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# DNA Methylation Differences in the Genes Responded to Photoperiod in Rice Cultivar KDML 105

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## ABSTRACT

Amplified Fragment Length Polymorphism (AFLP) technique was adapted using *Eco*RI and *Hpa*II restriction enzymes for detection of the gene responded to photoperiod by DNA methylation in rice cultivar KDML 105. Two primer combinations, HM-G/E-AAG and HM-G/E-AGG, could generate two AFLP markers from the genomic DNA of rice cultivar KDML 105 after exposure to short day (SD) photoperiod (11 hour per day). Two AFLP markers were PG-AG and PG-GG. The PG-AG marker was found in the rice sample at 15 days after exposure to SD photoperiod and the PG-GG marker was found in those of 9,10,12 and 15 days after exposure to SD photoperiod. Both AFLP markers were cloned and subsequently sequenced. DNA sequences of PG-AG and PG-GG markers showed 98-100% similarity to the sequences of rice genomic DNA, cDNA and mRNA in the database. This indicated that PG-AG and PG-GG markers could be a part of the expressed genes and their expression might be induced or inhibited by DNA methylation mechanism under short daylength condition. The PG-AG marker is also suspected to be the gene involves in the process of flower development in rice while PG-GG marker may attribute to the process of flower induction.

**Key words:** DNA methylation, photoperiod, rice, KDML 105, AFLP

## INTRODUCTION

Rice (*Oryza sativa* L.) is a main cereal for human consumption for centuries. There are three subspecies *japonica*, *indica* and *javanica*. Thai jasmine rice cultivar (Khao Dok Mali 105, KDML 105), an *indica*, is highly demanded due to its pleasant aroma, soft and tender texture after cooked. However, not only its yield per rai is quite low it is also a photoperiod sensitive cultivar. Therefore, the growth period is limited to only one season a year in the short day (SD) photoperiod. Sirigayon (2000) reported that the minimum induction cycle of 9 days (exposure to SD) is needed for KDML105 to change from vegetative to reproductive phase.

Genes controlling the flowering time became the target for rice development among rice researchers (Yano *et al.*, 2001; Hayama and Coupland, 2004). Flowering time is controlled by at least four pathways in *Arabidopsis* (Levy and Dean, 1998; Pineiro and Coupland,1998). Two of these pathways appear to monitor the endogenous developmental stages of the plant and the other two pathways mediate signals from the environment i.e., the photoperiodic promotion

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pathway and the vernalization promotion pathway. DNA methylation involves in the promotion of flowering by vernalization as reported in *Arabidopsis* (Finnegan *et al*., 1998; Sheldon *et al*., 1999; Genger *et al*., 2003) and in wheat (Horváth *et al*., 2002). DNA methylation was also known to have a control mechanism over genes expression and plant development (Finnegan *et al*.,2000) and a cause of transgene silencing in transgenic plant (Klöti *et al*., 2002). Finnegan *et al*. (2000) indicated that DNA methylation plays a role in the gene expression during flower development. It is, therefore, of interest to see whether DNA methylation of some genes occur or change during transition from vegetative to reproductive phase in KDML 105 and how they are controlled by short daylength. This will enable us to use the information for KDML 105 improvement and better management of rice production.

## MATERIALS AND METHODS

### Plant materials and photoperiod control

A photoperiod sensitive rice cultivar KDML 105 and a photoperiod insensitive rice cultivar Suphan Buri 1 (SP1) were grown under long day (LD) photoperiod (13 hour of light per day) for 55 days. A half of each cultivar was then transferred to grow under short day (SD) photoperiod (11 hour of light per day) for 15 days.

### DNA extraction

Genomic DNA was extracted from the youngest leaves of both KDML 105 and SP 1 on days 0, 5, 7, 8, 10 and 15 for control plants (LD) and days 0, 5, 6, 7, 8, 9, 10, 12 and 15 for SD-exposed plants. DNA extraction method was modified from that of Agrawal *et al*. (1992).

### Determination of DNA methylation by AFLP

AFLP technique was modified from the methods described by Peyachoknagul (2002) and Xiong *et al*.(1999). DNA was digested with *Eco*RI and *Hpa*II. The latter is a methylation sensitive restriction enzyme. Digested DNA fragments were ligated with adapters and pre-selective amplified with E+1 primer (E-A) and HM+1 primer (HM-G). The PCR reaction was performed for 20 cycles having 30s of denaturation at 94°C, 1 min annealing at 56°C, and 1 min extension at 72°C. The pre-selective product from pre-amplification was diluted twenty folds with TE buffer and further used as template DNA for selective amplification. Selective amplification was done using E+3 primer (E-AAC, E-AAG, E-ACA, E-ACT, E-AGC and E-AGG) and HM+1 primer (HM-G). The PCR products were separated on 6% denaturing polyacrylamide gel electrophoresis and detected by silver staining. DNA fingerprinting patterns of SD and LD exposed rice were compared and the DNA fragments only present or absent in SD condition were cloned and sequenced for further analysis.

### Cloning and sequencing of DNA fragments

The DNA bands showing their differences in SD and LD exposed plants were cut from the polyacrylamide gel and cloned into pGEM®T plasmid using the method described by the manufacturer (Promega, USA). They were then sequenced and compared with the database in the Genbank (http://www.ncbi.nlm.nih.gov).

## RESULTS AND DISCUSSION

### Differences in AFLP markers of SD and LD exposed plants

AFLP fingerprints of LD and SD rice were developed using six primer combinations (HM-G/E-AAC, HM-G/E-AAG, HM-G/E-ACA, HM-G/E-ACT, HM-G/E-AGC, and HM-G/E-AGG) as have been reported to give the different bands among SD (8 hours of light per days) and LD exposed rice samples (Thananantа,2004). Although Thananantа (2004) has found 12 markers from the genomic DNA of KDML 105 in the SD samples using methylation-sensitive amplified polymorphism (MSAP) technique, those markers seemed to be different from the markers found in this work. The possible cause of this different result might be the difference in growth condition and maturity of the rice plant at the day of treatment. However, in this experiment other two new markers could be found. Two distinct AFLP bands indicating the difference between SD and LD treated rice KDML105 were PG-AG of 197 bp from the primers HM-G and E-AAG (Figure 1), and PG-GG of 102 bp from HM-G and E-AGG primers (Figure 2). The band differences of PG-AG was found on the day 15 after the exposure of KDML 105 to the short daylength which corresponded to the change in gene expression from vegetative phase to flowering phase. As for PG-GG band, the differences were found on days 9, 10, 12 and 15 after SD exposure. This result agreed with that of Sirigayon (2000) on the

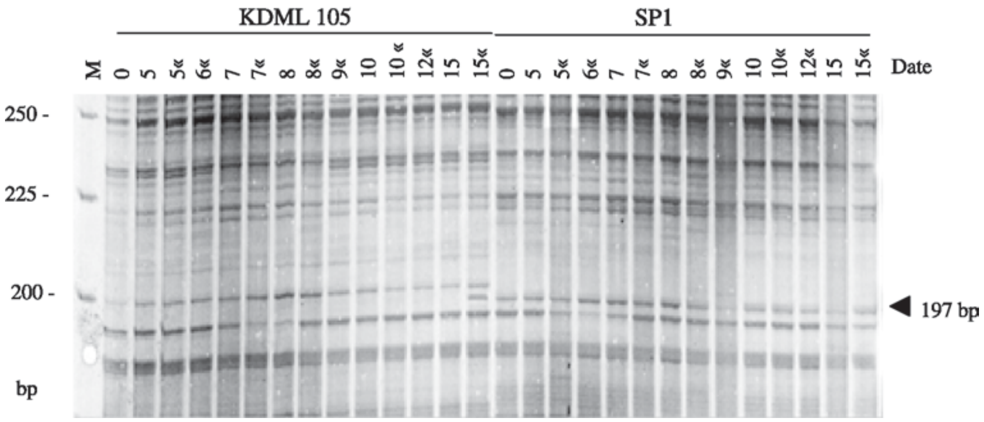

**Figure 1** AFLP patterns of KDML105 and SP1 showing the PG-AG band of 197 bp from primers HM-G and E- AAG (M= standard DNA of 25 bp DNA ladder, <<= DNA of SD exposed plants).

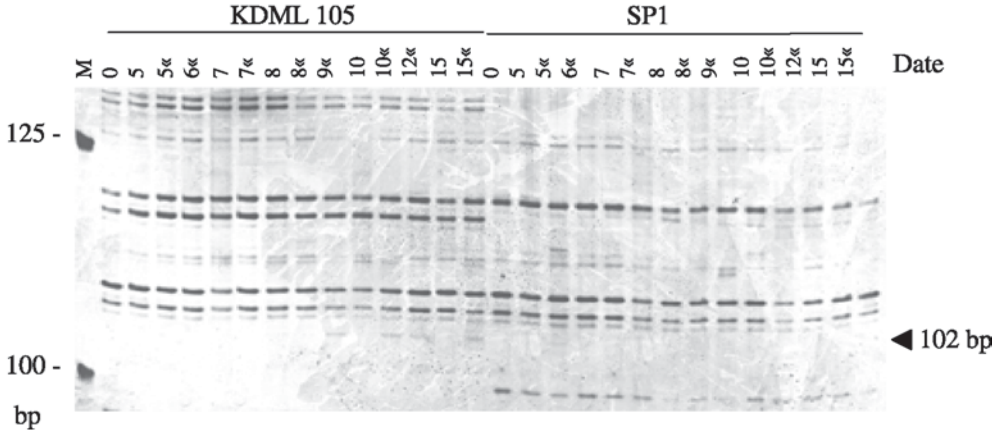

**Figure 2** AFLP patterns of KDML105 and SP1 showing the PG-GG band of 102 bp from primers HM-G and E- AGG (M= standard DNA of 25 bp DNA ladder, <<= DNA of SD exposed plants).

flowering induction of KDML 105 after 9 days of continuous short daylength exposure. In case of SP1, PG-AG band was present in all exposed days both in SD and LD conditions while PG-GG band was not found in any samples indicated that short day photoperiod did not cause DNA methylation change in SP1.

It is clearly seen from this work that the differences in PG-AG and PG-GG bands were the results of DNA methylation changes in KDML105 in response to the short daylength period and directly related to the flowering as well. These DNA methylation changes could act as a control mechanism on the gene expression as also reported by several groups of researchers (Bird,1978; Kl?ti *et al*.,2002; Lauria *et al*.,2004).

### Cloning and sequencing of DNA bands

AFLP markers of PG-AG and PG-GG were cloned and sequenced (Table 1). The base sequences of PG-AG and PG-GG were found to be 98% and 100% similar to the cDNA sequences in the database of Genbank, respectively (Table 2), which confirmed the existence of these DNA in the genomic composition of KDML105. Since DNA methylation is known to be a control mechanism on the gene expression involving flower induction in *Arabidopsis* (Finnegan *et al*.,1998; Sheldon *et al*.,1999; Genger *et al*.,2003), it is therefore reasonable to assume its role in flower induction of rice as well. This result is one of the first few attempts to report the association of DNA methylation mechanism with the short day photoperiod response of rice.

The close similarity of PG-AG and PG-GG markers to the mRNA and cDNA as reported in the database of Genbank also elucidated them as a part of the expressed genes in rice of photoperiod sensitive cultivar since they were found to show the differences only in KDML105 but not in SP 1 (photoperiod insensitive cultivar). Further study on the expression of these genes would enable us to know whether PG-AG and PG-GG markers were induced or inhibited by DNA methylation mechanism and thereby related to any level of flowering process of KDML105.

## CONC LUSION

After having been exposed to either short daylength (11 hour/day) or long daylength (13 hour/day), DNA of both rice cultivars, KDML 105 (photoperiod sensitive rice) and Suphan Buri 1 (photoperiod insensitive rice) were digested with restriction enzymes *Eco*RI and *Hpa*II, and their AFLP patterns were compared. There were two distinctively different bands of PG-AG and PG-GG found in the short daylength exposure of

**Table 1** Base sequence of AFLP markers responded to short daylength which related to DNA methylation mechanism in KDML 105.

| AFLP markers | Fragment size (bp) | Base sequences (5′ → 3′) |
|---|---|---|
| PG-AG | 167 | CCGGACAATCAGAGGCAGCTTGTTTATGTACATGAACAGCCTTCTGAGTTCTCTCTTGACACTGTCTCCATGTCAACCACATCTTGGTCCTCAACATAGATCCATAAGTCANCCAGGGTCAGTCTTCTTCAAGTTGTCACGGCAGAATGGACAGGACTGTGAATTC |
| PG-GG | 72 | CCGGGTTAGTCAGGATCTGGAAGGTCGGTTCGGGCTCAGGGGCTTTCTTCTCCGCCACACCATCGAGAATTC |

**Table 2** Comparing the degree of sequences similarity between AFLP markers and those of GenBank database.

| AFLP marker | Fragment size (bp) | Similarity of AFLP markers to sequences in the GenBank database |
|---|---|---|
| PG-AG | 167 | *O. sativa indica* genomic DNA, chromosome 10: gb\|AAAA02030250.1 (98%)<br>*O. sativa japonica* genomic DNA, chromosome 10: gi\|31432860 (98%)<br>*O.sativa japonica* mRNA: gi\|37535757 (98%)<br>*O. sativa japonica* cDNA: gi\|32995978 (98%) |
| PG-GG | 72 | *O. sativa indica* genomic DNA, chromosome 8: gb\|AAAA02023622.1 (100%)<br>*O. sativa japonica* genomic DNA, chromosome 8: gi\|38637170 (100%)<br>*O.sativa japonica* mRNA: gi\|17298152 (100%) and gi\|50942476 (100%)<br>*O. sativa japonica* cDNA: gi\|32970968 (100%) and gi\|37991377 (100%) |

KDML 105. These two bands had 98% and 100% similarity to the sequences of cDNA and mRNA as indicated in the database of Genbank and it was therefore proven to be a part of the expressed genes in KDML105. The possibility of the DNA methylation mechanism to induce or inhibit some genes expression under short daylength is high. PG-AG is suspected to involve in flower development of rice while PG-GG might have some induction effect on flowering.

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