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# Frameshift and a premature stop codon of *ClLL2* gene due to a single nucleotide deletion is responsible for a non-lobed leaf phenotype in watermelon

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## Abstract

**There are various leaf shape in plants, watermelon leaves are normally lobed leaves, lobed leaf was believed to be controlled primarily by a *ClLL1* gene (*Cla97C04G076510*) in previous research.The presence of a 27-bp and a 24-bp deletion in one coding region has been shown to be responsible for leaf non-lobed. In this study, in order to further explore the formation mechanism of lobed leaf, a $F_2$ population was constructed from the cross between Liu-2 with non-lobed leaf mutant and inbred line 63 to identify that the lobed trait is controlled by a single simple dominant gene designated as *ClLL2*. The *ClLL2* gene was mapped to a 2.5 Mb (Cla97Chr04:22,306,334-24,808,958 97103 V2) interval on chromosome 4 using 10 non-lobed and 10 lobed $F_2$ individuals by BSA-seq. Fine genetic map was conducted with 1082 $F_2$ plants and was narrowed down the lobed leaf locus to an 98.23 kb genomic region, which contains a total of 8 genes. Sequence alignment between parental lines identified a single nucleotide deletion (A/-) in the second exon of *Cla97C04G076510*, which encodes a homeobox leucine zipper protein. Alignment of predicted amino acid sequences of *Cla97C04G076510* between parent lines showed that the translation frameshift started at the 121 residue (T to L). A premature stop codon in *ClLL2* resulted in a truncated protein with only 149 amino acid residues. Translation frameshift mutations lead to mistranslation of the homeobox associated leucine zipper (HALZ) and interferes with the function of the *ClLL2* gene.The candidate gene is valuable resources for providing insight into the molecular mechanism underlying the leaf shape traits in watermelon.**

## Result

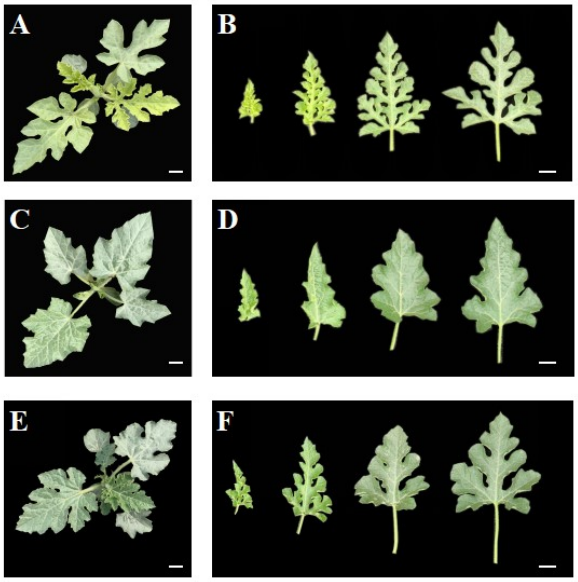


**Fig. 1 Phenotype of the parental lines and $F_1$ hybrid.**
A and B stand the 63; C and D stand the Liu-2; E and F stand the $F_1$ hybrid. Scale: 1cm.

**Table 1. The chi-square goodness-fit test ratios of segregation in $F_2$ populations.**

| Generation | Total no. of individuals | Lobed | Non-lobed | Expected ratio | $\chi^2$ ($\alpha = 0.05$) | $P$ value ($\alpha = 0.05$) |
|---|---|---|---|---|---|---|
| Liu-2 | 20 | 0 | 20 | – | – | – |
| 63 | 20 | 20 | 0 | – | – | – |
| $F_1$ | 20 | 20 | 0 | – | – | – |
| $F_2$(2020) | 196 | 154 | 42 | 3：1 | 0.680 | 0.248 |
| $F_2$(2021) | 886 | 657 | 229 | 3：1 | 0.339 | 0.561 |

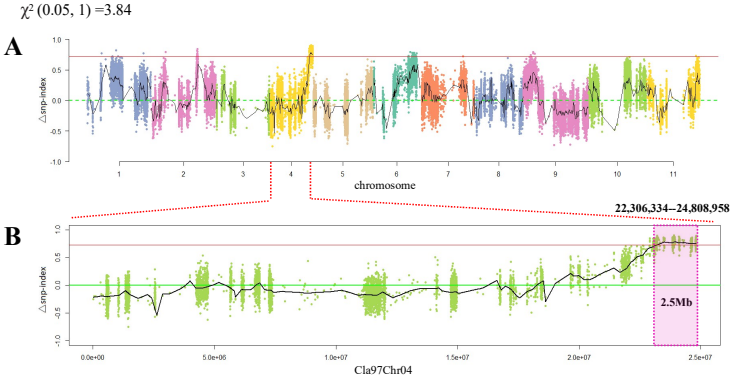


**Fig. 2 The calculated single-nucleotide polymorphism of single nucleotide polymorphisms ΔSNP-index plots.**
(A) ΔSNP-index plot of 11 chromosomes with statistical confidence intervals. (B) ΔSNP-index plot of Cla97Chr04 with statistical confidence intervals. The red line is the threshold value calculated by Loess regression (0.667). The pink region on Chr.4 ranged from 22.30-24,81Mb (approximately 2.51 Mb) and was identified as a candidate region, where the ΔSNP-index values were over threshold value.

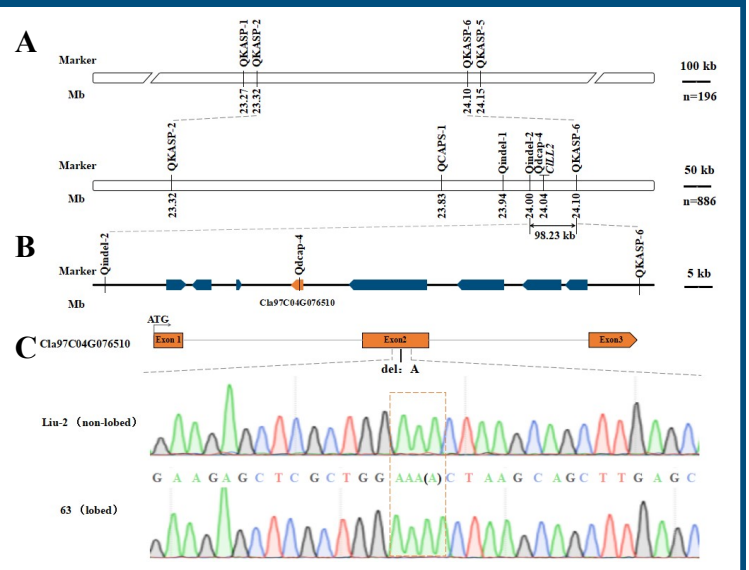


**Fig. 3 Genetic mapping and sequence alignment of the *ClLL2* gene.**
(A) *ClLL2* gene was limited to a 98.23kb region linked with Qindel-2 and QKAPS-6 using 1082 $F_2$ individuals. (B) A single nucleotide deletion occurred in *Cla97C04G076510*. (C) Gene structure of *Cla97C04G076510* and confirmation the mutantion site by sanger sequencing. Orange boxes represent exons, respectively. Black line indicates the 'A' deletion.

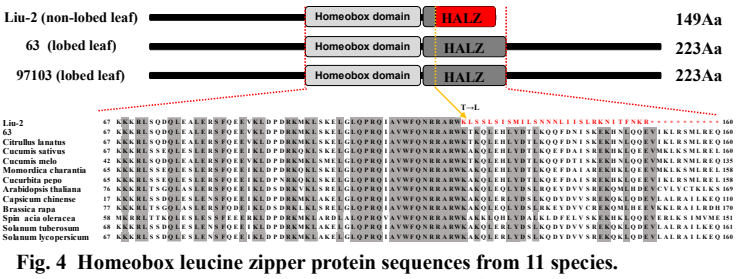


**Fig. 4 Homeobox leucine zipper protein sequences from 11 species.**
Only the sequences encoded by the homeobox domain and homeobox associated leucine zipper (HALZ) are shown. The amino acid sequence after translation frameshift mutation are red boldface typed and boxes.

## Conclusion

**A single nucleotide deletion (A/-) in the second exon of gene *Cla97C04G076510* that encodes a homeobox leucine zipper protein is responsible for non-lobed leaf in watermelon. The translation frameshift led to mistranslation of the homeobox associated leucine zipper (HALZ) and interfered with the function of the gene. The candidate gene is valuable resource for providing insight into the molecular mechanism underlying the leaf shape traits in watermelon.**

## Acknowledgments

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