Title :   Cloning of the *zinc finger protein CONSTANS-LIKE* gene from Thai longan varieties with different flowering behaviors

Author(s) :
1.   Sasithorn Siriwongjak   Student ID :   650510290
2.   Student ID :
3.   Student ID :

Major :   Biology

Advisor(s) :
1.   Assoc. Prof. Dr.   Pathrapol Lithanatudom
2.   Asst. Prof. Dr.   Suparat Lithanatudom
3.

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ABSTRACT

Longan is one of the most economically important fruit crops in Thailand, and flowering is a key factor influencing fruit yield. Transcriptomic analysis of longan samples exhibiting different flowering behaviors in a previous study revealed that the *zinc finger protein CONSTANS-LIKE (ZCOL)*, may play a regulatory role in longan flowering. Investigation of nucleotide sequence variation among cultivars with different flowering behavior, together with analysis of the effects of ZCOL overexpression in a model plant system, will provide a valuable information for a better understanding of the functional role of this gene. This study aimed to construct recombinant plasmids containing the *ZCOL* gene from three longan cultivars using the pGFPGUSPlus vector (Addgene #64401) to enable expression of the ZCOL protein fused with green fluorescent protein (GFP). Colony PCR and restriction enzyme digestion were performed to identify positive clones, which were subsequently submitted for nucleotide sequencing to evaluate genetic variation of the *ZCOL* gene among different longan cultivars. In this study, two recombinant clones each from 'Thao' and 'Phetsakorn' cultivars were successfully constructed. Sequencing analysis revealed that only one clones from 'Thao' cultivars showed the highest similarity to the reference sequence, differing

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by a single nucleotide substitution. Protein sequence analysis further identified a missense mutation at position 359, resulting in an alanine to threonine substitution (Ala359Thr), which was consistently observed in both recombinant clones derived from 'Thao' cultivar. These findings provide essential molecular insights to support future functional characterization of *ZCOL,* enhance understanding of the genetic mechanisms underlying flowering regulation in longan, and contribute to development of improved breeding programs.

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