การศึกษาความแตกต่างระหว่างข้าวพันธุ์กลายไม่ไวต่อช่วงแสงกับพันธุ์เดิมด้วยเทคนิคทางดีเอ็นเอ

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บทคัดย่อ

ข้าวขาวดอกมะลิ 105 พันธุ์กลายไม่ไวแสงสามารถให้ผลผลิตได้ทั้งในและนอกฤดูปลูกปกติ ได้ศึกษาการถ่ายทอดลักษณะกลายนี้โดยใช้วิธีทางพันธุศาสตร์ พบว่าเป็นลักษณะด้อย และได้ใช้เทคนิคการทำลายพิมพ์ดีเอ็นเอเปรียบเทียบการแสดงออกของยีนในใบที่ได้จากข้าวพันธุ์กลายสามสายพันธุ์และขาวดอกมะลิ 105 พบว่ามีความแตกต่างกันในระดับการแสดงออกของยีนในหลายตำแหน่ง แต่การตรวจสอบส่วนสำคัญของยีนความหอมในพันธุ์กลายไม่แสดงความแตกต่างจากพันธุ์ขาวดอกมะลิ 105 เดิม

คำสำคัญ: ขาวดอกมะลิ 105 พันธุ์กลายไม่ไวต่อช่วงแสง ลายพิมพ์ดีเอ็นเอ รังสีนิวตรอนเร็ว

Studies of Genetic Differences between KDML 105 and its Photoperiod-insensitive Mutants using DNA techniques

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Abstract

Photoperiod-insensitive mutants of KDML 105 could be planted for grains during and outside the regular cropping season. From genetic studies, the mutant characteristics appeared recessive. A DNA-fingerprinting technique was used to compare gene expression profiles in the leaves of mutants and KDML 105. Differences in the level of expression were found for several loci. Examination of the essential part of the gene for fragrance showed no differences between the mutants and the parental KDML 105.

Keywords: KDML 105, photoperiod-insensitive mutant, DNA fingerprint, fast neutron radiation

Introduction

Khao Dawk Mali 105 (KDML 105) is a fragrant variety of rice, which is widely popular in Thailand as well as in foreign countries. However, it is a short-day plant, meaning that it would only flower when daytime is shorter than nighttime. As a result, it can only be cultivated once a year, being planted in the rainy season to be harvested in mid-November.

Starting in 1996, the Office of Atoms for Peace together with the Department of Agriculture used fast neutron irradiation to induce mutations in KDML 105. Several photoperiod insensitive mutant lines were obtained that could flower in every month of the year.[5] Further studies were conducted to reveal genetic mechanisms underlying the mutations in these lines. Here, we report a genetic complementation study on non-photoperiodic mutant no. 4, a cDNA-AFLP differential display and an analysis of *BADH2* allele of mutants no. 1, 4 and 16 in comparison with KDML 105.

Methods

1. Plant materials and growing conditions

KDML 105 and non-photoperiodic mutant lines no. 1, 4 and 16 were seeded in pots on 14 December 2005. Plants were exposed to natural day light. Leaves were collected for RNA on 22 February 2006 and immediately frozen in liquid nitrogen. The plants were in their vegetative phase at the time of leaf collection.

2. cDNA synthesis and fingerprinting

Total RNAs were extracted from leaf samples using RNeasy Plant Mini Kit (QIAGEN Sciences, Maryland, USA), according to the manufacturer's protocol, and treated with DNaseI prior to cDNA synthesis. First-strand cDNA was synthesized from 3.5 μg total RNA using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas International Inc., Canada). Second-strand cDNA was synthesized with 1U Ribonuclease H and 30U *Escherichia coli* DNA Polymerase I. cDNA was precipitated with isopropanol and resuspended in water. For cDNA-AFLP, the obtained cDNA was digested sequentially with 5U *Taq*I and 10U VspI. The digested cDNA was then ligated to 5pmol *Ase*I adaptor and 50pmol *Taq*I adaptor. Pre-selective amplification of cDNA was conducted in the presence of 2 μM *Ase*I primer, 2 μM *Taq*I primer

with 30 cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 1 min. The selective amplification was conducted with *Ase*I and *Taq*I primers having two additional nucleotides at their 3' end. The PCR condition included 11 touchdown PCR cycles of 30 sec at 94°C, 30 sec at 65°C (reducing the temperature by 0.7°C per cycle), and 1 min at 72°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 56°C and 1 min at 72°C. The sequences for *Ase*I adaptor, *Taq*I adaptor, *Ase*I primer and *Taq*I primer were as in Bachem *et al*. (1998).[1]

3. Analysis of the fragrance gene, *BADH2*

DNA was extracted from leaves as described by Dellaporta, *et al*. (1983).[3] The initial PCR analysis of the *BADH2* gene was conducted by the laboratory of Dr. Apichart Vanavichitr, Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency. The experiment was repeated with additional non-fragrant rice controls using primers 5'-TGC TCC TTT GTC ATC ACA CC-3' and 5'-TCC ACA GAA ATT TGG AAA CAA-3', which were designed based on the mutation reported by Bradbury, et al. (2005).[2]

Results and Discussion

1. Inheritance of the photoperiod-insensitive mutant phenotype

To study the inheritance of photoperiod-insensitive phenotype, mutant no. 4 was crossed to Suphanburi 1 (SPR1), which is also photoperiod insensitive. F1s from the cross and their parents were seeded in April and their inflorescence dates were compared. Both mutant no. 4 and SPR1 first flowered in July, when photoperiod sensitive rice varieties do not normally flower. On the other hand, their F1 progeny of did not flower until late September, when the photoperiod was suitable for the sensitive varieties including KDML105 and Hangyi 71 (Table 1). Since the F1 plants behaved similarly to the photoperiod sensitive varieties, the photoperiod-insensitive trait of both mutant no.4 and SPR1 could be regarded as a recessive trait. Also, it can be concluded that mutant no.4 and SPR1 could genetically complement each other regarding photoperiod sensitivity. Therefore, the mutation that caused photoperiod insensitivity in mutant no.4 would not be located in the gene responsible for this trait in SPR1.

Table 1. Flowering dates of various rice varieties and the mutant line, planted on 1 April 2006.

Plant line	Flowering date
Mutant no.4	14 July 2006
SPR1	2 July 2006
Mutant no.4 x SPR1	20 September 2006
KDML105	12 October 2006
Hangyi71	1 October 2006

2. Comparison of gene expression profile between photoperiod-insensitive mutants and KDML 105

To understand the genetic mechanism of photoperiodic control of flowering in KDML105, we compared the cDNA-AFLP fingerprinting profiles that were obtained from KDML105 and three photoperiod-insensitive mutant lines, no.1, no.4 and no.16. The experiment was repeated twice. 12 cDNA bands were identified that exhibited differences in their intensity between KDML105 and the mutants (Fig. 1). The observed differences indicated that a set of genes exhibited differential expression between the mutants and KDML105. Ten cDNA fragments were found to show up regulations in their expression, while two fragments showed down regulations (Table 2). Similar experiment was conducted by Hayama, et al. (2002),[4] to identify differentially regulated genes between Norin 8 and its photoperiod insensitive *se5* mutant. In their experiment, they identified nine genes with diurnal expression, which were up-regulated in the mutant, and two genes that were down-regulated in the mutant. Genes that showed an alteration in their expression in our experiment might possibly be involved in the mechanism of photoperiodic response in rice.

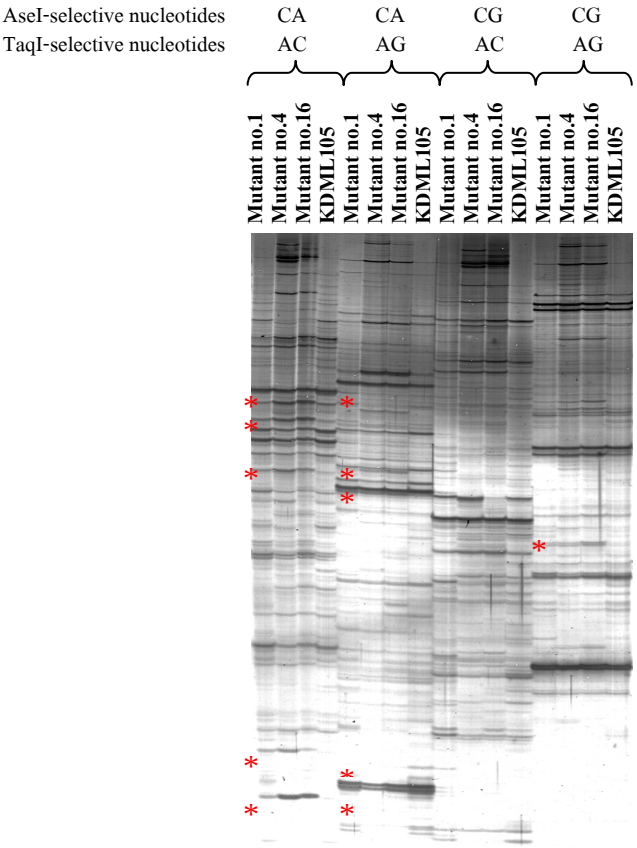

Fig. 1. cDNA-AFLP fingerprints of photoperiod insensitive mutants and KDML105. cDNA bands showing differential intensity are marked with asterisks.

Table 2. Size of cDNA fragments that exhibited differential intensity between the mutants and KDML105

Regulation	Primer pair	Fragment size (bp)
Up regulation	AseI-CA/TaqI-AC	310, 300, 290, 250, 130, 120
	AseI-CA/TaqI-AG	300, 250, 230
	AseI-CG/TaqI-AG	200
Down regulation	AseI-CA/TaqI-AG	125, 115

3. Non-photoperiodic mutants retained the mutation in *BADH2* gene

Non-photoperiodic mutants no. 1, 4 and 16 were tested for the presence of a mutation in *BADH2* gene, encoding betaine aldehyde dehydrogenase-like protein, which was essential for the production of characteristic fragrance of KDML 105.[2] PCR amplification of *BADH2* alleles showed that the three mutants had an allele of the same size as KDML 105 (Fig. 2), indicating that they had inherited the same mutation in *BADH2* as KDML 105. On the other hand, Hangyi 71 and Suphanburi 1, which are non-fragrant varieties of rice, carried an allele with a larger size (Fig. 2), presumably without the mutation in *BADH2* gene. The lack of the functional allele of *BADH2* indicated that, when planted in appropriate environment, the three mutants should produce fragrance that is characteristic of KDML 105.

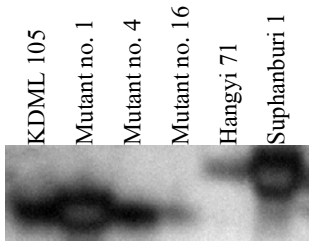

Fig. 2. PCR analysis of *BADH2* alleles of KDML 105, photoperiod-insensitive mutants no. 1, 4 and 16, and non-fragrant Hangyi 71 and Suphanburi 1.

Conclusion

Genetic studies revealed that the photoperiod insensitive trait in mutant no.4 was recessive and was not caused by a mutation in the same gene as in Suphanburi 1. Comparison of the gene expression profile between the mutants and KDML 105 revealed 10 potentially up-regulated cDNA products and 2 potentially down-regulated cDNA products. PCR analysis of *BADH2*, the fragrance gene, revealed that mutants no. 1, 4 and 16 retained the KDML 105 allele of this gene. Therefore, these mutants should be able to produce fragrance similarly to KDML 105 when grown under appropriate conditions.

Acknowledgement

The authors wish to thank Miss Sirilak Chookaew and Miss Walayaporn Tongpun for technical support.

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