

## Abstract

Periwinkle [*Catharanthus roseus* (L.) G. Don] is renowned for its diverse colors and resilience to harsh climates. Our study embarked on elucidating the inheritance patterns of wavy flower forms, aiming to refine periwinkle breeding strategies. Phenotypes of self-pollinated ( $S_1$ ) and cross-pollinated ( $F_1$ ,  $F_2$ ) populations further underscored their morphological distinctions. Specifically, the extreme-wavy type displayed elliptical leaves with pronounced pointy tips and a notably wrinkled surface. This type also bore intensely wavy petal margins and female sterility. Our findings suggest that the inheritance of these flower forms is regulated by an allele *WAVY* (*Wv*), which exhibits incomplete dominance. Specifically, the non-wavy (NW) form arises from *wvwv* genotype, the extreme-wavy (EW) from *WvWv* genotype, and the medium-wavy (MW) from *Wvwv* genotype. This study provides clarity on inheritance patterns, facilitating strategic breeding of diverse flower forms in periwinkle.

## Introduction

*Catharanthus roseus* (L.) G. Don, also known as periwinkle. Noted for its resilience to heat and drought, periwinkle's corolla typically features five flat lobes, with limited variation in flower shape. Wavy petals, which enhance ornamental value, are a key focus in breeding. While the wavy petal trait in *Primula* and *Cyclamen* is known to be governed by a dominant gene, similar studies in periwinkle are scarce. Our study aims to explore the inheritance patterns of wavy petals in periwinkle.

## Material and method

Seven *Catharanthus roseus* varieties were crossbred: non-wavy types 'Summer Sakura' (SS), 'Jams 'N Jellies Blackberry' (JJB), 'Mediterranean XP Rose Halo' (MRH), 'Tattoo Papaya' (TP), 'Tattoo Blackberry' (TB); and wavy types 'Hu Lo' (HL) and 'Pink Princess' (PP). HL (medium-wavy) and PP (extreme-wavy) served as pollen parents, while the non-wavy acted as seed parents. The resulting crosses produced inbred  $S_1$ , hybrid  $F_1$ , and self-pollinated  $F_2$  generations, totaling 6  $S_1$ , 5  $F_1$ , and 8  $F_2$ . Flower patterns were recorded, and the segregation ratio in offspring was analyzed using the chi-square test.

## Results and discussion

Significant morphological differences among NW, MW, and EW flower types includes leaf and floral organ size, weight, as well as calyx and corolla (Fig. 1 and 2, Table 1). The EW type exhibited petal-like ovary organs (Fig. 3). Flower development timing did not significantly differ across types (Table 2). All wavy flower types displayed loosely arranged petals at later stages (Fig. 4). The  $S_1$  generation of the MW type segregated into NW, MW, and EW types in a 1:2:1 ratio (Table 3).  $F_1$  hybrids between NW and MW types showed only these phenotypes (1:1 ratio), and  $F_2$  of NW type crosses with the EW type mirrored the ratios of  $S_1$  of MW type. This inheritance pattern suggests that the wavy flower trait is controlled by an incompletely dominant allele, *WAVY* (*Wv*). The genotype *WvWv* results in EW flowers, *Wvwv* in MW, and *wvwv* in NW flowers.

**Table 1. The morphological differences of corolla and leaf between non-wavy, medium-wavy, and extreme-wavy types of *Catharanthus roseus*.**

Phenotype (cultivar)	Leaf			Flower				
	Length (cm)	Width (cm)	Length : width ratio <sup>x</sup>	Diameter (cm)	Wave height of petal (cm)	Fresh weight of corolla (g)	Dry weight of corolla (g)	Diameter : fresh weight ratio <sup>w</sup>
NV (JJB) <sup>z</sup>	7.39±0.85 a <sup>y</sup>	3.05±0.29 b	2.44 a	4.77±0.56 a	0.36±0.11 c	0.23±0.03 b	0.30±0.07 b	21.6 a
MW (HL)	7.46±0.39 a	3.56±0.36 a	2.11 b	4.27±0.31 b	1.21±0.19 a	0.27±0.05 a	0.41±0.04 a	16.16 b
EW (PP)	6.45±0.55 b	3.74±0.37 a	1.73 c	3.23±0.43 c	1.02±0.19 b	0.21±0.03 b	0.25±0.02b	17.11 b

<sup>z</sup> NW = non-wavy; MW = medium-wavy; EW = extreme-wavy. JJB = Jams 'N Jellies Blackberry; HL = Hu Lo; PP = Pink Princess.

<sup>y</sup> Means within each column followed by the different letter(s) are significantly different at  $P < 0.05$  by Fisher's LSD test.

<sup>x</sup> Means are averaged from the ratio of each examined corolla.

<sup>w</sup> Means are averaged from the ratio of each examined leaf.

**Table 2. The relative growth of flower bud of non-wavy, medium-wavy and extreme-wavy flower forms of *Catharanthus roseus*.**

Phenotype (cultivar) <sup>z</sup>	Day(s) after flower bud size in 0.5 cm length							
	1	2	3	4	5	6	7	8
Length of flower bud (cm)								
NW (JJB)	0.52	0.57	0.80	0.97	1.25	1.71	2.16	3.06
MW (HL)	0.50	0.61	0.87	1.08	1.49	2.21	2.73	3.92
EW (PP)	0.56	0.60	0.83	0.97	1.08	1.54	1.89	2.45
Relative growth (cm)								
NW (JJB)	-	0.05	0.24	0.16	0.28	0.46	0.45	0.90
MW (HL)	-	0.11	0.27	0.21	0.41	0.72	0.52	1.18
EW (PP)	-	0.05	0.23	0.13	0.11	0.46	0.34	0.56

<sup>z</sup> NW = non-wavy; MW = medium-wavy; EW = extreme-wavy. JJB = Jams 'N Jellies Blackberry; HL = Hu Lo; PP = Pink Princess.

**Table 3. Segregation of non-wavy, medium-wavy and extreme-wavy petal types in progeny of self-pollinated and crossed *Catharanthus roseus* cultivars.**

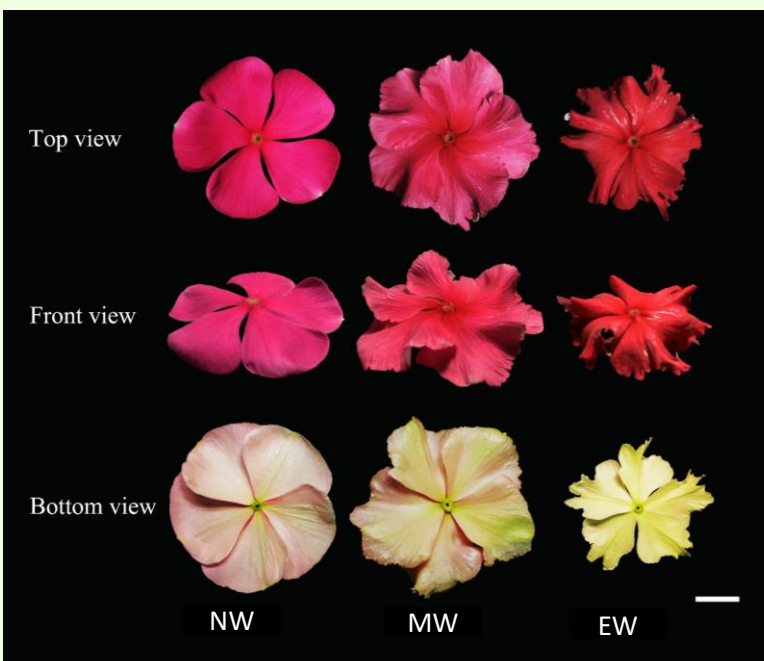
Parents/crosses/generation	Petal type <sup>y</sup>			Test ratio	X <sup>2</sup>	P
	NW	MW	EW			
Cultivar <sup>z</sup> (petal form)						
HL ⊗ (MW)	25	30	22	1:2:1	3.987	0.136
PP ⊗ (EW)	- <sup>x</sup>	-	-	-	-	-
SS ⊗ (NW)	200	0	0	1:0:0		all NW
JJB ⊗ (NW)	175	0	0	1:0:0		all NW
MRH ⊗ (NW)	158	0	0	1:0:0		all NW
TP ⊗ (NW)	100	0	0	1:0:0		all NW
TB ⊗ (NW)	136	0	0	1:0:0		all NW
$F_1$						
SS × HL	33	37	0	1:1:0	0.229	0.633
JJB × HL	49	44	0	1:1:0	0.269	0.604
TP × HL	28	17	0	1:1:0	2.689	0.101
TB × HL	37	23	0	1:1:0	3.267	0.071
MRH × PP	0	87	0	0:1:0		all MW
$F_2$						
SS × HL F1-23 <sup>w</sup> ⊗ (MW)	14	24	11	1:2:1	0.388	0.824
JJB × HL F1-9 ⊗ (MW)	4	8	1	1:2:1	2.077	0.354
TP × HL F1-21 ⊗ (MW)	13	28	20	1:2:1	2.016	0.365
TB × HL F1-57 ⊗ (MW)	20	52	15	1:2:1	3.897	0.143
MRH × PP F1-80 ⊗ (MW)	10	29	13	1:2:1	1.038	0.595
JJB × HL F1-7 ⊗ (NW)	53	0	0	1:0:0		all NW
TP × HL F1-14 ⊗ (NW)	67	0	0	1:0:0		all NW
TB × HL F1-32 ⊗ (NW)	126	0	0	1:0:0		all NW

<sup>z</sup>HL = Hu Lo; PP = Pink Princess; SS = Summer Sakura; JJB = Jams 'N Jellies Blackberry; MRH = Mediterranean XP Rose Halo; TP = Tattoo Papaya; TB = Tattoo Blackberry.

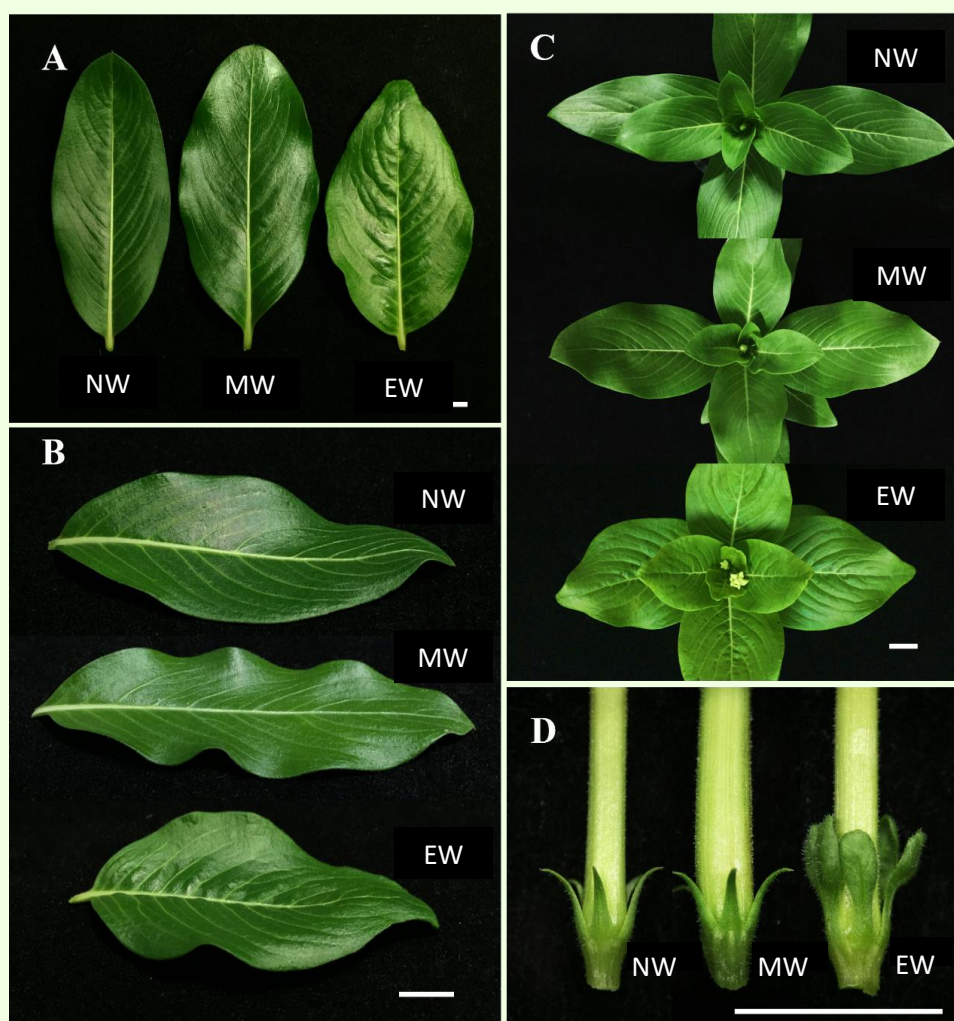
<sup>y</sup> NW = non-wavy; MW = medium-wavy; EW = extreme-wavy.

<sup>x</sup> extreme-wavy petal phenotype is unable to self-pollinate due to abnormal ovary.

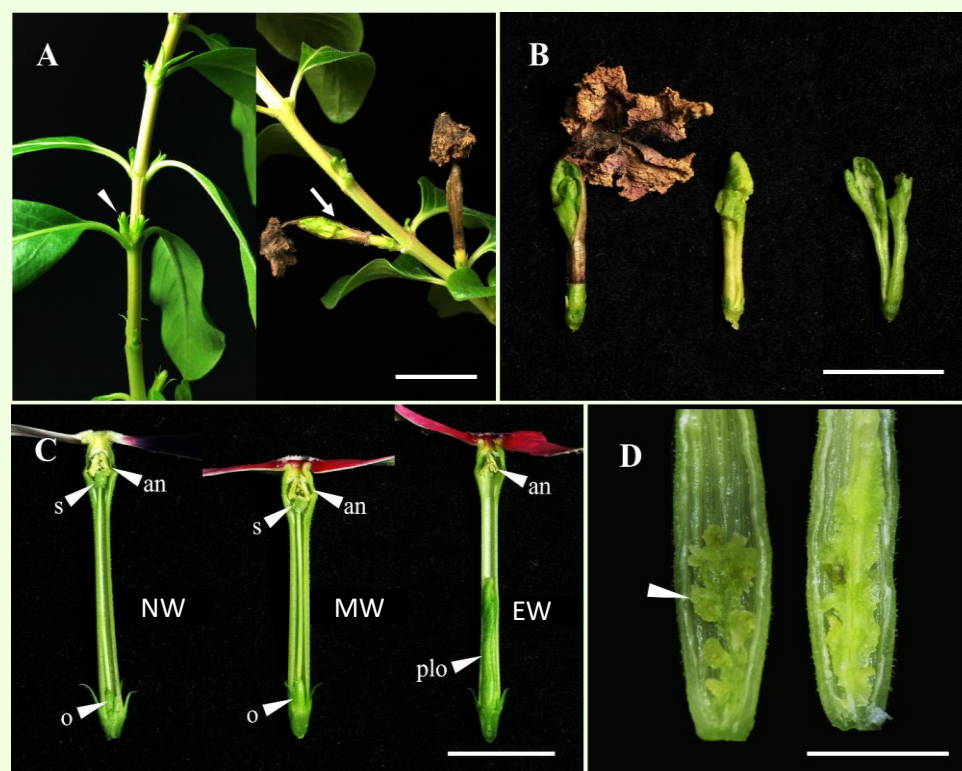
<sup>w</sup> code number of selected progeny.



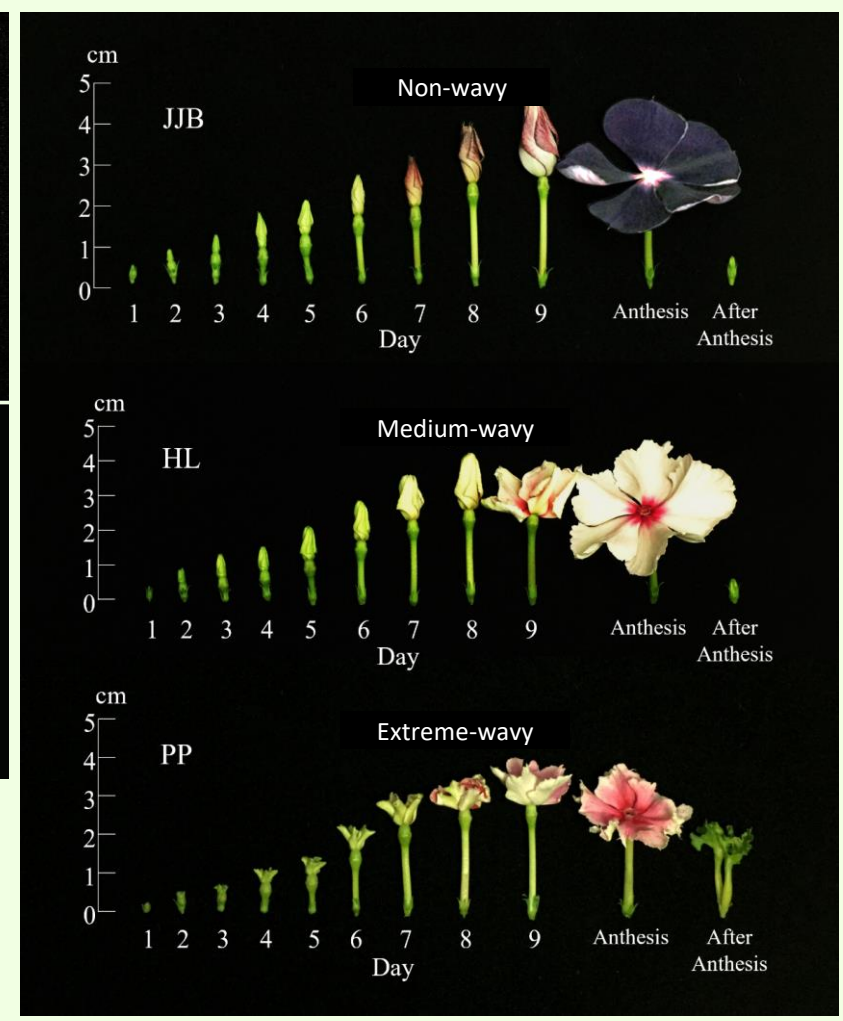
**Fig. 2. The corolla top view, front view and bottom view of non-wavy, medium-wavy and extreme-wavy flower forms of *Catharanthus roseus*; NW = non-wavy; MW = medium-wavy; EW = extreme-wavy; Bar = 1 cm.**



**Fig. 1. The top view(A), front view(B), juvenile plant top view(C), and sepal(D) of non-wavy, medium-wavy and extreme flower forms of *Catharanthus roseus*; NW = non-wavy; MW = medium-wavy; EW = extreme-wavy; Bar = 1 cm.**



**Fig. 3. The morphological difference between non-wavy, medium-wavy and extreme-wavy flower forms of *Catharanthus roseus*. (A) Unpollinated and wilted flower of non-wavy (left; arrowhead) and extreme-wavy flower form (right; arrow). (B) The corolla cannot fall off due to the abnormal ovary. After removing the wilted corolla, the interior has a green structure similar to petals and leaves, with many vascular bundles. (C) The non-wavy and wavy flower form has normal anthers (an), pistil (s), and ovary (o), while the extreme-wavy flower form has normal stamens but lacks pistils, and its ovary is a petal-like organ. (D) The petal-like organs near the sepals of extreme-wavy flower form have several underdeveloped embryos (arrow) and placental structures. NW = non-wavy; MW = medium-wavy; EW = extreme-wavy; Bar = 1 cm.**



**Fig. 4. The floral development records of non-wavy, medium-wavy, and extreme-wavy flower form in *Catharanthus roseus*, from 0.5 cm flower bud to blooming appearance and residual organ appearance after withering; JJB = Jams 'N Jellies Blackberry; HL = Hu Lo; PP = Pink Princess.**