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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.



Fig. 2 The calculated single-nucleotide polymorphism of single nucleotide polymorphisms Δ SNP-index plots.

(A) Δ SNP-index plot of 11 chromosomes with statistical confidence intervals. (B) Δ SNP-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 Δ SNP-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.

Liu-2 (non-lobed leaf) 63 (lobed leaf)								Homeobox domain							HALZ														1	49	ЭA	Aa													
							ſ	Homeobox domain							HALZ				J								223Aa																		
97103 (lobed leaf)								ſ	Homeobox domain						HALZ												223 Aa																		
																											T→I	L						••••		·····									
Lin-2	61	к	КK	RL:	s o r	QL	EA	L E	RS	EQ	E E	vк	L D	P D	RK	мк	LS	K E I	G	LQP	RQ	AV	WFO	QNE	R.	RW	KL:	5 S L	8.1	ям і	L S	N N I	N L I	115	LR	IK N	I T I	END	c R +					Ξ.,	160
63	63	к	КΚ	RL :	s o r	QL	EA	LE	RS	EQ.	εE	vк	L D	P D	RK	мк	L S	K E I	. G I	LQP	RQ	AV	WE	QNE	ER/	RW	КТ	QL	EH	LYD	TL	ĸġ	9 F I	D N I	SK	EK	HN	iloc	EN	lικ	LR	S M	LR	E Q I	60
Citrullus lanatus	63	к	КΚ	RL :	s o r	QL	EA	LE	RS	EQ.	εE	vк	L D	P D	RK	мк	L S	K E I	. G I	LQP	RQ	AV	WE	QNE	ER/	NRW	кті	CQ L	EН	LYD	ΤL	ĸġ	9 F I	D N I	SK	EK	HN	loc	28.3	IК	LR	S M	LR	E Q I	60
Cucumis sativus	61	K	КK	RL :	5 S E	QL	ES	L E	RS	FQ	εE	I K	L D	P D	RK	QK	L S	K E I	. G .	LQP	RQ	AV	WF	QNE	kR/	N RW	КΛΙ	¢Q L	ΕH	LYB	ΤL	KQI	EFI	д л I	SR	EK	нкі	01	E E N	мк	LK	S M	LRF	6 L 1	60
Cucumis melo	-43	K	КΚ	RL:	s q r	QL	EA	L E	RS	FQ	εE	vк	L D	P D	RK	мκ	L SI	4 E	. G I	LQP	RQ	AV	WF	QNE	kR/	NRW	КΤΙ	¢Q L	ΕH	LYB	ΤL	ĸQ	Q F I	от і	SK	EK	HN	00	28.3	мк	LR	NM	LRF	e Q 1	35
Momordica charantia	65	K	КK	RL :	5 S E	QL	ESI	L.E.	RS	FQ	εE	1 K	L D	P D	RK	QK	LS	K E I	. G I	LQP	RQ	AV	WFO	QNE	kR/	RW	КΛΙ	¢ Q L	ΕH	LYB	ΤL	KQI	EFI) A I	AR	EK	нкі	101	2 E N	мк	LK	S M	LRI	2 L 1	58
Cucurbita pepo	65	K	КΚ	RL :	8 S E	QL	ES	L E	RS	FQ	εE	1 K	L D	P N	RK	QК	L S	K E I	G	LQP	RQ	AV	WF	QNE	kR/	NRW	КЛІ	¢Q L	ΕH	LYB	ΤL	ĸQI	EFI	ð A I	SR	EK	нкі	01	E E N	IК	LR	S M	LRF	6 L 1	58
Arabidopsis thaliana	76	K	КK	RL 1	r s c	QL	AS	L E	RS	FQ	εE	I K	L D	S D	RK	VК	L S	RE	. G .	LQP	RQ	AV	WF	QNE	kR/	N RW	КΛΙ	¢Q L	ΕQ	LYB	S L	RQI	EYI	o v v	/ S R	EK	QMI	6н і	OE V	C V	LY	ст	KLF	68.1	69
Capsicum chinense	17	K	КΚ	RL :	sst	QL	ES	L E	NS -	FQ	εE	1 K	L D	P D	RK	мκ	LΛ	K E I	. G I	LQP	RQ	AV	WF	QNE	kR/	NRW	КЛІ	¢Q L	ER	LYB	S L	ĸQI	DYI	o v v	/ S R	EK	QK	01	0 E N	L A	LR	AI	LKF	e Q 1	10
Brassica rapa	71	K	КK	RL 1	r s c	QL	AS	L E	RS	FQ	DE	I K	L D	S D	RK	L K	L S	RE	. G .	LQP	RQ	AV	WF	QNE	kR/	N RW	КΛΙ	¢Q L	ΕQ	LYB	S L	RKI	EYI	o v v	/ C R	EK	QM	ÉН I	E E N	KK	L R	AI	LRF	он 1	70
Spin acia oleracea	58	м	KR	RL 1	гть	QL	ES	L E	S S -	FE	εĒ	RK	L D	P D	RK	мк	LА	R D	A	LQP	RQ	AV	WF	QNE	kR/	NRW	КΛΙ	ς κ L	QН	LYB	ΛL	KLI	DFI	e L V	/ SK	EK	нк	00	2 E 3	ER	LK	8 I.	MVM	4E 1	51
Solanum tuberosum	68	K	КK	RL :	sst	QL	ES	LE	N S	FQ	εĒ	1 K	L D	P D	RK	мк	LΛ	KE	G	LQP	RQ	AV	WFO	QNE	RR/	RW	КЛ	¢Q L	ER	LYB	S L	ĸqi	DYI	o v v	/ S R	EK	QK	101	DEN	L A	LR	AI	LKF	e Q 1	61
Sohnum beonervieum	63	K	RE	R I -	a s r	OL	ie sk	I.E.	NR	EO.	e 🗐	1 12	E D.	P D	RE	MK	il a i	c eli	G	LOP	ROI	A N	WEI	ONE	2.10.1	N R W	R A I	601	ER	LVD	\$1.	601	οv ι	0 V V	18.0	10.00	oxi	llo i	DEA	dt. A	LR	Δ.1	LKC	60.1	164

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.

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