CLONING OF THE ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR 2-LIKE GENE FROM THAI LONGAN VARIETIES WITH DIFFERENT FLOWERING BEHAVIORS

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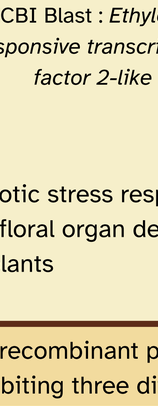
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ABSTRACT

Longan (*Dimocarpus longan*) is an economically important fruit crop in Thailand. Therefore, investigating genes involved in flowering regulation represents a promising approach to enhance longan production potential. The aim of this study was to construct the recombinant DNA to examine the effects of overexpression of an *ethylene-responsive transcription factor 2-like* (*ERT*) gene in a model plant, as this gene is likely involved in regulating longan flowering. The *ERT* gene was amplified from two longan cultivars exhibiting distinct flowering behaviors: the natural-independent flowering ("Thao") and continuous flowering ("Phetsakorn"). The amplified *ERT* fragments were ligated into the two types of plasmids, pBlueScript II SK (+) and pGFPGUSPlus vector (Addgene #64401) for sequence verification and overexpression construct development for gene transfer into a model plant system, respectively. The result demonstrated the successful construction of *ERT* genes inserts in pBlueScript II SK (+) plasmid, comprising three and two positive clones from 'Thao' and 'Phetsakorn', respectively. All clones derived from the 'PhetSakhon' cultivar exhibited a nucleotide substitution resulting in an amino acid change from aspartic acid to cysteine (Asp11Cys) in both clones. In addition, one positive *ERT* clone from 'Phetsakorn' cultivar was successfully subcloned into the pGFPGUSPlus overexpression vector and submitted for nucleotide sequencing. The recombinant DNA constructs generated in this study will be further used to investigate the role of *ERT* in regulating flowering behavior in a model plant system. The findings provide essential baseline information for advancing genetic knowledge of longan and supporting future breeding efforts to improve longan production efficiency.

INTRODUCTION

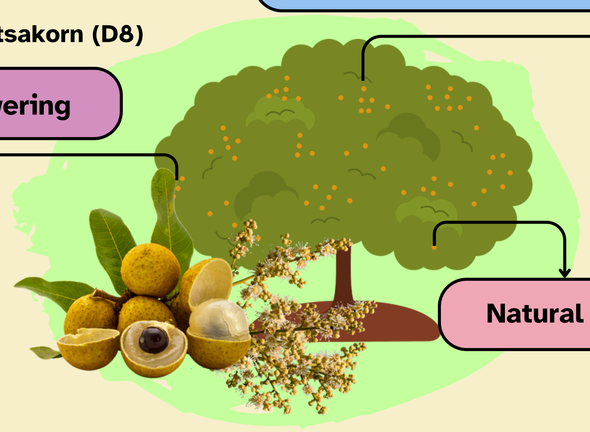


- Depend on environmental conditions, especially low temperature
- Example cultivar: **E-Daw**

Seasonal or Natural Dependent Flowering

- Year-round
- Not synchronized among individual shoots
- Example cultivar: **Phetsakorn (D8)**

Continuous Flowering



- 1-2 times/year
- Synchronized the entire tree
- Example cultivar: **Thoa (D6)**

Natural Independent Flowering

Cluster-495.35092 → ***ERT*** → NCBI Blast : *Ethylene-responsive transcription factor 2-like* → **Expression Non-flowering bud** → **Act as Inhibitor**

AP2/ERF superfamily

- Plant growth
- Biotic and abiotic stress responses
- Regulation of floral organ development
- Flowering in plants

→ **Studies on Thai longan cultivars, particularly comparative analyses among the three groups, remain limited.**

OBJECTIVE

To construct recombinant plasmids carrying the *ERT* gene from Thai longan cultivars exhibiting three different flowering behaviors.

METHODOLOGY

1 Gene preparation

cDNA → Design Primer DLERT_35092 F / DLERT_35092 R → **D6** / **D8** → PCR → 1% Agarose Gel Electrophoresis

2 Plasmid preparation



LB agar & LB broth + Ampicillin

E. *coli* DH5α that contains a plasmid



Plasmid Purification Kit

OD_{260}/OD_{280} (ng/µl)

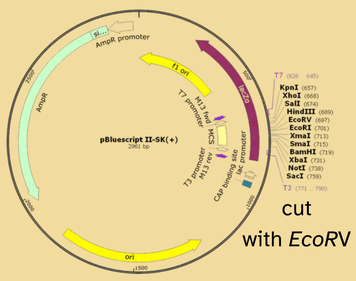


Gel Purification Kit

Gel Purification & OD measurement

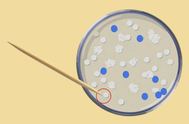
Pfu + mgFPDLERT_35092 modify the gene

3 Ligation & Transformation in pBlue



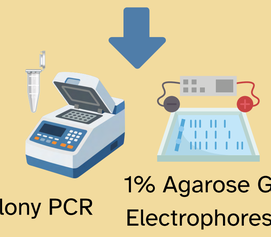
cut with *EcoR*V

Transformation (Heat Shock) E. *coli* DH5α



LB agar+ Ampicillin+ IPTG+X-gal

blue-white colony selection

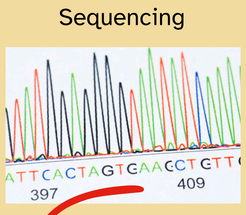


Colony PCR

1% Agarose Gel Electrophoresis

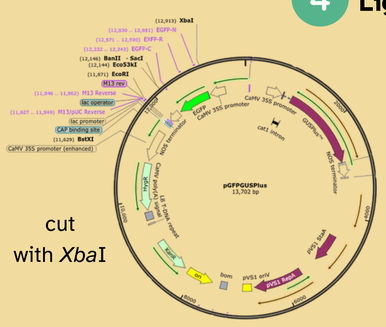


Cut Check with *BamH*I & *Hind*III



cut *ERT* gene from the pBlue plasmid with *Xba*I

4 Ligation & Transformation in GUSPlus



cut with *Xba*I

Do the same 3 as in pBlue

But some are different

- Change Ampicillin to Kanamycin
- Cut Check with *EcoR*I & *Xho*I

MAIN RESULTS

1. *ERT* gene fragment preparation

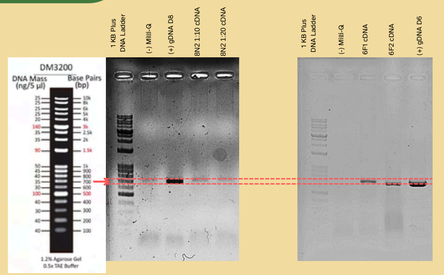


Fig. 1 D8 & D6 DLERT_35092 HOT FIREPol® PCR

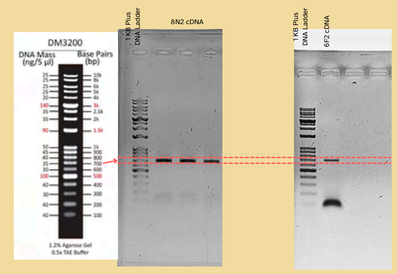


Fig. 2 D8 & D6 DLERT_35092 Pfu PCR

2. Multiple Sequence Alignment

D8 **Selected Clone A4 for the downstream experiment**

Fig. 3A All clones of D8 Multiple DNA Alignment in pBluescript II SK (+) vector by Clustal Omega





Fig. 3B Three clones of D8 Multiple DNA Alignment in pBluescript II SK(+) vector by Clustal Omega

Base No. 1087 Clone A3 **SNP: G to C**

Base No. 1120-1121 Clone A3 **2 nucleotide substitutions: CT to AC**

Base No. 1321 Clone A3 & 4 **SNP: G to T**

Base No. 1374 Clone A3 & 4 **SNP: A to G**

Asp11Cys Missense Mutation

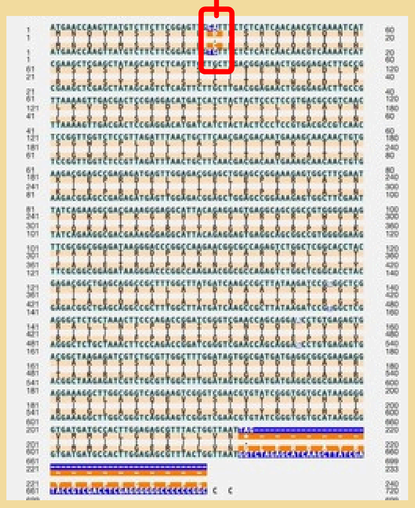


Fig. 3C Protein Alignment of D8 Ckone A4 in pBluescript II SK (+) vector by Clustal Omega

D6 **Selected Clone A2 for the downstream experiment**

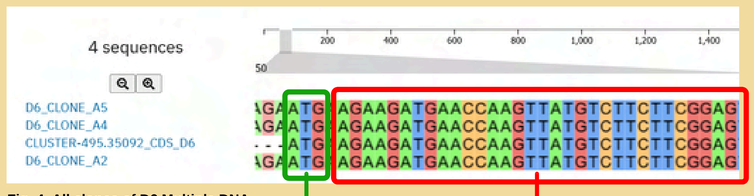


Fig. 4 All clones of D6 Multiple DNA Alignment in pBluescript II SK (+) vector by Clustal Omega

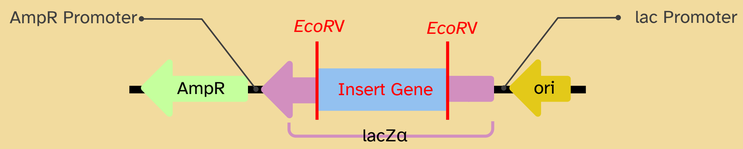
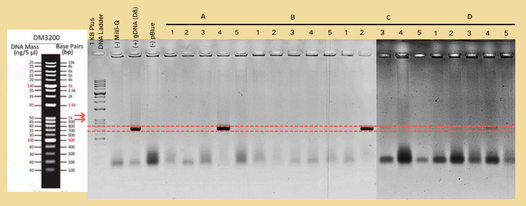


Fig. 5 pBluescript II SK (+) vector model structrue with insert gene (D6 or D8)

3. Cloning of the *ERT* gene into the pGFPGUSPlus plasmid



Fig,6 D8 DLERT_35092 in pGFPGUSPlus

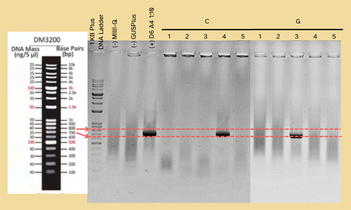


Fig.7 D6 DLERT_35092 in pGFPGUSPlus

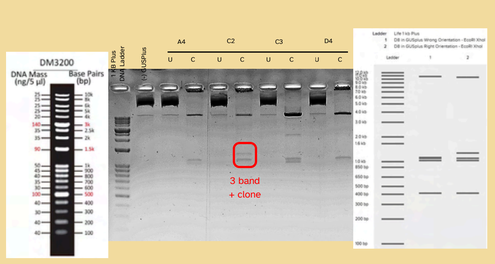


Fig.8 D8 in pGFPGUSPlus cut with *EcoR*I & *Xho*I
One positive clone was obtained

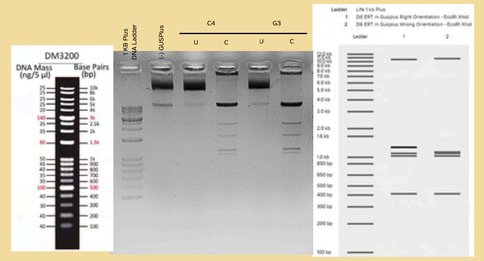


Fig.9 D6 in pGFPGUSPlus cut with *EcoR*I & *Xho*I
No positive clones were obtained

CONCLUSION

This study successfully amplified and cloned the *ERT* gene from both D6 and D8 samples. All D6 clones matched the reference sequence, whereas D8 clones contained silent and missense mutations in the carried carried by the pBluescript II SK(+) vector. Cloning into the pGFPGUSPlus vector yielded a correctly oriented *ERT* insert in only one D8 clone, and no correctly oriented D6 transformants were obtained. Further screening and selection of positive clones are required to obtain correctly oriented constructs for both genotypes. Once verified constructs are obtained, functional characterization of *ERT* through overexpression analysis in a suitable model plant system should be conducted to evaluate its potential role in flowering regulation.

KEY REFERENCES

1. Lithanatudom SK, Pipatchananan P, Jaitan N, Tiansawat P, Lithanatudom P (2024) Microevolution of Longan flowering behaviors from primitive characteristic to KClO. Induction Is Driven Artif Selection Agricultural Benefit Curr Plant Biology 40:100427. https://doi.org/10.1016/j.cpb.2024.100427
2. Jiang Q, Wang Z, Hu G, Yao X (2022). Genome-wide identification and characterization of AP2/ERF gene superfamily during flower development in Actinidia eriantha. BMC Genomics 23(1):1-16. https://doi.org/10.1186/s12864-022-08871-4

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