

Mapping a double flower phenotype associated gene *DcAP2L* in *Dianthus chinensis* by using Reduced-Representation Genome Sequencing and BSR-seq analysis

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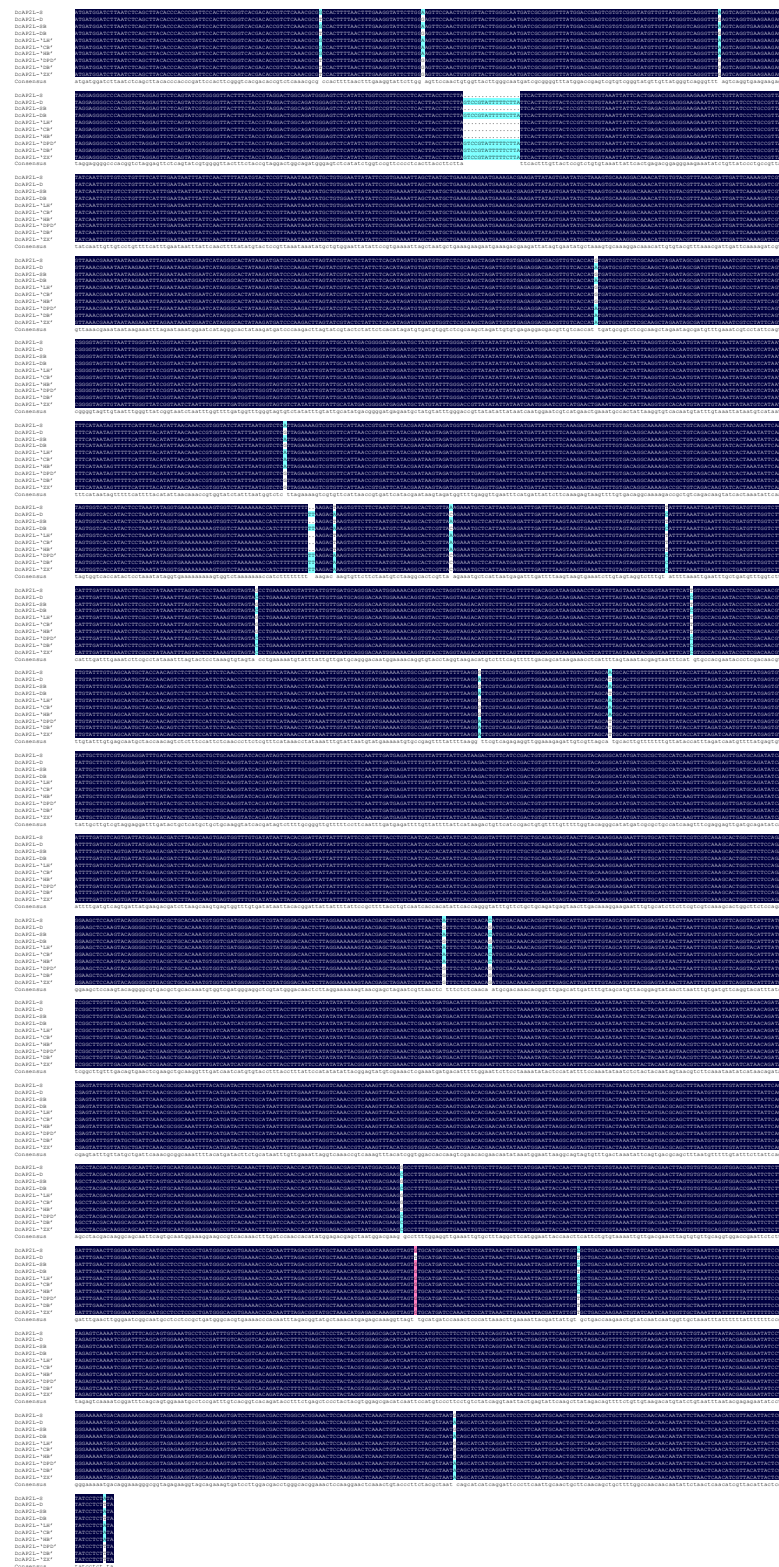
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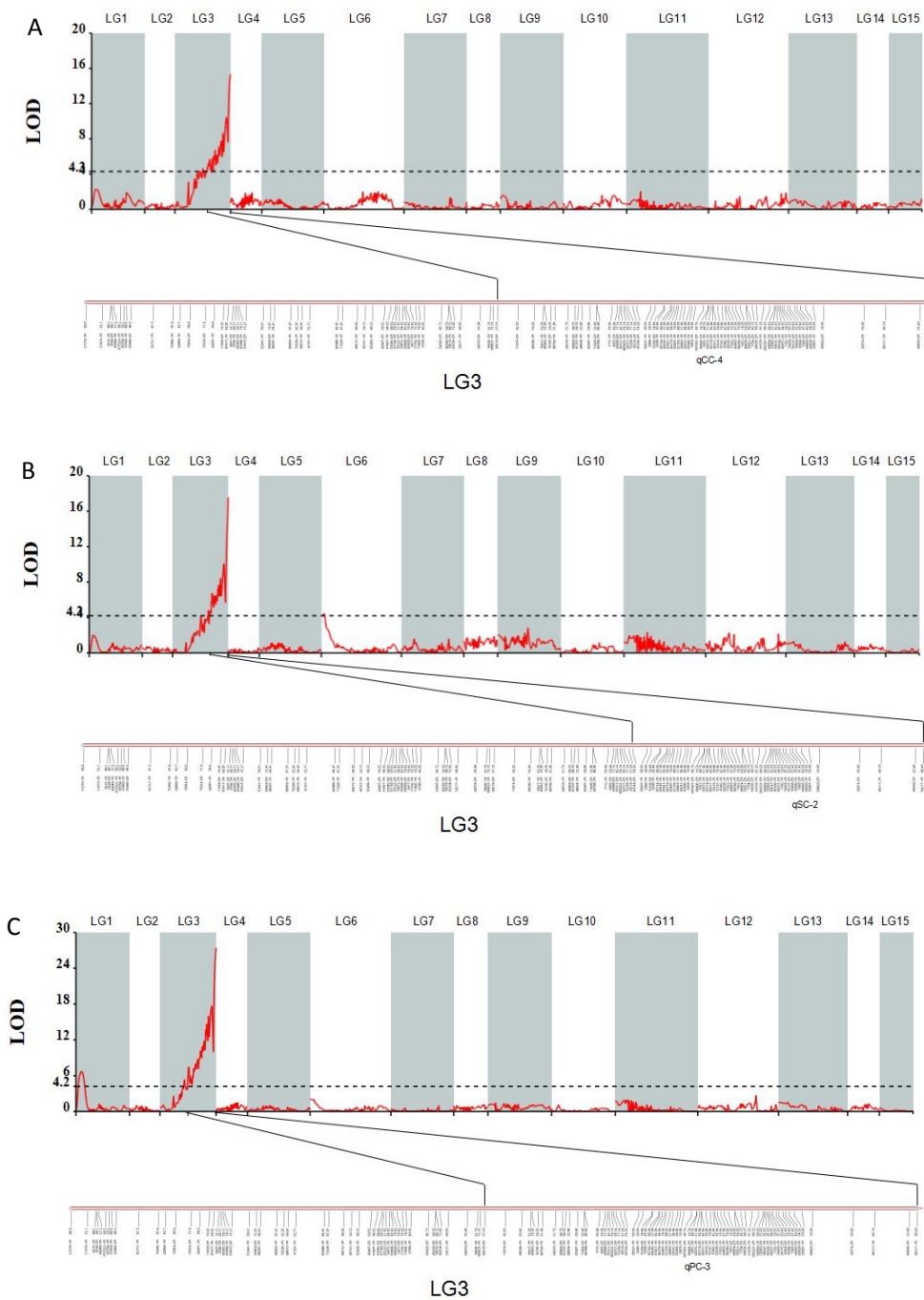
Supplementary Figure S1: Sequence alignment of full-length sequence of *DcAP2L*. *DcAP2L-S*, *DcAP2L-D*, *DcAP2L-SB*, *DcAP2L-DB*, *DcAP2L-LH*, *DcAP2L-CB*, *DcAP2L-HB*, *DcAP2L-DPD*, *DcAP2L-DB*, and *DcAP2L-ZX* represent full-length sequence of *DcAP2L* in ‘MH’, ‘X4’, single flower bulk of F₂, double flower bulk of F₂, ‘LH’, ‘CB’, ‘HB’, ‘DPD’, ‘DB’, and ‘ZX’, respectively. Sequence differences are highlighted with light blue.

DcAP2L-S	ATGATGGATCTTAATCTCAGCTTACACCCACCCGATTCCACTTCGGGTCACGACACCGTCTCAAACCGG	100
DcAP2L-D	ATGATGGATCTTAATCTCAGCTTACACCCACCCGATTCCACTTCGGGTCACGACACCGTCTCAAACCGG	100
DcAP2L-SB	ATGATGGATCTTAATCTCAGCTTACACCCACCCGATTCCACTTCGGGTCACGACACCGTCTCAAACCGG	100
DcAP2L-DB	ATGATGGATCTTAATCTCAGCTTACACCCACCCGATTCCACTTCGGGTCACGACACCGTCTCAAACCGG	100
Consensus	atgatggatcttaatctcagcttacacccacccgattccacttcgggtcacgacacccgtctcaaacggg	
DcAP2L-S	GTTCACACTGTGGTACTTGGGCAATGATCGCGGGGTTATGGACCGAGTCTGTCGGGTATGTTGTTATGGGTCAGGGTT	200
DcAP2L-D	GTTCACACTGTGGTACTTGGGCAATGATCGCGGGGTTATGGACCGAGTCTGTCGGGTATGTTGTTATGGGTCAGGGTT	200
DcAP2L-SB	GTTCACACTGTGGTACTTGGGCAATGATCGCGGGGTTATGGACCGAGTCTGTCGGGTATGTTGTTATGGGTCAGGGTT	200
DcAP2L-DB	GTTCACACTGTGGTACTTGGGCAATGATCGCGGGGTTATGGACCGAGTCTGTCGGGTATGTTGTTATGGGTCAGGGTT	200
Consensus	gttccaactgtggttacttgggcaatgatcgcggggttatggaccgagtcgtcgggtatgttgttatgggtcagggttt	
DcAP2L-S	TAGGAGGGGCCACGGTCTAGGAGTTCCTCAGTATCGTGGGTTACTTCTACCGTAGGACTGGCAGATGGGAGTCTCATATCTGGGCAATGGAAAACAG	300
DcAP2L-D	TAGGAGGGGCCACGGTCTAGGAGTTCCTCAGTATCGTGGGTTACTTCTACCGTAGGACTGGCAGATGGGAGTCTCATATCTGGGCAATGGAAAACAG	300
DcAP2L-SB	TAGGAGGGGCCACGGTCTAGGAGTTCCTCAGTATCGTGGGTTACTTCTACCGTAGGACTGGCAGATGGGAGTCTCATATCTGGGCAATGGAAAACAG	300
DcAP2L-DB	TAGGAGGGGCCACGGTCTAGGAGTTCCTCAGTATCGTGGGTTACTTCTACCGTAGGACTGGCAGATGGGAGTCTCATATCTGGGCAATGGAAAACAG	300
Consensus	taggaggggccacggcttaggagttctcagtatcgtggggttacttctaccgtaggactggcagatgggagttctcatatctgggcaatggaaaacag	
DcAP2L-S	GTGTACCTAGGAGGATTTGATACTGCTCATGCTGCTGCAAGGGCATATGATCGCGCTGCCATCAAGTTTCGAGGAGTTGATGCAGATATCAATTTTGATG	400
DcAP2L-D	GTGTACCTAGGAGGATTTGATACTGCTCATGCTGCTGCAAGGGCATATGATCGCGCTGCCATCAAGTTTCGAGGAGTTGATGCAGATATCAATTTTGATG	400
DcAP2L-SB	GTGTACCTAGGAGGATTTGATACTGCTCATGCTGCTGCAAGGGCATATGATCGCGCTGCCATCAAGTTTCGAGGAGTTGATGCAGATATCAATTTTGATG	400
DcAP2L-DB	GTGTACCTAGGAGGATTTGATACTGCTCATGCTGCTGCAAGGGCATATGATCGCGCTGCCATCAAGTTTCGAGGAGTTGATGCAGATATCAATTTTGATG	400
Consensus	gtgtacctaggagatttgatactgctcatgctgctgcaagggcatatgatcgcgctgccatcaagtttcgagagttgatgcagatatacaattttgatg	
DcAP2L-S	TCAGTGATTTAAGACGATCTTAAGCAAAATGAGTAACCTGCACAAGGAAGAATTTGTCGATCTTCTTCGTCGTCAAAGCACTGGCTTCTCCAGAGGAAG	500
DcAP2L-D	TCAGTGATTTAAGACGATCTTAAGCAAAATGAGTAACCTGCACAAGGAAGAATTTGTCGATCTTCTTCGTCGTCAAAGCACTGGCTTCTCCAGAGGAAG	500
DcAP2L-SB	TCAGTGATTTAAGACGATCTTAAGCAAAATGAGTAACCTGCACAAGGAAGAATTTGTCGATCTTCTTCGTCGTCAAAGCACTGGCTTCTCCAGAGGAAG	500
DcAP2L-DB	TCAGTGATTTAAGACGATCTTAAGCAAAATGAGTAACCTGCACAAGGAAGAATTTGTCGATCTTCTTCGTCGTCAAAGCACTGGCTTCTCCAGAGGAAG	500
Consensus	tcagtgattatgaagacgatcttaagcaaatgagtaacctgcacaaggaagaatttgtgcatcttcttcgctgcaagcactggcttctccagaggaag	
DcAP2L-S	CTCCAAGTACAGGGGCGTGACGCTGCACAAATGTGGTGCATGGGAGGCTCGTATGGGACAACCTCTTAGGAAAAAGTACATTTATCTCGGCTTGTTTGAC	600
DcAP2L-D	CTCCAAGTACAGGGGCGTGACGCTGCACAAATGTGGTGCATGGGAGGCTCGTATGGGACAACCTCTTAGGAAAAAGTACATTTATCTCGGCTTGTTTGAC	600
DcAP2L-SB	CTCCAAGTACAGGGGCGTGACGCTGCACAAATGTGGTGCATGGGAGGCTCGTATGGGACAACCTCTTAGGAAAAAGTACATTTATCTCGGCTTGTTTGAC	600
DcAP2L-DB	CTCCAAGTACAGGGGCGTGACGCTGCACAAATGTGGTGCATGGGAGGCTCGTATGGGACAACCTCTTAGGAAAAAGTACATTTATCTCGGCTTGTTTGAC	600
Consensus	ctccaagtacagggcgctgacgctgcacaaatgtggtgcatagggagctcgatgggacaactcttaggaaaaagtacatttatctcggcttgtttgac	
DcAP2L-S	AGTGAAGCTGGAAGCTGCAAGAGCCTACGACAAAGGAGCAATTCAGTGCATGGAAAGGAAGCGCTCACAAACTTTGATCCAACCATATGGAGACGAGC	700
DcAP2L-D	AGTGAAGCTGGAAGCTGCAAGAGCCTACGACAAAGGAGCAATTCAGTGCATGGAAAGGAAGCGCTCACAAACTTTGATCCAACCATATGGAGACGAGC	700
DcAP2L-SB	AGTGAAGCTGGAAGCTGCAAGAGCCTACGACAAAGGAGCAATTCAGTGCATGGAAAGGAAGCGCTCACAAACTTTGATCCAACCATATGGAGACGAGC	700
DcAP2L-DB	AGTGAAGCTGGAAGCTGCAAGAGCCTACGACAAAGGAGCAATTCAGTGCATGGAAAGGAAGCGCTCACAAACTTTGATCCAACCATATGGAGACGAGC	700
Consensus	agtgaactggaagctgcaagagcctacgacaaaggagcaattcagtgcatggaaaggaagcgtcacaaactttgatccaaccacatattggagacgagc	
DcAP2L-S	TAATGGACGAAGCGCCTTTTGGAGTGGACCGAATTCCTCTGATTTGAACTTGGGAATCGGCAATGCCCTCCGCTGATGGGCAAGTGAAGCCACAA	800
DcAP2L-D	TAATGGACGAAGCGCCTTTTGGAGTGGACCGAATTCCTCTGATTTGAACTTGGGAATCGGCAATGCCCTCCGCTGATGGGCAAGTGAAGCCACAA	800
DcAP2L-SB	TAATGGACGAAGCGCCTTTTGGAGTGGACCGAATTCCTCTGATTTGAACTTGGGAATCGGCAATGCCCTCCGCTGATGGGCAAGTGAAGCCACAA	800
DcAP2L-DB	TAATGGACGAAGCGCCTTTTGGAGTGGACCGAATTCCTCTGATTTGAACTTGGGAATCGGCAATGCCCTCCGCTGATGGGCAAGTGAAGCCACAA	800
Consensus	taatggacgaagcgcttttggagtggaacccaatctctctgatttgaaacttgggaatcggcaatgctcctccgctgatgggcaagtgaaacccacaa	
DcAP2L-S	TTTAGACGGTATGCTAAACATGAGAGCAAAGAGTCAAATCGGATTTACAGAGTGGAAATGCCCTCCGATTTGTACGGTACAGATACCTTTCTGAGCTC	900
DcAP2L-D	TTTAGACGGTATGCTAAACATGAGAGCAAAGAGTCAAATCGGATTTACAGAGTGGAAATGCCCTCCGATTTGTACGGTACAGATACCTTTCTGAGCTC	900
DcAP2L-SB	TTTAGACGGTATGCTAAACATGAGAGCAAAGAGTCAAATCGGATTTACAGAGTGGAAATGCCCTCCGATTTGTACGGTACAGATACCTTTCTGAGCTC	900
DcAP2L-DB	TTTAGACGGTATGCTAAACATGAGAGCAAAGAGTCAAATCGGATTTACAGAGTGGAAATGCCCTCCGATTTGTACGGTACAGATACCTTTCTGAGCTC	900
Consensus	tttagacggtatgctaaacatgagagcaaaagatcaaatcggatttacagagtggaatgccctccgatttgtacgggtacagatacctttctgagctc	
DcAP2L-S	CCTACTACGTGGAGCGACATCAATTCATGTCCTTCCCTGCTATCAGGAAAGGGCGGTAGAGAAGGTAGCAGAAAAGTATCCTTGGACGACCTGGGCAC	1000
DcAP2L-D	CCTACTACGTGGAGCGACATCAATTCATGTCCTTCCCTGCTATCAGGAAAGGGCGGTAGAGAAGGTAGCAGAAAAGTATCCTTGGACGACCTGGGCAC	1000
DcAP2L-SB	CCTACTACGTGGAGCGACATCAATTCATGTCCTTCCCTGCTATCAGGAAAGGGCGGTAGAGAAGGTAGCAGAAAAGTATCCTTGGACGACCTGGGCAC	1000
DcAP2L-DB	CCTACTACGTGGAGCGACATCAATTCATGTCCTTCCCTGCTATCAGGAAAGGGCGGTAGAGAAGGTAGCAGAAAAGTATCCTTGGACGACCTGGGCAC	1000
Consensus	cctactacgtggagcgacatcaattccatgctccttccctgctatcaggaaagggcggtagagaaggttagcagaaaagtgatccttggacgacctgggcac	
DcAP2L-S	GGAAACTCCAAGGAACCTCAAACCTGTACCCTTCTACGCTAATA	1100
DcAP2L-D	GGAAACTCCAAGGAACCTCAAACCTGTACCCTTCTACGCTAATA	1100
DcAP2L-SB	GGAAACTCCAAGGAACCTCAAACCTGTACCCTTCTACGCTAATA	1100
DcAP2L-DB	GGAAACTCCAAGGAACCTCAAACCTGTACCCTTCTACGCTAATA	1100
Consensus	ggaactccaaggaactcaaacctgtacccttctacgcttaata	
DcAP2L-S	ACAATATTCTAACTCAACATCGTTACATTACTCCTATCCTCTATA	1145
DcAP2L-D	ACAATATTCTAACTCAACATCGTTACATTACTCCTATCCTCTATA	1145
DcAP2L-SB	ACAATATTCTAACTCAACATCGTTACATTACTCCTATCCTCTATA	1145
DcAP2L-DB	ACAATATTCTAACTCAACATCGTTACATTACTCCTATCCTCTATA	1145
Consensus	acaatattctaaactcaacatcgtttacattactcctatcctctata	

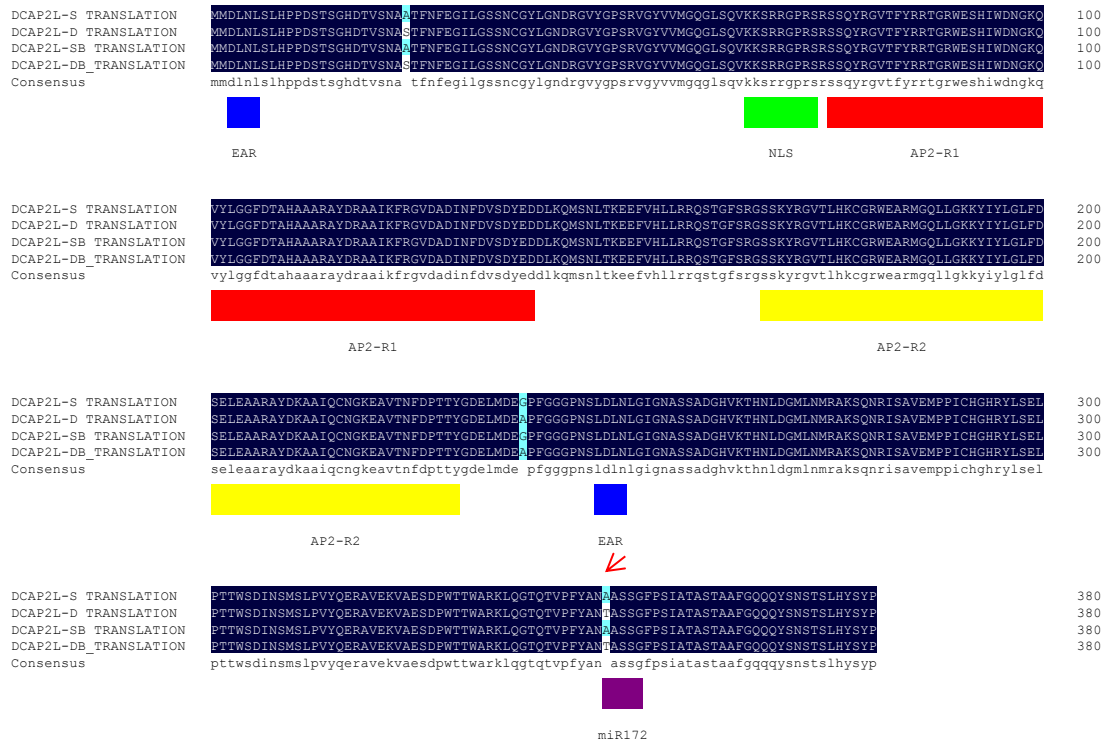
Supplementary Figure S2: Sequence alignment of CDS sequence of *DcAP2L*. *DcAP2L-S*, *DcAP2L-D*, *DcAP2L-SB* and *DcAP2L-SB* represent CDS sequence of *DcAP2L* in ‘MH’, ‘X4’, single flower bulk and double flower bulk of F₂, respectively. Sequence differences are highlighted with light blue.



Supplementary Figure S3: Flower and stem color characteristics of the two parents.
A, 'MH' shows calyxes and stem with fuchsia.
B, 'X4' shows green calyxes and stem which are different from 'MH'.



Supplementary Figure S4: Genetic location of QTLs for color related trait. A, B and C indicate genetic location of QTLs for calyxes color, stem color and style color of pistil, respectively. The horizontal dashed line represents a linkage group-wise logarithm of odds (LOD) significance threshold of 4.3 for calyxes color trait, and 4.2 for stem color and style color of pistil trait. qCC-4, qSC-2 and qPC-3 are most significant QTL regions for three color-related traits with the same peak position correspond the highest LOD value.



Supplementary Figure S5: Sequence alignment of putative amino acid sequence of *DcAP2L*. *DcAP2L-S* translation, *DcAP2L-D* translation, *DcAP2L-SB* translation and *DcAP2L-DB* translation represent putative amino acid sequence of *DcAP2L* in ‘MH’, ‘X4’ and single flower and double flower bulk of F₂ population, respectively. The missense mutation that lies within the miR172 target site of *DcAP2* is marked by red arrow. Color boxes beneath the sequence indicate the position of the two AP2 domains (AP2-R1 and AP2-R2, in red and yellow respectively), of the EAR motif-like sequences (in dark blue), of the nuclear localization signal (NLS, in green), and the sequence derive from miR172 target site (in purple).

Supplementary Table S1: Evaluation criteria for agronomic traits and assignment for qualitative characters of *D.Chinensis*.

Trait	Abbreviation	Evaluation criterion	Assignment
Double flower trait (petal number)	DFT	Average petal number of nine flowers in each plant	/
Stamen number	SN	Average stamen number of nine flowers in each plant	/
Stem color	SC	The stem color with green and fuchsia (part or all with fuchsia)	1 represent stem color with green; 2 represent stem color with fuchsia
Branch number	BN	Number of branches on main stem	/
Leaf with wax	LWW	It's actually a white appendage on the leaf, we wiped the leaves with a black cloth to judge whether the leaf have this white appendage	1 represent the leaf have this white appendage; 2 represent the leaf have no this white appendage
Leaf width	LW	Average width and length of two leaves which located at the fifth pair	/
Leaf length	LL	from the top down	/
Plant height	PH	One plant measured three times	/
Plant width	PW	One plant measured three times	/
Stem diameter	SD	Measured three times for the stem diameter of main stem which next to the soil	/
Calyx color	SC	The calyx color with green and fuchsia (part or all with fuchsia)	1 represent calyx color with green; 2 represent calyx color with fuchsia
Style color of the pistil	PC	The style color with white and fuchsia(part or all with fuchsia)	1 represent style color with white; 2 represent style color with fuchsia

Supplementary Table S2: Primers used for quantitative PCR and cloning.

Name	Application	Forward primer (5'-3')	Reverse primer (5'-3')
<i>DcAP2L</i>	qRT-PCR	GATGGGAGGCTCGTAT GG	CCGTCTAAATTGTGGGTTTT
<i>DcAGa</i>	qRT-PCR	GCATTAGTAGAATCCG ATCAA	TCGTAGTCACCACCACCCG
<i>DcAGb</i>	qRT-PCR	CGAATCCGATCCAAAA AGAATG	TCGTACTCGCTGCTACCTCC
<i>DcGAPDH</i>	qRT-PCR	CGGAAAGTTGACTGGT ATGGC	CATCCTCGGTGTAGCCCAA AT
<i>DcAP2L</i>	cloning	TGTATTCCCCTTTGTAC ACTCAC	GACACGATTCAGACTATGAA CACT
miR172a-pr ecursor	cloning	TGTGGTCTCAAATGTG CTGTGGCATCATCAAG ATTCAC	TGTGGTCTCAAAGCGCCGAT GCAGCATCATCAAGA

Supplementary Table S3: Number of floral organs of ‘MH’, ‘X4’ and F₁ plants in *D. chinensis*.

Floral organ	‘MH’	‘X4’	F ₁
Sepal	5.00±0.00	5.00±0.00	5.00±0.00
Petal	5.00±0.00	25.43±3.18	9.52±1.65
Chimera petal-stamen	0.00±0.00	3.43±1.93	2.34±1.58
Stamen	10.00±0.00	13.62±2.26	8.54±2.33
Style	2.00±0.00	2.60±0.69	2.00±0.00
Total	22.00±0.00	50.08±4.95	27.4±2.22

Nine flowers in each plant and six plants in each material are measured.

Deviation values represents standard deviation.

Supplementary Table S4: Summary of sequence data from parents and 140 F₂ progenies.

Sample ID	Raw reads	Clean reads	GC rate(%)	Q30 rate(%)	Average Depth
'X4'	5223010	5222674	41.27	92.59	24.22
'MH'	5287378	5287064	40.96	92.74	24.92
Average of F ₂	2652103	2651894	41.44	90.05	9.60

Supplementary Table S5: Type of variation of the identified 2353 SNP markers for constructing the genetic linkage map.

Type of variation	NO. of SNP	Rate (%)
A/C	230	9.77
C/G	164	6.97
G/A	686	29.15
T/A	324	13.77
C/T	747	31.75
G/T	202	8.59
Total	2353	100

Supplementary Table S6: Significance test of agronomic characters between parents and character statistics of parents and offspring.

Trait	Generation			
	‘MH’	‘X4’	F ₁	F ₂
Double flower trait (petal number)**	5.00±0.00	25.43±3.18	9.52±1.65	11.89±6.21
Stamen number	10.00±0.00	13.62±2.26	8.54±2.33	9.51±1.89
Stem color**	2.00±0.00	1.00±0.00	1.00±0.00	1.54±0.50
Branch number*	12.25±2.38	10.25±1.64	19.25±1.48	12.26±3.78
Leaf with wax**	1.00±0.00	2.00±0.00	1.00±0.00	1.18±0.38
Leaf width(mm)	6.82±0.84	6.20±0.72	8.47±0.53	7.21±1.83
Leaf length(cm)	7.32±0.56	6.05±0.58	8.03±0.90	6.55±1.06
Leaf length(cm)	16.75±0.80	17.27±1.46	36.02±3.22	22.74±5.61
Plant width**	21.77±3.05	14.77±2.05	25.67±1.41	22.81±4.71
Stem diameter	2.74±0.46	2.23±0.31	3.61±0.22	3.03±0.71
Calyx color**	2.00±0.00	1.00±0.00	1.00±0.00	1.54±0.50
Style color of the pistil**	1.00±0.00	2.00±0.00	2.00±0.00	1.36±0.48

Note: DPS7.05 software is used for character statistics, and *t*-test is used to test significant difference of agronomic characters between two parents. **indicates $P < 0.01$; *indicates $P < 0.05$. Deviation value represents standard deviation.

Supplementary Table S7: The sequence of fragment containing the sequence of pre-miR172a of *D.chinensis*.

>seq-pre-miR172a

GGTAAGGCTTTTCTTTCAAGATTCTCTCTGCCGACAGTGGTCCCAAAGATGGACCCCC
ACCCACGAGGAGCATCGTGGA AAAAGAAGAGGTTCCAACCACGTCTACAAAGCAAGT
GGATTGATGTGACATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGC
AAGACCCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGGACACGCTCGAGTATA
AGAGCTCATTTTTACAACAATTACCAACAACAACAACAACAACAACAACAATTACAATTA
CATTTACAATTATCGATAACAATGTGCTGTGGCATCATCAAGATTCACATGCCTCACTTCC
ATTGACCTTTCTTTCCCACTTATTTTTTTGTTATTTTGTAAAAAAAATTGTTTTTTTTTTT
TTTTAATATTATTAGATGTGAAGCTTTCATATATGTGGTCCTTCAAATAATTGTATCAGAA
AATATTATTGTGTTTTTATTATTATTATTGGGTCCATTTATTTTTATTTTTGTGGGAAGAG
GTCAAGAGGAAAACAAATGATATGAGAATCTTGATGATGCTGCATCGGCGCTTGTCTTG
CTTTAATGAGATATGCGAGAAGCCTATGATCGCATGATATTTGCTTTCAATTCTGTTGTG
CACGTTGTAAAAAACCTGAGCATGTGTAGCTCAGATCCTTACCGCCGGTTTCGGTTCAT
TCTAATGAATATATCACCCGTTACTATCGTATTTTTATGAATAATATTCTCCGTTCAATTTA
CTGATTGTACCCTACTACTTATATGTACAATATTTAAAATGAAAACAATATATTGTGCTGAA
TAGGTTTATAGCGACATCTATGATAGAGCGCCACAATAACAAACAATTGCGTTTTATTAT
TACAAATCCAATTTTTAAAAAAGCGGCAGAACCGGTCAAACCTAAAAGACTGATTACA
TAAATCTTATTCAAATTTCAAAAGTGCCCCAGGGGCTAGTATCTA