano. 2008. *Ehd2*, a Rice

Matsubayashi. M., R. Ito, T. Nomoto, T. Takase and N. Yamada (eds.). 1965. Ripening of rice plant. In Theory and Practice of Growing Rice. pp.139-149.

MOAI [Ministry of Agricultural and Irrigation]. 2014. Myanmar Agriculture in Brief. Ministry of Agriculture and Irrigation, Myanmar.

Molecular mapping of quantitative trait loci for plant growth, yield and yield related traits across three diverse locations in a doubled haploid rice population. Euphytica. 125: 207–214.

Morinaga, T., H. Kurlyama and H. Kudo. 1955. properties long grain rice varieties of Sri Lanka. *Res.* J. Chemic. Sci., 8: 29-35.

Nishida, H., Y. Okumoto, H. Nakagawa, K. Ichitani, H. Inoue and T. Tanisaka 2001. Analysis of tester lines for rice (*Oryza sativa* L.) heading-time genes using reciprocal photoperiodic transfer treatments. *Annals of Botany* 88, 527–536.

Ortholog of the Maize *INDETERMINATE1* Gene, Promote Flowering by Up-Regulating *Ehd1*Plant Physiol.148(3): 1425–1435.

Platten, J.D., J.N. Cobb and R.E. Zantua.2019. Criteria for evaluating molecular markers: comprehensive quality metrics to improve marker assisted selection. PloS ONE. 14:e0210529.

Putterill, J., F. Robson, K. Lee, R. Simon and G. Coupland. 1995. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell. 80: 847–857.

QTL mapping (Raleigh, N. C. Department of Statistics, North Carolina State University).

Septiningsih, E.M., J. Prasetiyono, E. Lubis, T.H. Tai, T. Tjubaryat, S. Moeljopawiro and S.R. McCouch. 2003. Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor. Appl. Genet. 107: 1419–1432.

Shibaya, T., K. Hori, E. Ogiso-Tanaka, U. Yamanouchi, K. Shu, N. Kitazawa, A. Shomura, T. Ando, K. Ebana and J.Wu.2016. *Hd18*, encoding histone acetylase related to Arabidopsis FLOWERING LOCUS D, is involved in the control of flowering time in rice. Plant Cell Physiol. 57: 1828–1838.

Song, Y.L, Z.C. Gao and W.J. Luan. 2012. Interaction between temperature and photoperiod in regulation of flowering time in rice. Life Sciences. 3:24- 249.

Takahashi, Y., and K. Shimamoto. 2011. Heading date 1 (*Hd1*), an ortholog of Arabidopsis CONSTANS, is a possible target of human selection during domestication to diversify flowering times of cultivated rice. Genes & Genetic Systems 86: 175–182.

Thein, M.S. 2011. Marginal land vs productive land: A case study on cultivating Myanmar local quality rice (*Oryza sativa* L.) morphotypes. Phetchaburi, Thailand: P148.LDD.

Umehara and Y. Nagamura et al. 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. The Plant Cell 12: 2473–2484.

Velasco, J. R. and F.R. K. Dela. 1958. The response of forty six rice varieties to photoperiod. Philip. Agric. 42: 12-17.

Vergara, B.S., S. Puranabhavung and R.Lilis. 1966. Studies on the response of the rice plant to photoperiod. I. Flowering response, insensitive phase and photoinductive cycles needed for flowering. Philipp. Agric. 50: 1-8.

Wei, F.J., Y.C. Tsai, H.P. Wu, L.T. Huang, Y.C. Chen, Y.F. Chen, C.C. Wu, Y.T. Tseng and Y.C. Hsing. 2016. Both *Hd1* and *Ehd1* are important for artificial selection of flowering time in cultivated rice. Plant Sci. 242: 187–194.

Win, U.K. 1991. A century of rice improvement in Burma. International Rice Research Institute, Manila, ISBN: 971-22-0024-8, Pages: 162.

Xue, W., Y. Xing, X. Weng, Y. Zhao, W. Tang, L. Wang, H. Zhou, S. Yu, C. X, X. Li and Q. Zhang. 2008. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. Nat. Genet. 40:761-767.

Yano, M., K.Shoko, T. Yuji, L. Hongxuan and S.Takuji .2001. Genetic control of flowering time in rice, a short-day plant. Plant Physiol 127:1425-1429

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y.

Zhou, Z.L., L.Jiang, K. Liu, X. Da- yong, Z. hu- qu, and W. Jian- min. 2011. Crop genetics and germplasm enhancement, Nanjing agricultural university, Nanjing , Rice Science , 18(4): 287-296.