Relationship between Phloem Fiber and Trailing Habit, and Independent Inheritance of Growth Habit and Flower Form in Periwinkle

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ABSTRACT. Stem anatomy and modulus of elasticity (MOE) were compared between upright and trailing cultivars of periwinkle [Catharanthus roseus (G.) Don.]. The inheritance of growth habit and flower form was also studied. Internode cross sections revealed that phloem fiber was distributed at the inner cortex in upright cultivars but not in trailing cultivars. Except the youngest internode, the upright ‘Vitesse Pink’ had the highest MOE throughout the 1st–13th internodes above the cotyledon. The more trailing ‘Cora Cascade Strawberry’ had consistently lower MOE than a less trailing ‘Cora Cascade Polka Dot’. All F1 plants between upright and trailing cultivars were upright, and the F2 generation derived from self-pollinating F1 fit a 3 upright : 1 trailing segregation ratio. All F1 plants between upright/double-flower and trailing/single-flower exhibited upright and single-flowers, whereas plants in the F2 generation fitted a 9 upright/single-flower : 3 trailing/single-flower : 3 upright/double-flower : 1 trailing/double-flower ratio. New double-flowered periwinkle selections with trailing growth habit were successfully developed from the F2 population.

Periwinkle, a member of the Apocynaceae family, has long-lasting flowering periods under warm climates, with many flower color variations. Periwinkle has become an important bedding plant or potted flowering plant in the world owing to its tolerance to heat and drought, and well adaptation (Curry, 2012). Periwinkle is also known for its use of terpenoid indole alkaloids for treating cardiac diseases and certain tumors in mammals (Zhou et al., 2009).

Most periwinkle cultivars have an upright growth habit, although trailing cultivars are desirable as they have increased their utility as ornamental plants for uses in hanging baskets and mixed containers, and as groundcover in the landscape. Trailing habits can be related to less or gradual loss of fiber tissue within stems (Evert, 2006). Fibers provide much of the mechanical support; therefore, shrubs are expected to have a higher portion of fibers than climbers (Crivellaro et al., 2012). In non-self-supporting Clematis vitalba L. and Clematis maritima L., primary phloem fiber and collenchyma are displaced outward by plant development, which led to disrupting the mechanical integrity, but not in semifluctually supporting Clematis recta L. (Rowe et al., 2004).

Modulus of elasticity measures the resistance of an object or substance to being deformed elastically when a mechanical force is applied to it. Biomechanical properties, such as stem stiffness or elasticity can be quantitatively compared with MOE (Niklas, 1995). MOE decreased significantly after loss of fibers in C. vitalba and C. maritima, but not in C. recta, which remained higher MOE (Rowe et al., 2004). Stems of upright periwinkle contained phloem fiber (Kartmazova, 1972), but no information is available on stem anatomy or MOE of trailing cultivars.

Genetic control of growth habit varies among plant species. For example, growth habit of cowpea [Vigna unguiculata (L.) Walp.] is governed by two allelic pairs, A4 and BB. The genotype AB has the prostrate growth habit; the genotype aabb has the climbing growth habit, and AAbb has the bushy habit (Uguru and Uzo, 1991). In red clover (Trifolium pretense L.), a dominance × dominance epistasis controls prostrateness, with prostrate being partially dominant to erect (Mirzaie-Nodoushan et al., 1999). Egyptian clover (Trifolium alexandrinum L.) is erect, whereas Trifolium apertum Bobrov is prostrate, and all their F1 plants were erect, indicating thereby dominance of erect habit over the prostrate (Malaviya et al., 2004). Growth habit of coleus [Solenostemon scutellarioides (L.) Codd] is controlled by a single gene (U) with additive effects, and upright is dominant to trailing. Upright growth habit is designated with a UUUU genotype and trailing with a uuuu genotype (Nguyen et al., 2008). Information on the genetics for the trailing growth habit of periwinkle is presently limited.

Flower doubleness generally increases the ornamental value of floriculture crops. We have released double-flowered periwinkle cultivars (Chen and Yeh, 2012) and proposed that a nuclear recessive gene controls the double-flowering phenotype, and the dominant allele conditions single flowers in either the homozygous or heterozygous state (Chen et al., 2012).
Presently, there appears no double-flowered periwinkle cultivar with a trailing growth habit.

The objectives of this study were to 1) compare the stem anatomy and MOE between upright and trailing cultivars, 2) determine the genetics and inheritance of trailing habit and flower form, and 3) develop new periwinkle progeny with trailing habit and double flowers.

Materials and Methods

All the plants were raised from seeds, and young plants were transplanted into 9-cm pots containing 2 peatmoss (Fafard No. 1; Conrad Fafard, Agawam, MA) : 1 vermiculite : 1 perlite (by volume). The plants were placed in a greenhouse with day/night temperatures of 32/25 °C under 11.1–13.2 h daylengths, with an average noontime photosynthetic photon flux of 1382 μmol·m⁻²·s⁻¹. Plants were fertilized with water-soluble 20N–8.7P–16.6K (20–20–20 General Purpose; JR Peters, Allentown, PA) at 1 g·L⁻¹ and irrigated as needed.

STEM ANATOMY AND MOE BETWEEN UPRIGHT AND TRAILING CULTIVARS. Stem anatomy study was conducted between the upright cultivar Vitesse Pink and trailing cultivar Cora Cascade Polka Dot when the plants had two flowers. The lowest internode of the main stem was cross-sectioned by hand and stained with 0.5% safranin O (Sigma-Aldrich, St Louis, MO) and 1% alcian blue (in 3% acetic acid; Sigma-Aldrich). Stem tissues were observed with a light microscope (Eclipse E600; Nikon, Tokyo, Japan). In addition, stem segments of upright ‘Vitesse Pink’ were immersed in H₂O₂ solution (30% H₂O₂ : distilled water : glacial acetate = 1:4:5) at 60 °C for 3 d. Then, rinsed with distilled water thoroughly and stained with 1% safranin O (in 50% ethanol). After rinsing, the sample was examined under a microscope for compositions of tissues.

Apart from ‘Vitesse Pink’ and ‘Cora Cascade Polka Dot’, a more trailing cultivar Cora Cascade Strawberry was added for the comparison of their stem tissues and MOE. The 1st, 5th, 8th, 11th, and 13th (the youngest) internodes above cotyledons were sectioned. Proportions of cortex, phloem fiber, phloem, xylem, and pith area were analyzed by ImageJ (National Institutes of Health, Bethesda, MD) after taking microscopic photos (Optronics Microfire True Color Firewire Digital CCD; Meyer Instruments, Houston, TX). MOE was measured after the process described by Jacobsen et al. (2005). Plants were watered thoroughly at the day before MOE test and sealed in plastic bags to prevent water evaporation. Stem segments were cut and the test was conducted within 20 min. A four-point bending test was conducted under 25 °C using a microtester (Microtest M200; Deben, East Grinstead, UK).

The experiment was arranged in a completely randomized design and replicated three times with three-to-five stem samples per replication for each cultivar. Least significant differences were determined at P < 0.05 to compare among the means. The relationship between proportions of various tissue areas and internode position was determined by using regression analysis using SigmaPlot (version 10.0; Systat Software, San Jose, CA). The relationship between proportions of various tissue areas and MOE was determined with correlation analysis.

INHERITANCE OF TRAILING HABIT AND FLOWER FORM. Upright, double-flowered cultivars Taoyuan No. 1-Rose Girl, Taoyuan No. 4-Summer Snow, and Taoyuan No. 5-Red Lady and trailing, single-flowered cultivars Cora Cascade Strawberry, Cora Cascade Polka Dot, and Cora Cascade Magenta were self-pollinated and cross-pollinated. Because periwinkle is a self-pollinating species, we performed emasculation and cross-pollination following the method described by Miyajima (2004). F₁, F₂, and backcross family were produced. Plants were raised as described earlier. Growth habit and flower form were recorded when the plants had two open flowers, following the International Union for the Protection of New Varieties of Plants guidelines. Data were subjected to
chi-square test for goodness of fit to compare actual ratios with expected ratio.

Table 1. Proportion of tissue structure and modulus of elasticity (MOE) of different internode positions in periwinkle cultivars Vitesse Pink (VP), Cora Cascade Polka Dot (CCP), and Cora Cascade Strawberry (CCS).

| Internode position | Cortex area (%) | Phloem fiber area (%) | Phloem area (%) | Xylem area (%) | Pith area (%) | MOE (N·mm⁻²) |
|--------------------|-----------------|-----------------------|-----------------|----------------|--------------|--------------|
| 1                  |                 |                       |                 |                |              |              |
| VP                 | 31.0 b⁻         | 3.0 a                 | 33.1 a          | 30.3 b         | 2.5 b        | 251.27 a     |
| CCP                | 35.5 a          | 0.0 b                 | 28.8 b          | 33.3 a         | 2.4 b        | 244.96 a     |
| CCS                | 36.9 a          | 0.0 b                 | 31.5 a          | 27.5 c         | 4.1 a        | 140.31 b     |
| 5                  |                 |                       |                 |                |              |              |
| VP                 | 41.9 b          | 5.5 a                 | 21.6 c          | 17.1 b         | 13.8 a       | 145.77 a     |
| CCP                | 45.3 a          | 0.0 b                 | 24.2 b          | 19.6 a         | 11.0 b       | 124.65 b     |
| CCS                | 43.0 b          | 0.0 b                 | 26.1 a          | 16.7 b         | 14.2 a       | 73.15 c      |
| 8                  |                 |                       |                 |                |              |              |
| VP                 | 45.5 a          | 4.1 a                 | 20.3 a          | 13.9 a         | 16.2 a       | 105.93 a     |
| CCP                | 45.9 a          | 0.0 b                 | 22.0 a          | 16.2 a         | 15.9 a       | 89.36 a      |
| CCS                | 48.9 a          | 0.0 b                 | 22.2 a          | 13.5 a         | 15.4 a       | 50.90 b      |
| 11                 |                 |                       |                 |                |              |              |
| VP                 | 48.9 a          | 2.8 a                 | 18.9 a          | 10.8 a         | 19.5 a       | 65.8 a       |
| CCP                | 48.0 a          | 0.0 b                 | 19.9 a          | 10.6 a         | 21.5 a       | 50.90 a      |
| CCS                | 51.0 a          | 0.0 b                 | 18.8 a          | 8.6 a          | 21.5 a       | 33.00 b      |
| 13                 |                 |                       |                 |                |              |              |
| VP                 | 52.6 a          | 0.2 a                 | 18.4 a          | 8.6 a          | 20.2 a       | 24.01 a      |
| CCP                | 50.7 a          | 0.0 b                 | 17.8 a          | 8.5 a          | 23.0 a       | 25.61 a      |
| CCS                | 52.3 a          | 0.0 b                 | 18.1 a          | 8.3 a          | 21.4 a       | 18.93 a      |

Significance

| Internode position | L*** | NS | L***Q* | L***Q** | L***Q* | L*** |
|--------------------|------|----|--------|---------|--------|------|
| 1                  |      |    |        |         |        |      |
| 5                  |      |    |        |         |        |      |
| 8                  |      |    |        |         |        |      |
| 11                 |      |    |        |         |        |      |
| 13                 |      |    |        |         |        |      |

Notes:
1. Internode position from the base, as counted above the cotyledon.
2. Means within columns followed by different letters per internode position are significantly different (P < 0.05) based on Fisher’s protected least significant difference.
3. Nonsignificant or significant at P < 0.05, 0.01, or 0.001, respectively; L = linear; Q = quadratic.

Table 2. Correlation coefficient between modulus of elasticity and tissue area proportion of different internodal positions in periwinkle cultivars Vitesse Pink, Cora Cascade Polka Dot, and Cora Cascade Strawberry.

| Internode position | Cortex area | Phloem fiber area | Phloem area | Xylem area | Pith area | MOE |
|--------------------|-------------|-------------------|-------------|------------|-----------|-----|
| 1                  | –0.71*      | 0.55              | 0.08        | 0.79*      | –0.72*    |     |
| 5                  | –0.08       | 0.70*             | –0.57       | 0.34       | 0.17      |     |
| 8                  | –0.58       | 0.76*             | 0.12        | 0.44       | –0.13     |     |
| 11                 | –0.77*      | 0.67*             | 0.53        | 0.14       | 0.10      |     |

Notes:
1. Internode position from the base, as counted above the cotyledon.
2. Significance at P < 0.05 level based on the table of critical values for Pearson’s correlation coefficients (r) with df = 14.

Table 3. Phenotype of six periwinkle cultivars used as parents for crosses in this study and their self-pollinated progenies.

| Cultivar (abbreviation) | Growth habit | Flower form |
|-------------------------|--------------|-------------|
| Taoyuan No. 1-Rose Girl (TY1) | Upright | Double |
| Taoyuan No. 4-Summer Snow (TY4) | Upright | Double |
| Taoyuan No. 5-Red Lady (TY5) | Upright | Double |
| Cora Cascade Polka Dot (CCP) | Trailing | Single |
| Cora Cascade Strawberry (CCS) | Trailing | Single |
| Cora Cascade Magenta (CCM) | Trailing | Single |
| TY1 ⊗TY1 | Upright | Double |
| TY4 ⊗TY4 | Upright | Double |
| TY5 ⊗TY5 | Upright | Double |
| CCP ⊗CCP | Trailing | Single |
| CCS ⊗CCS | Trailing | Single |
| CCM ⊗CCM | Trailing | Single |

Notes:
1. Thirty progenies were examined.

Results and Discussion

**STEM ANATOMY AND MOE BETWEEN UPRIGHT AND TRAILING CULTIVARS.** Internode cross sections of the upright ‘Vitesse Pink’ revealed the existence of phloem fiber in ‘Vitesse Pink’ (Fig. 2A and C) but not in ‘Cora Cascade Polka Dot’ (Fig. 2B). Hand-broken stem segments and macerated stem segments showed the existence of phloem fiber in ‘Vitesse Pink’ (Fig. 2A and C) but not in ‘Cora Cascade Polka Dot’ (Fig. 2B). Kartmazova (1972) also showed the existence of stem phloem fiber in upright periwinkle. We dissected the stem and observed the phloem fiber in four other upright cultivars (including the Taoyuan series and Jams ‘N Jellies Blackberry) but not in 18 other trailing cultivars (including the Cora Cascade, Mediterranean, and Boa series) in another experiment (data not shown). Results indicate that phloem fiber affects the growth habit of periwinkle in that trailing cultivars lack phloem fiber. Gorshkova et al. (2012) have demonstrated that fibers are important in establishing plant architecture, as a source of mechanical support. Wang and Gregg (1989) reported that ‘Jane Cowl’ hibiscus (Hibiscus L.) produced trailing, instead of upright, shoots after the treatment with uniconazole because of the decreased cell size and cell wall thickness, as well as the greatly diminished phloem fiber and fibers that are hollow.
Table 4. Segregation for upright and trailing growth habit in progeny of 21 periwinkle crosses.

| Crosses (cross no.) | Growth habit | Test ratio | χ² | P  |
|---------------------|--------------|------------|----|----|
|                     | Upright      | Trailing   |     |    |
| F1                  |              |            |    |    |
| TY1 × CCS (1)      | 12           | 0          | 1:0| 0  | 1 |
| TY4 × CCP (2)      | 23           | 0          | 1:0| 0  | 1 |
| TY5 × CCM (3)      | 14           | 0          | 1:0| 0  | 1 |
| CCM × TY1 (4)      | 31           | 0          | 1:0| 0  | 1 |
| CCM × TY5 (5)      | 13           | 0          | 1:0| 0  | 1 |
| CCP × TY1 (6)      | 18           | 0          | 1:0| 0  | 1 |
| CCP × TY5 (7)      | 38           | 0          | 1:0| 0  | 1 |
| CCS × TY1 (8)      | 77           | 0          | 1:0| 0  | 1 |
| CCS × TY5 (9)      | 13           | 0          | 1:0| 0  | 1 |
| F2                  |              |            |    |    |
| TY1 × CCS F1-1 × (10) | 23  | 5          | 3:1| 0.76| 0.38|
| TY4 × CCP F1-2 × (11) | 40  | 12         | 3:1| 0.10| 0.75|
| TY4 × CCP F1-4 × (12) | 28  | 5          | 3:1| 1.71| 0.19|
| TY4 × CCP F1-6 × (13) | 51  | 10         | 3:1| 2.41| 0.12|
| TY5 × CCP F1-15 × (14) | 52  | 17         | 3:1| 0  | 0.94|
| TY5 × CCM F1-1 × (15) | 138 | 45         | 3:1| 0.02| 0.90|
| TY5 × CCM F1-2 × (16) | 93  | 21         | 3:1| 2.63| 0.10|
| TY5 × CCM F1-3 × (17) | 152 | 57         | 3:1| 0.58| 0.45|
| TY5 × CCM F1-9 × (18) | 243 | 99         | 3:1| 2.84| 0.09|
| CCP × TY1 F1-3 × (19) | 31  | 5          | 3:1| 2.37| 0.12|
| CCP × TY5 F1-24 × (20) | 71  | 28         | 3:1| 0.57| 0.45|
| CCP × TY5 F1-31 × (21) | 136 | 33         | 3:1| 2.70| 0.10|

2TY1 = Taoyuan No. 1-Rose Girl; TY4 = Taoyuan No. 4-Summer Snow; TY5 = Taoyuan No. 5-Red Lady; CCP = Cora Cascade Polka Dot; CCM = Cora Cascade Magenta.

Table 5. Joint segregation of growth habit (upright and trailing) and flower form (single and double) in progeny of 15 periwinkle crosses.

| Crosses (cross no.) | Growth habit/flower form | Test ratio | χ² | P  |
|---------------------|--------------------------|------------|----|----|
|                     | US | TS | UD | TD |     |    |
| F1                  |   |    |    |    | 1:0:0:0 | 0  | 1 |
| TY1 × CCS (22)      | 14 | 0  | 0  | 0  | 1:0:0:0 | 0  | 1 |
| TY4 × CCP (23)      | 38 | 0  | 0  | 0  | 1:0:0:0 | 0  | 1 |
| TY5 × CCM (24)      | 14 | 0  | 0  | 0  | 1:0:0:0 | 0  | 1 |
| CCP × TY5 (25)      | 38 | 0  | 0  | 0  | 1:0:0:0 | 0  | 1 |
| F2                  |   |    |    |    | 1:0:0:0 | 0  | 1 |
| TY1 × CCS F1-1 × (26) | 55 | 17 | 15 | 6  | 9:3:3:1 | 0.50| 0.92|
| TY4 × CCP F1-6 × (27) | 43 | 5  | 8  | 5  | 9:3:3:1 | 7.23| 0.07|
| TY4 × CCP F1-15 × (28) | 35 | 12 | 17 | 5  | 9:3:3:1 | 1.83| 0.61|
| TY5 × CCM F1-3 × (29) | 107 | 43 | 45 | 14 | 9:3:3:1 | 2.25| 0.52|
| TY5 × CCM F1-9 × (30) | 174 | 75 | 69 | 24 | 9:3:3:1 | 4.29| 0.23|
| CCP × TY5 F1-24 × (31) | 63 | 21 | 8  | 7  | 9:3:3:1 | 7.40| 0.06|
| CCP × TY5 F1-31 × (32) | 106 | 24 | 30 | 9  | 9:3:3:1 | 3.44| 0.33|

Backcross

TY1 × CCS F1-1) × TY1 (33) | 14 | 0  | 12 | 0  | 1:0:1:0 | 0.15| 0.69|
| CCM × (TY1 × CCS F1-1) (34) | 98 | 98 | 0  | 0  | 1:1:0:0 | 0  | 1 |
| (TY5 × CCM F1-1) × TY5 (35) | 58 | 0  | 41 | 0  | 1:0:1:0 | 2.92| 0.08|
| CCM × (TY5 × CCM F1-1) (36) | 67 | 63 | 0  | 0  | 1:1:0:0 | 0.12| 0.73|

2TY1 = Taoyuan No. 1-Rose Girl; TY4 = Taoyuan No. 4-Summer Snow; TY5 = Taoyuan No. 5-Red Lady; CCP = Cora Cascade Polka Dot; CCM = Cora Cascade Magenta.

Phloem fibers were observed in the upright ‘Vitesse Pink’ throughout the internodes sampled, but not in the trailing ‘Cora Cascade Polka Dot’ and ‘Cora Cascade Strawberry’ (Table 1). Trailing periwinkle cultivars lack stem phloem fiber, regardless of internode position. This differs to prostrate C. vitalba and C. maritima that phloem fibers are displaced outward as by plant development, which led to disrupted mechanical integrity (Rowe et al., 2004).

Regardless of cultivars, the basal internodes had lower proportions of cortex and pith but higher proportions of phloem and xylem, and MOE (Table 1). Rowe et al. (2004) also reported that biomechanical properties of the plant stem can change markedly during growth where young apical parts are relatively compliant and the basal and older stages are relatively stiff.

Except that MOE of the youngest internode did not differ among the cultivars; the upright Vitesse Pink had the highest MOE (Table 1). Our results are consistent with previous reports that mechanical properties of the upright stems are stiffer than horizontal axes in terms of both flexural stiffness and structural Young’s modulus (Rowe and Speck, 1998).

Except for the youngest internode, anatomical observation and correlation analysis showed that ‘Cora Cascade Strawberry’ had consistently lower MOE and proportion of basal xylem area than ‘Cora Cascade Polka Dot’ (Tables 1 and 2). This may explain why ‘Cora Cascade Strawberry’ exhibits a more trailing growth habit than ‘Cora Cascade Polka Dot’. Rowe et al. (2004) showed sclerenchyma area correlates well with the changes in MOE; i.e., stem stiffness or elasticity for other plants. Results in this study show that phloem fiber appeared in the upright periwinkle cultivars but not in trailing cultivars, whereas xylem is crucial in determining mechanical strength in trailing cultivars.

Inheritance of upright and trailing growth habits.

Three upright/double-flowered cultivars (Taoyuan No. 1-Rose Girl, Taoyuan No. 4-Summer Snow, and Taoyuan No. 5-Red Lady) and three trailing/single-flowered cultivars (Cora Cascade Strawberry, Cora Cascade Polka Dot, and Cora Cascade Magenta) were used as parents. Progeny from self-pollination of these cultivars exhibited the same phenotype as their parents (Table 3), indicating the homozygosity status of their growth habit and flower form.

All F1 plants of upright × trailing (cross no. 1–3) and trailing × upright crosses (cross no. 4–9), including reciprocal crosses between ‘Taoyuan No. 1-Rose Girl’ and ‘Cora Cascade Strawberry’, ‘Taoyuan No. 5-Red Lady’ and ‘Cora Cascade Magenta’ were upright (Table 4). The F2 generation derived from self-pollinating F1 (cross no. 10–21) fit a 1:3:3:1:1 model of segregation ratio (χ² = 2.84, P = 0.09–0.94). These results indicate that growth habit seems to be controlled by one single nuclear locus with two alleles and that upright allele was dominant over the trailing allele. Similar examples for...
supports that two independent loci control the two traits of growth habit and flower form. Plants in the F2 generation fitted a 9 upright/single-flower : 3 trailing/single-flower : 3 upright/double-flower : 1 trailing/double-flower ratio (Table 5). Results are consistent with Table 4 that upright is dominant over trailing and support the previous observation that single-flower is dominant over double-flower (Chen et al., 2012).

The F2 progeny from self-pollinating F1 plants (cross no. 26–32) exhibited four phenotypic types (Table 5), indicating that the F1 plants were heterozygous for growth habit and flower form. Plants in the F2 generation fitted a 9 upright/single-flower : 3 trailing/single-flower : 3 upright/double-flower : 1 trailing/double-flower ratio (χ² = 0.50–7.40, P = 0.06–0.92). The observed ratio supports that two independent loci control the two traits of growth habit and flower form. Backcrosses of the F2 plants to upright, double-flowered parents (cross no. 33, 35) resulted in all upright plants but were segregated into 1 single : 1 double-flowered progeny (χ² = 0.15–2.92, P = 0.08–0.69). Backcrosses with trailing, single-flowered parents (cross no. 34, 36) produced all single-flowered progeny, but were segregated into 1 upright : 1 trailing ratio (χ² = 0–0.12, P = 0.73–1). Segregation data obtained from F1, F2, and backcross families (Table 5) confirm previous observations (Chen et al., 2012) that the double-flowered phenotype is controlled by a recessive allele in periwinkle. Similar examples for a monogenic recessive gene conditioning flower doubling have been reported in

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