Genetic variation analysis of longan using primers designed from nucleotide sequence data of genes responding to flowering induction by potassium chlorate

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Abstrack

Longan is an important economic fruit crop in Thailand. Flowering and fruit setting are crucial factors affecting longan yield. Previous studies comparing differential gene expression between RNA samples extracted from control and potassium chlorate-induced flowering longan samples at different time points used Next Generation Sequencing (NGS) technology to generate nucleotide sequence data of genes associated with the longan flowering pathway. These sequence data can be applied to various aspects of longan research, particularly in developing molecular markers associated with specific traits or studying the genetic diversity of different longan cultivars in Thailand. In this study, four primers consisted of *NAD(P)H dehydrogenase (NADPH)*, *Myb transcription factor (MYB)*, *Cytochrome P450 (P450)*, and *UDP-glucosyltransferase (UGT)* were used to analyze PCR product patterns in DNA samples extracted from 32 different longan cultivars. All DNA samples were tested for their suitability as templates for Polymerase Chain Reaction (PCR) using an actin primer. The experimental results revealed that the UGT and P450 primers produced different PCR bands among longan samples, ranging from 1,000 to 1,500 base pairs and 1,500 to 2,000 base pairs, respectively. However, to improve clarity, further experiments should be conducted using agarose gel electrophoresis with a higher agarose concentration or polyacrylamide gel electrophoresis, which offers higher resolution for DNA band separation. The results from these analyses could be applied to develop UGT and P450 primers as molecular markers for identifying longan cultivars in the future.

Introduction

Longan (Dimocarpus longan) is an economically significant fruit crop. The flowering of longan is a crucial factor in longan production [1]. A previous study reviewed three different flowering behavior of Thai longan: seasonal flowering, natural independent flowering, and continuous flowering [2]. Molecular genetic studies on the gene(s) involved in longan flowering induction will provide valuable information that can be applied to longan breeding programs, particularly for developing new longan cultivars capable of flowering off-season or year-round. The cultivar "E-Daw" is the most popular longan cultivar grown in Thailand. It required dry and cold conditions for seasonal flowering induction, whereas off-season flowering can be artificiallyinducted using potassium chlorate ($KClO_3$) [3]. A study based on next-generation sequencing technology (RNA sequencing) has identified differentially expressed genes between control and $KClO_3$-treated longan RNA samples [2]. The sequences of these genes can be further studied to develop molecular markers, which can be used for trait-linked markers development and longan genetic diversity studies. Therefore, the aim of this study is to use the set of primers designed from RNA sequencing data to identify genetic diversity among 32 Thai longan cultivars. This may lead to the development of new molecular markers associated with flowering behavior, traits, or specific cultivars, which could beneficial for longan breeding programs in the future.

Materials and Methods

- **DNA concentration and quality checking**

Nanodrop spectrophotometer → Actin PCR → Gel electrophoresis
- gDNA
- Actin PCR product

1. PCR amplification

DNA Extraction (from senior)

	Conc.	Final conc.	Volumn
1.) 5X PCR Master Mix (ExcelTaq)	5x	1x	2
2.) Forward primer	10 mM		0.5
3.) Reverse primer	10 mM		0.5
4.) DW water			5
5.) DNA templates	10 (ng/ μl)		2
Total			10

	Temperature (°C)	Time (sec)	Cycle
1.) Initial Denaturation	95	120	1
2.) Denaturation	95	30	
3.) Annealing	Depends on primers	30	39
4.) Extension	72	60 – 120 (Depends on PCR product size)	
5.) Final extension	72	60	1

4 pairs of Primers : **UGT, MYB, P450, NADPH** (from seniors)

- Identified the suitable PCR condition of each primer (annealing temperature) using gDNA of 3 cultivars: E-Daw (D1), Thoa (D6) and Phetsakorn (D8)
- Amplified the PCR product from remaining 29 longan gDNA samples.
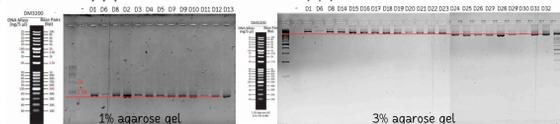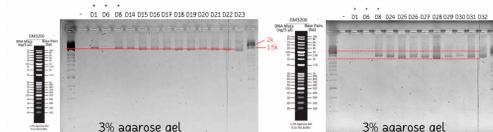
2. Agarose Gel Electrophoresis

Gel electrophoresis → Gel documentation

3. Data analysis

Results

UGT

- 45 minutes

1% agarose gel   3% agarose gel

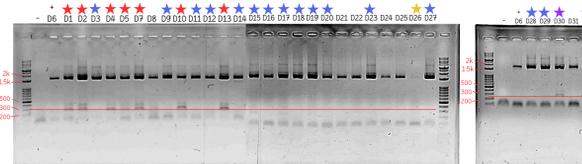- 90 minutes

3% agarose gel   3% agarose gel

Result
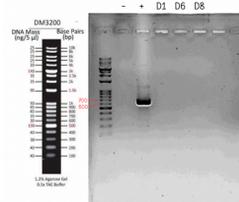- No polymorphism was detected among the 32 longan samples

P450

Result
- Polymorphism was detected among the 32 longan samples
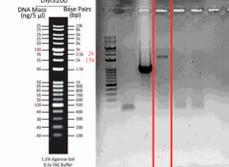
NADPH

Result
- No PCR product was amplified from the genomic DNA samples, possibly due to the presence of an intron

MYB

Annealing temperature testing

Result
- The PCR reaction conditions need to be optimized to generate a more specific band of the PCR product.

Conclusion

Three out of four primer pairs (UGT, P450, and NADPH) successfully amplified the PCR product. Among these, the P450 and UGT primers demonstrated polymorphism among 32 longan cultivars. However, to clarify the results, higher concentrations of agarose gel electrophoresis or polyacrylamide gel electrophoresis, might be used to detect polymorphism, offering higher resolution for DNA band separation.

Acknowledgement

References