Attempt to Classify *Petunia* Species on the Basis of Corolla Shape

Chika AOKI and Kazumi HATTORI

Faculty of Agriculture, Nagoya University, Nagoya, 464-01

Four parameters, namely, corolla diameter (CD), corolla tube length (TL), corolla length (CL) and corolla tube diameter (TD) of *Petunia* were measured. These measurements and ratios of TL/CD, CL/CD and TL/TD were compared in order to study the possibility of using them as corolla shape indices in the classification of *Petunia*. The values of CD, CL, TD and ratios of TL/CD, CL/CD were affected by the temperature conditions controlled at 30/24, 25/19, 20/14°C (day/night). With the decrease of the temperature, the CD and TD values clearly increased. Since the changes in CD were drastic, the values of the TL/CD and CL/CD ratios tended to decrease with decreasing temperature. Measurements of CD, TL and CL were significantly different between white-flowered species (*P. axillaris* and *P. parodii*) and purple-flowered *P. inflata*. Flowers of *P. parodii* and *P. axillaris* resembled each other, whereas the values of TL and the three ratios at the same temperature were distinct. In *P. inflata*, all the measurements and ratios did not overlap with those of white-flowered species, and the values of the ratios were the smallest among the species and lines investigated in this study. In the reciprocal F₁ hybrids of *P. axillaris* and *P. inflata*, the measurements and ratios were intermediate between those of the parental species. There was no significant difference between the ratios of reciprocal F₁ hybrids. Moreover, the ratios of *P. hybrida* were intermediate between those of *P. axillaris* and *P. inflata*. In *P. hybrida*, the values of the measurements and ratios of 'W166K' were slightly larger than those of 'Rose'. The CL/CD ratio showed the largest differences among the species and lines. The results of the present study suggest that the three ratios are very useful as indices of the corolla shape for the classification of *Petunia* species.

KEY WORDS: *Petunia*, corolla diameter, corolla tube length, corolla length, corolla tube diameter, corolla shape, temperature effect

Introduction

Petunia is one of the most important ornamental plants as it is cultivated in many areas of the world. It is a very convenient genus as a model material, because plants can be readily grown from seeds and can flower in about 3 months. Yields of about 100-1000 seeds per capsule can be attained. The plant can be propagated by rooted cuttings or plantlets regenerated from cultured tissues. Therefore, *Petunia* has been used as material for studies on self-incompatibility, chromosomal behavior and biochemistry of flower color pigmentation, etc., since the 19th century (SINK 1984). Recently, it has often been used in the field of biotechnology (BINDING 1976, FREARSON *et al.* 1973) or molecular biology (BOESHORE *et al.* 1985, BOVENBERG *et al.* 1980, COLIJN *et al.* 1979, KOES *et al.* 1989, MACLEAN *et al.* 1988).

Contemporary *Petunia* taxonomy began with FRIES' monograph in 1911, and it is thought that the genus *Petunia* contains more than 30 species (SINK 1984). In these species, putative ancestors for *P. hybrida* Vilm. as a commercial plant are *P. axillaris* (Lam.) B. S. P., *P. parodii* STEERE, *P. integrifolia* (Hook). SCHINZ and THELLUNG (*P. violacea* R. E. FRIES), *P. inflata* R. E. FRIES and *P. occidentalis* R. E. FRIES (WIJSMAN 1982). *Petunia axillaris* and *P. parodii* are closely related. Both species are white-flowered and their corolla tubes are slender. Simi-

Received July 30, 1990.

larly, *P. integrifolia*, *P. inflata* and *P. occidentalis* are also closely related. They are purple (or magenta)-flowered and their corolla tubes are short and funnel-shaped. As shown in Fig. 1, the corolla shape of the F_1 hybrid of *P. inflata* $\times$ *P. axillaris* is intermediate between that of the parents. According to WIJSMAN (1982), CABRERA (1977) considered that *P. axillaris* and *P. parodii* were conspecific. WIJSMAN (1982) who considered that the three purple-flowered species were also conspecific, concluded that the two white-flowered species belonged to one species, *P. axillaris* (with subspecies (ssp.) *axillaris* and *parodii*), while the three purple-flowered species belonged to another species, *P. integrifolia* (with ssp. *integrifolia*, *inflata* and *occidentalis*).

The genus *Petunia* contains two groups of species based on the chromosome number: either $2n=14$ or $2n=18$. (WIJSMAN, *et al.* 1983). Considerable differences between both groups were observed, for example, in the corolla size, corolla color and leaf shape, etc. According to SINK (1984), FRIES (1911) classified the species in the genus *Petunia* based on the corolla color (white or purple) and morphological differences in corolla tube (slender or funnel-shaped). On the other hand, SMITH and DOWNS (1966) classified them based on the morphology of the calyx and the plant type, whether herb or shrub. The classification of WIJSMAN *et al.* (1983, 1985) based on the putative number of chromosomes corresponded to that of SMITH and DOWNS (1966). WIJSMAN and DE JONG (1985) who suggested that the species with $2n=14$ chromosomes belong to a different genus from that of the species with $2n=18$ chromosomes assigned the former group of species to the genus *Stimoryne*. However, in the present paper, the species *axillaris*, *parodii* and *inflata* with a chromosome number $2n=14$ have been considered to belong to the genus *Petunia*.

WIJSMAN (1982) stated that the distinction between ssp. *axillaris* and ssp. *parodii* was possible by comparing the differences in the corolla tube length and the ratios of corolla tube length to corolla diameter. In this paper, the corolla diameter (CD), corolla tube length (TL), corolla length (CL) and corolla tube diameter (TD) of some species, cultivars and hybrids were measured and compared. Then, the three ratios derived from the measurements of these parameters were compared to consider their usefulness as corolla shape indices. Moreover, flowers opening under three temperature conditions were examined to determine whether the temperature conditions affected the corolla shape.

Fig. 1. *Petunia* flowers. From left to right, *P. axillaris*, F_1 hybrid of *P. inflata* $\times$ *P. axillaris*, *P. inflata*. a: seen from above, and b: lateral view.

Materials and Methods

Flowers of three wild species, two cultivars of *P. hybrida* and reciprocal F_1 hybrids of *P. axillaris* and *P. inflata* were used for this study. Seeds of *P. axillaris*, *P. parodii* and *P. inflata* were given by Dr. SINK, Professor, Michigan State University in 1978. The characteristics of the plant materials are as follows:

P. axillaris (Lam.) BRITTON, STERN and POGGENBURG—Corolla color - white; Corolla tube - slender and subcylindric (Fig. 1); Stems - rigid.

P. parodii STEERE—Corolla color - white; Corolla shape and plant type similar to those of *P. axillaris*; Corolla tube - longer than that of *P. axillaris*.

P. inflata R. E. FRIES—Corolla color - purple (or magenta); Corolla tube - short and funnel shaped (Fig. 1); Stems - thinner and more flexible than those of *P. axillaris* and *P. parodii*.

P. × hybrida 'W166K'—Corolla color - dark red or dark purple; Stems - rather rigid.

P. × hybrida 'Rose'—Corolla color - bright pink; Dwarf.

To avoid segregation within a single entry, all the materials were propagated from rooted cuttings and grown in the greenhouse. After 2-3 flowers opened, the pots in which *Petunia* grew were transferred to the phytotron under natural light conditions at controlled temperatures 30/24 (high temperature), 25/19 (middle temperature), 20/14 (low temperature) °C day/night. Flowers which opened in the greenhouse were not used as samples, and only those which opened in the phytotron were used. Measurements were performed for the flowers of *P. parodii*, *P. axillaris*, *P. × hybrida* 'W166K' and 'Rose' in September to October 1988 and for those of *P. inflata* and reciprocal F_1 hybrids in January to March 1989.

Measurements of CD, TL, CL and TD were taken as indicated in Fig. 2. In all the entries, the measurements involved 20 flowers at full opening and anther dehiscence. However, only 10 flowers in 'Rose' and 6 flowers in 'W166K' fully opened under the 30/24 °C treatment.

In the measurements for each flower, three ratios, TL/CD, CL/CD and TL/TD were calculated.

Fig. 2. Determination of the flowering parameters. CD: Corolla diameter, TL: Corolla tube length, CL: Corolla length, TD: Corolla tube diameter

Results

Measurements of CD, TL, CL and TD under three temperature conditions are shown in Table 1. The values of CD, CL and TD were affected by the temperature conditions, unlike those of TL (Tables 2a-d). In most entries, the values of CD and TD tended to increase with decreasing temperature. However, in the F_1 hybrid of *P. inflata* $\times$ *P. axillaris*, the values of CD did not exhibit this tendency. The values of CL were the largest under the middle temperature conditions in most entries. In the F_1 hybrid of *P. axillaris* $\times$ *P. inflata* and 'Rose', the values of CL were the largest under the low temperature conditions, whereas under the high temperature conditions, the values of CL were not the largest in all cases (Table 1, Figs. 3, 4).

Corolla of *P. parodii* and *P. axillaris* was similar in shape, but the TL value of *P. parodii* was larger than that of *P. axillaris*. In *P. inflata*, the values of CD, TL and CL were the smallest among the entries, whereas the values of TD were larger than those of the white-flowered species (*P. parodii* and *P. axillaris*). The values in the F_1 hybrids were intermediate between those of the parental species except for TD. In the reciprocal F_1 hybrids, the TD values were larger than that of *P. inflata*. The values of CD did not differ significantly in 'W166K' from that of *P. axillaris*, but those of TL and CL were smaller, and those of TD were larger, when flowering occurred at the same temperature. In 'Rose', all the values were a little smaller than those of 'W166K' under the same temperature conditions (Table 1).

The values of the three ratios of *P. parodii* were larger than those of *P. axillaris*. As shown in Fig. 5, the values of the TL/CD ratios of *P. parodii* and *P. axillaris* were differ-

Table 1. Mean values of corolla characteristics in *Petunia* species and lines grown under three different temperature regimes.

Entry	CD (cm)			TL (cm)			CL (cm)			TD (cm)		
	H	M	L	H	M	L	H	M	L	H	M	L
<i>P. parodii</i>	5.19a	6.28a	6.35a	5.63a	6.52a	5.79a	8.35a	9.66a	8.94a	0.58a	0.64a	0.69a
<i>P. axillaris</i>	4.64bc	5.67b	6.07b	4.37b	4.83b	4.56b	6.81b	7.63b	7.50b	0.50b	0.60b	0.62b
<i>P. inflata</i>	2.89d	3.56c	3.86c	1.38c	1.55c	1.61c	2.64c	3.15c	3.20c	0.76c	0.83c	0.88c
<i>P. axillaris</i> $\times$ <i>P. inflata</i>	4.61b	4.78d	5.40d	2.41de	2.35d	2.44d	5.11d	5.16d	5.54d	0.81d	0.83c	0.94de
<i>P. inflata</i> $\times$ <i>P. axillaris</i>	4.38e	5.13e	4.75e	2.29d	2.39d	2.31e	4.73e	5.29d	5.05e	0.74c	0.86c	0.91cd
<i>P. \times hybrida</i> 'W166K'	4.78c	5.72b	5.94b	3.33f	3.46e	3.40f	5.75f	6.32e	6.28f	0.92e	1.02d	1.05f
<i>P. \times hybrida</i> 'Rose'	3.96f	4.73f	5.33d	2.49e	2.63f	2.58g	4.49g	4.95f	5.08e	0.78cd	0.87c	0.98e

H: High temperature, M: Middle temperature, L: Low temperature

In one character of an entry, sizes between different temperature conditions are significantly different at least of 5% level except for ns (not significant).

CD: corolla diameter, TL: corolla tube length, CL: corolla length, TD: corolla tube diameter.

Means within a column followed by the same letter are not significantly different at 1% level by Duncan's multiple range test.

Table 2. Tables of analysis of variance in given floral characteristics.

(a) Corolla diameter (CD)

Factors	DF	SS	MS	F	P
Temperature	2	4.06	2.03	34.9898	<0.01
Entry	6	11.78	1.96	33.8316	<0.01
Error	12	0.70	0.06		
Total	20	16.54			

(b) Corolla tube length (TL)

Factors	DF	SS	MS	F	P
Temperature	2	0.24	0.12	3.8692	>0.05, ns
Entry	6	42.94	7.16	230.1110	<0.01
Error	12	0.37	0.03		
Total	20	43.55			

(c) Corolla length (CL)

Factors	DF	SS	MS	F	P
Temperature	2	1.54	0.77	16.4925	<0.01
Entry	6	66.56	11.09	237.1350	<0.01
Error	12	0.56	0.05		
Total	20	68.67			

(d) Corolla tube diameter (TD)

Factors	DF	SS	MS	F	P
Temperature	2	0.07	0.03	59.7887	<0.01
Entry	6	0.38	0.06	110.8870	<0.01
Error	12	0.01	0.00		
Total	20	0.46			

ent by about 0.85 under the low temperature conditions, 0.95 under the middle temperature and about 1.00 under the high temperature conditions. The values of the CL/CD ratios differed by about 1.35, 1.45 and 1.50 under the low, middle and high temperature conditions, respectively (Fig. 6). However, when the TL/CD and CL/CD ratios were compared between *P. parodii* at low temperatures and *P. axillaris* at high temperatures, the values of both ratios overlapped. The values of all the ratios of *P. inflata* were the smallest among the three species, and they did not overlap with the values of the white-flowered species. In *P. inflata*, most of the values of the TL/CD ratios were less than 0.5 and those of the CL/CD ratios were less than 1. In the white-flowered species, all the values of the CL/CD ratios were more than 1. Ratios of the F₁ hybrids were intermediate between those of the paren-

Fig. 3. Flowers of *P. inflata* under different temperature conditions. From left to right, flowering at high, middle, low temperature. a: seen from above, and b: lateral view.

Fig. 4. Flowers of F₁ hybrids. a: *P. inflata* × *P. axillaris* and b, c: *P. axillaris* × *P. inflata*. From left to right, flowering at high, middle, low temperature.

tal species. There was no difference in the three ratios between reciprocal F₁ hybrids at the same temperature. In *P. hybrida*, the three ratios were distributed near the range of the ratios of the F₁ hybrids. The values of all the ratios of 'Rose' were slightly smaller than those of 'W166K' (Figs. 5, 6, 7). The CL/CD ratios showed the most distinct difference out of the three ratios among entries. Among the white-flowered species, *P. inflata* and others at the same temperature, the values of the CL/CD ratios did not overlap (Fig. 6). Moreover, the values of the TL/CD ratios did not overlap, in the white-flowered species and the other 5 entries at the same temperature also (Fig. 5). The values of the TL/TD ratios were larger

Fig. 5. TL/CD ratio in *Petunia* species and lines grown under three temperature regimes.

Fig. 6. CL/CD ratio in *Petunia* species and lines grown under three temperature regimes.

in *P. parodii* and *P. axillaris* than those in the other entries. There was no significant difference among entries except for the white-flowered species, under all three temperature conditions, but, in *P. inflata*, the values were a little smaller than those of the other entries (Fig. 7).

Discussion

In the taxonomical classification of *Petunia* based on morphology, SMITH and DOWNS (1966) reported a detailed analysis of sizes and shapes of plant parts (corolla tube length, calyx tube length, calyx lobe length, leaf shape and leaf length, etc.). WIJSMAN (1982) stated that the corolla tube length and the TL/CD ratio were different between *P. parodii* and *P. axillaris*, as observed in this study.

The duration of the anthesis period of many *Petunia* species which grow wild is about one to three months in late spring to early autumn (SMITH and DOWNS 1966), although, the plants cultivated in the greenhouse can flower at about 10-40°C. Therefore, the effect of the

temperature on flowering must be considered, if species or lines are characterized by the size and ratios of the corolla shape. Table 1 shows that the corolla size, especially the corolla diameter, sensitively responds to the temperature. It was reported by ROBERTS and STRUCKMEYER (1938a, b) that the vegetative growth and flowering of many plants were affected by the temperature in rudbeckia. PIRINGER and CATHEY (1960) studied the influences of environmental factors on the growth and flowering of petunia. They stated that the shoot length increased and the plant flowered earlier at high temperature in *P. × hybrida* 'Ballerina', when the temperature was controlled at 50-80°F. Within the range of temperatures set up in the present study, the corolla diameter increased with decreasing temperature.

WIJSMAN (1982) reported that the corolla shape of *P. axillaris* and *P. parodii* could be distinguished by TL measurements and TL/CD ratio, that is, a TL value of less than 46mm and TL/CD ratio of less than 1 in *P. axillaris*, a TL value of more than 46mm and a TL/CD ratio of more than 1 in *P. parodii*. In the present study, based on the results listed in Tables 1 and 2b, TL of *P. parodii* was longer than that of *P. axillaris*. Comparison of the TL/CD ratio between both species at the same temperature revealed that the values of the ratios of *P. parodii* were larger than those of *P. axillaris*. However, the values of the ratios of *P. parodii* at low temperatures and those of *P. axillaris* at high temperatures overlapped, suggesting that the temperature conditions in the flowering period must be uniform when corolla shapes are compared.

In the present study, the CL/CD, TL/TD and TL/CD ratios, were compared to classify the species and lines of *Petunia*. It was evident that the larger the values of the ratios, the more 'slender' the corolla shape. A corolla with large ratio values corresponded to the shape of the white-flowered species, whereas a corolla with small values corresponded to that of *P. inflata*. All the three ratios were useful to distinguish between *P. parodii* and *P. axillaris* at the same temperature. The values of the ratios of *P. inflata* were smaller, that is, *P. inflata* exhibited a corolla shape different from that of the white-flowered species. The corolla of the F₁ hybrids and *P. hybrida* gave intermediate values for the ratios between those of *P. axillaris* and *P. inflata*.

Fig. 7. TL/TD ratio in *Petunia* species and lines grown under three temperature regimes.

The variation of the values of the CL/CD ratios was the most conspicuous out of the three ratios. The TL/CD ratios did not clearly show differences among *P. inflata*, F₁ hybrids and 'Rose'. There was distinct difference in the TL/TD ratios between the white-flowered species and the other five entries. It was thus considered that the TL/TD ratio is a useful index to determine whether a corolla tube shape resembles that of *P. parodii* or *P. axillaris*.

There was no difference in the three ratios between reciprocal F₁ hybrids, but there was a slight difference between the two *P. hybrida* cultivars. The values of all the ratios of 'W166K' were slightly larger than those of the F₁ hybrids, whereas the values of 'Rose' were closer to them or slightly smaller. The shape of the corolla limb of 'Rose' resembled that of *P. inflata*. It was thus concluded that the corolla shape of *Petunia* could be quantitatively evaluated using these three ratios.

Consequently, it is considered that the ratios are useful as indices of the corolla shape for the classification of species and lines, and analysis of their relationships at the same temperature.

Literature Cited

- BINDING, H. 1976. Somatic hybridization experiments in solanaceous species. *Mol. Gen. Genet.* **144**: 171~175.
- BOESHORE, M. L., M. R. HANSON and S. IZHAR 1985. A variant mitochondrial DNA arrangement specific to *Petunia* stable sterile somatic hybrids. *Plant Mol. Biol.* **4**: 125~132.
- BOVENBERG, W. A., A. J. KOOL and H. J. J. NIJKAMP 1981. Isolation, characterization and restriction endonuclease mapping of *Petunia hybrida* chloroplast DNA. *Nucl. Acid. Res.* **9**: 503~517.
- COLIJN, C. M., A. J. KOOL and H. J. J. NIJKAMP 1979. An effective chemical mutagenesis procedure for *Petunia hybrida* cell suspension cultures. *Theor. Appl. Genet.* **55**: 101~106.
- FREARSON, E. M., J. B. POWER and E. C. COOKING 1973. The isolation, culture and regeneration of *Petunia* leaf protoplasts. *Dev. Biol.* **33**: 130~137.
- KOES, R. E., C. E. SPELT and J. N. M. MOL 1989. The chalcone synthase multigene family of *Petunia hybrida* (V30): differential, light-regulated expression during flower development and UV light induction. *Plant Mol. Biol.* **12**: 213~225.
- MACLEAN, M., W. V. BAIRD, A. G. M. GREATS and R. B. MEAGHER 1988. Determination of copy number and linkage relationships among five actin gene subfamilies in *Petunia hybrida*. *Plant Mol. Biol.* **11**: 663~672.
- PIRINGER, A. A. and H. M. CATHEY 1960. Effect of photoperiod, kind of supplemental light and temperature on the growth and flowering of petunia plants. *Proc. Am. Hortic. Sci.* **76**: 659~660.
- ROBERTS, R. H. and B. E. STRUCKMEYER 1938a. The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. *J. Agric. Res.* **56**: 633~677.
- and ——— 1938b. Photoperiod, temperature and some hereditary responses of plants. *J. Hered.* **29**: 95~98.
- SINK, K. C. 1984. "Monographs on Theoretical and Applied Genetics 9. *Petunia*" SINK, K. C. (ed.) Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1~9.
- SMITH, L. B. and R. J. DOWNS 1966. *Petunia* Juss. In "Solanaceae; Flora ilustrada Catarinense" Cons. Nac. Presq. Itajai, Sa. Cat., Brasil, 261~291.
- WIJSMAN, H. J. W. 1982. On the interrelationships of certain species of *Petunia*. I. Taxonomic notes on the parental species of *Petunia hybrida*. *Acta Bot. Neerl.* **31**: 477~490.
- , J. H. DE JONG and T. M. PEDERSEN 1983. ———. III. The position of *P. linearis* and *P. calycina*. *Acta Bot. Neerl.* **32**: 323~332.
- and ——— 1985. ———. IV. Hybridization between *P. linearis* and *P. calycina* and consequence in the petunia group. *Acta Bot. Neerl.* **34**: 337~349.

花型を用いたペチュニア属植物の分類の試み

青木千佳・服部一三

(名古屋大学農学部, 名古屋市, 〒464-01)

ペチュニア属の種, 品種, 系統の花の外部形態による分類を試みるため, 花の直径(CD), 花筒の長さ(TL), 花の全長(CL), および花筒の直径(TD)を測定した(Fig. 2). これらの測定値をもとに, TL/CD, CL/CDおよびTL/TDの三つの比率を計算し, 測定値とともに比較した. また, 開花時の温度が花型に及ぼす影響を調べるため, 30/24, 25/19, 20/14°C (いずれも昼温/夜温)のもとで開花させ, 比較した. 材料は *P. parodii*, *P. axillaris* (以上白花), *P. inflata* (赤紫花) の3野生種と, 栽培種 *P. hybrida* より 'W 166 K', 'Rose' の2品種, さらに *P. axillaris* と *P. inflata* との正逆 F₁ の4種7系統を供試した.

測定した4つの形質で系統間差異が認められたが(Table 2), CDはいずれの種, 系統とも温度が低くなるほど大きくなる傾向を示した(Table 1, 2a, Fig. 3). CLは中温区が最長のものが多かったが, CDほどはっきりした傾向は認められなかった. TDは温度が低くなるほど大きくなる傾向を示し, *P. parodii* と *P. axillaris* が他の5系統より小さかった. F₁は, TD以外はいずれも両親の中間の大きさであった. また, *P. hybrida* では 'W166K' が 'Rose' より全体に大きな値を示した(Table 1, 2c-d). TLは種, 系統間差異は認められたが, 分散分析の結果, 温度の影響は認められなかった(Table 2b).

本実験で比較した三つの比率は, いずれも数値が大きいほど細長い花型であることを示している. 三つの野生種のうち *P. parodii* と *P. axillaris* は花の外見が似ているが, 同じ温度のもとで開花させた場合, *P. parodii* が *P. axillaris* より TL と CL が有意に長く, したがって三つの比率も *P. parodii* が大きくなった. ただし, 低温区の *P. parodii* と高温区の *P. axillaris*, というように異なった温度区で比較すると, 比率の差がなくなったり, 逆転したりした. *P. inflata* の比率は, 本実験の供試材料中最小であった(Figs. 5, 6, 7). *P. hybrida* (栽培種) および *P. axillaris* と *P. inflata* の正逆 F₁ の比率は *P. axillaris* と *P. inflata* との中間で, 正逆 F₁ の間には差がなかった(Figs. 4-7). *P. hybrida* 2品種間では, 'W166K' がいずれの値も 'Rose' より大きくなった. したがって, この二品種間では, 'Rose' がより *P. inflata* に花型に近いといえる. 外部形態的にも 'W166K' より 'Rose' が *P. inflata* に似ているが, 指数で表しても同様な結果になった. なお, これら三つの比率の中で, 最も明確な系統間差異が得られたのは CL/CD であった(Figs. 5, 6, 7). 以上により, 同じ温度のもとで比較すれば, これらの比率を花型の指標として, *Petunia* 属の種, 品種, 系統の区別や, 類縁関係の推定に有効であると考えられる.