

Petunia

Tom Gerats · Judith Strommer
Editors

Petunia

Evolutionary, Developmental
and Physiological Genetics

Second Edition

 Springer

Editors

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*To Mel, Sarah and Wiert
and to Mike and Miriam*

Preface

The first edition of this monograph, edited by Kenneth Sink and published in 1984, effectively summarized nearly a century of research. It has provided a guide of inestimable value to the family of *Petunia* researchers for more than 20 years, over which time the nature of plant research has undergone a revolution. The fairly small but dedicated group of the mid-20th century was interested in *Petunia* primarily as a model system for physiological, biochemical, and genetic research. 1984 was still fairly early in the molecular era, particularly for plant research, but the timing was good for a comprehensive text covering areas from which molecular biology could grow. And so it grew: as evidenced in every chapter of this book, which summarizes the progress in *Petunia*-based research of the past two and a half decades, the tools of molecular biology are now standard in the biologist's tool box.

In the mid-1980s the search for “the *E. coli* of plants,” the organism to serve as the fundamental plant model system, had begun. In 1985 a meeting was organized at the University of Georgia in Athens, followed in 1987 by an EMBO-sponsored course at the Vrije Universiteit in Amsterdam on *Petunia* as a Model System. The general feeling, for reasons that emerge over and over in the present book, was that *Petunia* would be a superb choice as a plant model system. Early researchers like Bianchi, Cornu, Wiering, Maizonnier, de Vlaming and Farcy, and later ones like Benninck and Schram, had done their parts to prepare the way for using *Petunia* in molecular studies. Their work in genetics, cytogenetics, biochemistry, and physiology provided a strong foundation for molecular studies, especially on flavonoid synthesis and genome structure.

The *Arabidopsis* tide, however, had been building since the early 1980s, and by 1990 it was clear that the small, fast-cycling weed with its small genome was the model system of choice. A great number of young researchers became experts in *Arabidopsis*, and both the body of information and the available tools for *Arabidopsis*-based studies grew exponentially. The humble plant has served its role admirably and allowed for an incredible rate of progress in our understanding of many aspects of plant biology. Now there is a general surge of interest in comparative biology, and thus new model systems are again being sought. *Petunia*, as this work demonstrates, continues to offer many advantages.

Not the least of these is the spirit of the *Petunia* research community. The “First World *Petunia* Day,” a small one-day meeting pretty much restricted to researchers

from Amsterdam, Wageningen and Ghent, initiated a worthwhile tradition of bringing together *Petunia* research and researchers. Though initially a small group, the spirit was good, the thinking was large, and over the years, the meetings attracted more and more scientists. By the “Ninth WPD,” held in October 2007, more than 60 delegates joined in the informal “formal presentations” and the relaxed eating, drinking, and talking that still typify WPD meetings. It was at the “Eighth WPD,” in October 2006, that the idea for a new edition of the monograph was proposed and accepted. (A check of the *Petunia* Platform website [www.petuniaplatform.net] will provide the reader a quick introduction to the *Petunia* groups and their ongoing work.)

The fresh ideas and approaches to research, together with a continuing readiness to share, have been key to the impressive progress documented in this second edition of the monograph. A cursory look at authors and references in this work will give a hint of the extent of the collaborative spirit in the *Petunia* community. As members continue to work together to exploit the strengths of this model system, they will continue to contribute much to the development and evolution of science, particularly in the discipline of comparative biology.

We hope that this edition of the *Petunia* monograph will serve the current members of the *Petunia* research community well and help to attract yet more excellent and collaborative workers to this elegant plant system and to the community that has made it so.

The first edition of *Petunia* will be available via Springer.com!

Guelph
Nijmegen

Judith Strommer
Tom Gerats

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Abbreviations

ABA	abscisic acid
ABC	model for genes specifying floral organ identity
AFBA	after flower bud appearance
AFLP	amplified fragment length polymorphism
AM(S)	arbuscular mycorrhiza (symbiosis)
AN	anthocyanin
ATP	adenosine triphosphate
AVG	2-amino-ethoxyvinyl-glycine
BA	benzoic acid
BAC	bacterial artificial chromosome
BAP	6-benzaminopurine
bc	backcross
BHLH	basic helix-loop-helix
BIBAC	binary bacterial artificial chromosome
BIL	backcross inbred line
bp	base pairs (DNA)
BSTFA	N,O bis(trimethylsilyl) trifluoroacetamide
C6	6-carbon metabolite(s)
CA	cinnamic acid
CaMV	cauliflower mosaic virus
cDNA	complementary DNA
CFP	cyan fluorescent protein
Ch, ch	chromosome
CHX	cycloheximide
cm	centimeter(s)
cM	centiMorgan(s)
CMS	cytoplasmic male sterility
CP	cysteine Protease
CYT	cytochrome

d	day(s)
D	dimensional (1D, 2D, etc.)
DAG	diacylglycerol
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
ds	double-stranded
ePVCV	endogenous Petunia Vein-Clearing Virus
EM	ectomycorrhiza
EMS	ethane methyl sulfonate
EPRV	endogenous pararetrovirus
ER	endoplasmic reticulum
EST	expressed sequence tag
ETR	ethylene resistant
EXL	expansin-like
EXP	expansin protein
FISH	fluorescence in situ hybridization
FLIM	fluorescence lifetime imaging microscopy
FM	floral meristem
FRET	fluorescence resonance energy transfer
FTIR	Fourier transform infrared (spectroscopy)
g	gram
GA	gibberellic acid
GC	gas chromatography
GFP	green fluorescent protein
GMS	genic male sterility
GSI	gametophytic self-incompatibility
GUS	β -glucuronidase
h	hour(s)
hap	hours after pollination
HDGS	homology-dependent gene silencing
HFD	his-phe-asp amino-acid signature
HV	hypervariable
IAA	indole-3-acetic acid
Ig	immunoglobulin
IM	inflorescence meristem
IPT	Isopentenyltransferase
K	potassium
kb	kilobase(s)
kDa	kiloDalton(s)

LB	Luria broth
LIS	linalool synthase
LTR	long terminal repeat
m	meter(s)
MADS	family of transcription factors with specific motifs
MCS	multiple cloning site
MeBA	methyl benzoate
MD	Mitchell Diploid (Petunia Mitchell)
MFS	major facilitator superfamily
Mg	magnesium
mm	millimeter(s)
mM	millimolar
MP	movement protein
MS	mass spectrometry
mt	mitochondrial
MYA	million years ago
MYB	million years before; transcription factor with specific motif
myc	mycorrhiza
MYC	transcription factor with specific motif
MW, mw	molecular weight
N	nitrogen
NAD	nicotinamide adenine dinucleotide
NLS	nuclear localization signal
nod	nodulation
P	phosphorus
P _i	orthophosphate
PA	phosphatidic acid
PAGE	polyacrylamide gel electrophoresis
PAM	periarbuscular membrane
PCD	programmed cell death
PCF	petunia CMS-associated fused gene
PCR	polymerase chain reaction
PDMS	polydimethylsiloxane
pI	isoelectric point
PIP ₂	phosphatidyl inositol 4,5-bisphosphate
pm	picomole(s)
PPA	prepenetration apparatus
PPR	pentatricopeptide repeat
PR	pathogenesis related
PRV	pararetrovirus
PSC	pseudo-self-compatibility

PTGS	post-transcriptional gene silencing
PT	phosphate transporter
PVX	Potato virus X
QTL(s)	quantitative trait locus (loci)
RAM	root apical meristem
RAPD	random amplification of polymorphic DNA
rDNA	ribosomal DNA
RFLP	random fragment length polymorphism
RH	RNase H
RIHC	RNA-induced silencing complex
RIL	recombinant inbred line(s)
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
RNS	root nodule symbiosis
ROS	reactive oxygen species
RT	reverse transcriptase
SAM	shoot apical meristem; S-adenosyl-L-methionine
SC	self-compatibility
SCF	ubiquitin E3-ligase complex
SGN	Solanaceae Genomics Network
SI	self-incompatibility
siRNA	small interfering RNA
SLF	S-locus encoded F box
sp(p)	species (plural)
SPME	solid phase micro-extraction
ss	single-stranded
ssp; subsp(p)	subspecies (plural)
SYM	symbiosis
TD	Transposon Display
tDNA	transgene DNA

TGMV	tobacco golden mosaic virus
TGS	transcriptional gene silencing
TIGR	The Institute for Genomic Research
TMCS	trimethylchlorosilane
TMV	tobacco mosaic virus
TRV	tobacco rattle virus
TSD	target site duplication
TUNEL	terminal transferase-mediated dUTP nick end-labeling
UI	unilateral incompatibility
UV	ultraviolet
VIGS	virus-induced gene silencing
VLP	virus-like particle
WT	wild type
YFP	yellow fluorescent protein
YEP	yeast extract bacto-peptone
μm	micron(s)

Chapter 1

The Genus *Petunia*

João Renato Stehmann, Aline P. Lorenz-Lemke, Loreta B. Freitas,
and João Semir

Abstract The common garden petunia, *Petunia hybrida*, is derived from *P. integrifolia* and *P. axillaris*, two of many *Petunia* species endemic to South America. The geographic distribution includes temperate and subtropical regions of Argentina, Uruguay, Paraguay, Bolivia, and Brazil, with a center of diversity in southern Brazil. The presence of seven chromosomes and a number of morphological, anatomical, and biochemical characteristics differentiate the genus from its sister taxon, *Calibrachoa*. Included in this chapter is a taxonomic guide for the 14 currently recognized species, some of them restricted to very small geographic areas. Species diversity is in danger of diminishing significantly due to human intervention, particularly in the form of grassland destruction.

1.1 Historical Review

Petunia Jussieu (Solanaceae) is best known for the garden petunia, an ornamental hybrid widely cultivated around the world. There are many cultivars with a broad range of flower color and size, and the market of their seeds represents an important economic resource for many countries. Its generic epithet comes from “petum” or “betum,” an indigenous name given to the tobacco, *Nicotiana tabacum* L., that roughly resembles one of the first two species described in *Petunia*, *P. nyctaginiflora* Juss. (= *P. axillaris* [Lam.] Britt. et al.) (Fries 1991). Due to some morphological similarities, such as annual growth habit, five stamens, capsular fruits, and small seeds, *Petunia* and *Nicotiana* G. Don were historically included in the same infra-familiar taxonomic groups (Wettstein 1895; D’Arcy 1991; Hunziker 1979, 2001). However, recent phylogenetic studies based on molecular data suggest that *Nicotiana* and *Petunia* are not so closely related and should be placed in different subfamilies (Olmstead and Palmer 1992; Olmstead, Sweere, Spangler, Bohs, and Palmer 1999; Olmstead and Bohs 2007).

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Petunia was originally described by Jussieu (1803) based on material collected in Montevideo, Uruguay, by Commerson. Two very distinct species, *P. parviflora* and *P. nyctaginiflora*, were described in the same paper, the first with short funnelform corolla and the second salverform with a long corolla tube. Earlier, in 1793, Lamarck described the latter species as *Nicotiana axillaris* Lam. During the next four decades, species of *Petunia* were variably described in different genera of the Solanaceae such as *Nicotiana* (Lehmann 1818), *Fabiana* Ruiz & Pav. (Saint-Hilaire 1824), *Calibrachoa* La Llave & Lex. (La Llave and Lexarza 1825), *Salpiglossis* Ruiz & Pav. (Hooker 1831), and *Nierembergia* Ruiz & Pav. (Graham 1833).

In 1846, Miers published a revision of the South American Solanaceae in which he recognized 10 species of *Petunia*, half of them described for the first time. His illustrations of the species were presented later, in 1850. In the same year, but two months later, Sendtner published his revision of Solanaceae in the *Flora Brasiliensis*, with 13 species of *Petunia*, nine of which were new. In de Candolle's *Prodromus*, published in 1852, Dunal presented 16 species of *Petunia*, transferred another three from *Petunia* to *Fabiana*, and described a new genus, *Leptophragma*, now considered a synonym of *Calibrachoa*.

Fries (1911) published the first monograph of *Petunia*, accepting 27 species, nine of which were new. The taxonomic treatment provided a detailed discussion of the morphology, circumscription, geographic distribution, and relationships of *Petunia* with other genera. Fries' monograph remains the latest complete revision of the genus available. During the following five decades, few new species were described (Sandwith 1926; Steere 1931; Morton 1944). The number of species in *Petunia* increased significantly only when Smith and Downs (1964, 1966) described nine new species among the flora of Santa Catarina, Brazil.

The study of the garden petunia, termed *Petunia x hybrida* (Hook.) Vilm. and now commonly known as *Petunia hybrida*, was decisive for the future of the taxonomy of the genus. The garden petunia was first obtained by hybridization in 1834 by Atkins of Northampton, a British nurseryman, and it soon spread to European gardens (Sink 1984). Today it is cultivated all over the world and is one of the most important Solanaceae utilized for ornamental purpose.

Many authors have investigated the origin of this garden hybrid and the mechanisms of genetic incompatibility found in *Petunia* (Ferguson and Ottley 1932; Mather 1943; Mather and Edwardes 1943; Stout 1952; van der Donk 1974; Linskens 1975; Sink 1984; see Chapter 5), and different species have been suggested as parents of the garden petunia.

In 1982, Wijsman considered the origin of the hybrid and concluded that it was obtained from breeding of only two biological species, one with white flowers [*P. axillaris* (Lam.) Britton et al.], and the other with purple flowers [*P. integrifolia* (Hook.) Schinz & Thell.], each one with geographic subspecies. Later, Wijsman (1983) tried to breed other species (*P. calycina* and *P. linearis*, $2n = 18$) with the parents of the garden petunia ($2n = 14$). While hybrids were obtained by crossing species with equivalent chromosome number, all crossings between species with different chromosome numbers failed. Wijsman and de Jong (1985) concluded that these groups distinguished by chromosome number (and some morphological

characters) must be treated as different genera and proposed to keep in *Petunia* only the species related to *Petunia parviflora* Juss. ($2n = 18$), the type species of the genus as established by Britton and Brown (1913) using the mechanical method of typification (first species cited in the protologue). Species of *Petunia* with $2n = 14$ were transferred to *Stimoryne* Rafin., the next generic available synonym.

As the taxonomic proposal of Wijsman and de Jong (1985) would change the name of the garden petunia, Wijnands and Bos (1986) proposed to conserve *Petunia nycaginiflora* Juss. ($2n = 14$) as the type of *Petunia*. This proposal was accepted (Greuter et al. 1994), and those species related to *Petunia parviflora* ($2n = 18$) were transferred to *Calibrachoa* (Wijsman 1990; Stehmann and Semir 1997; Stehmann and Bohs 2007).

Further cytotaxonomic, reproductive, anatomical, and chemical studies related to species of *Petunia* and *Calibrachoa* have corroborated Wijsman's decision to split *Petunia* into two genera. All species investigated in *Petunia* have a chromosome number of $n = 7$ (Watanabe et al. 1996a), while in all *Calibrachoa* species examined to date, $n = 9$ or 18 (Stehmann, Semir, Dutilh, and Forni-Martins 1996; Watanabe et al. 1996b). Chromosome counts assign the basic numbers of $x = 7$ and $x = 9$ to *Petunia* and *Calibrachoa*, respectively. Interspecific cross-incompatibility between *Petunia* species with different chromosome numbers, as reported by Wijsman (1983) and Watanabe et al. (1996a), demonstrates that the groups are genetically isolated and they could not hybridize in natural conditions, even if they share the same pollinators. Anatomical features are useful to distinguish the genera. Reis, Sajo and Stehmann (2002) studied leaf anatomy in 16 species of *Calibrachoa* and seven species of *Petunia*. Fourteen *Calibrachoa* species have endodermis surrounding the vascular bundles, formed by well-developed parenchymatic cells very distinct from surrounding mesophyll. Only two species, *C. parviflora* (Juss.) D'Arcy and *C. pygmaea* (R.E. Fries) Wijsman, do not show differentiated endodermis. In none of the analyzed species of *Petunia* is the endodermis morphologically differentiated. Chemical evidence also supports Wijsman and de Jong (1985). Ellinger, Wong, Benson, Gaffield, and Waiss (1992) reported that *C. parviflora* yielded none of the ergostanoids that are associated with *Petunia* species. However, only a few species were studied and further work is needed.

Phylogenetic relationships have been clarified by the results of recent molecular studies. The separation into two genera was supported by RFLP chloroplast DNA (Ando et al. 2005b) and ITS, cpDNA and mtDNA analyses (Kulcheski et al. 2006). In both analyses, *Petunia* and *Calibrachoa* showed a close phylogenetic relationship and, for this reason, are considered sister groups.

1.2 Morphological Circumscription of *Petunia* and *Calibrachoa*

Some species of *Petunia* and *Calibrachoa* have sympatric distributions and share similar vegetative and floral attributes, making it difficult to recognize the genus to which they belong. To help with the recognition of each genus, we compare the principal diagnostic morphological traits below (Table 1.1).

Table 1.1 Comparative morphological traits between *Petunia* Juss. and *Calibrachoa* La Llave & Lex. (Solanaceae)

Trait	<i>Petunia</i>	<i>Calibrachoa</i>
Habit	Herbs with non-woody stems	Small shrubs or herbs, woody stems
Duration	Usually annual	Annual or perennial
Brachyblasts	Absent	Present or absent
Leaves	Ovate, elliptic, oblong, or obovate, rarely linear; flat margin	Ovate, elliptic, obovate, oblong, or linear; flat or revolute margin
Inflorescence	Monochasial with opposite leaf-like bracts	Monochasial with opposite leaf-like bracts
Aestivation	Imbricate	Reciprocal (except in <i>C. pygmaea</i>)
Symmetry	Actinomorphic or zygomorphic	Zygomorphic (except in <i>C. pygmaea</i>)
Calyx	Ribs usually not conspicuous; deeply lobed (except in <i>P. atiplana</i> and some coastal populations of <i>P. integrifolia</i>); lobes linear or enlarged toward the apex	Five or ten ribs; lobed usually to the middle, lobes usually narrowed toward the apex
Corolla	Funnelform, campanulate, or salverform; purple, red (bright or orange), pink, or white	Funnelform (salverform, ventricose, and apically constricted in <i>C. pygmaea</i>); purple, red, pink, or whitish
Anthers	Yellow, bluish, or violet	Yellow
Seed coat	Cells with wavy anticlinal walls	Cells with straight anticlinal walls

In nature, *Petunia* species are mostly annual with herbaceous stems and brachyblasts absent or poorly developed. It is difficult to get information about life history from herbarium material; additional information can be obtained by growing plants in greenhouses, but there they do not behave as they do in nature, especially in the subtropical regions where most species of *Petunia* grow. In southern Brazil the winter is very cold, with minimum temperatures below 0°C; thus, frost and snow must affect the survival of individuals. Since *Petunia* species are usually not lignified and they lack any special underground system (except for coastal populations of *P. integrifolia*), we can assume that most species, such as *P. axillaris*, *P. integrifolia*, *P. reitzii* L.B. Sm. & Downs, and *P. bonjardinensis* T. Ando & Hashim., are annuals. *Calibrachoa* species are annual or perennial. The perennials have a shrubby habit with basal woody stems, often bearing brachyblasts.

Leaf morphology in *Petunia* is more uniform than in *Calibrachoa*. In *Petunia* the leaves are usually sessile, more rarely petiolate, ovate, obovate, or elliptic, rarely linear, and the surface and margin are flat. In *Calibrachoa*, leaves are also commonly sessile, but their form is extremely variable. There are elliptic, ovate, obovate, oblong, and linear leaves, with flat or revolute margins. The revolute margin is not found in any *Petunia* species. Reis et al. (2002) showed that leaves of

Calibrachoa are quite diversified, both externally and internally, and its species can be separated according to the type of margin, distribution of stomata on leaf surfaces, organization of the mesophyll, and morphology of trichomes.

The inflorescences in both genera are sympodial, with monochasial growth, whereby each flower is always associated with two opposite, leaf-like bracts (one sympodial unit). The sympodial pattern is typical for almost all species of Solanaceae (Bell and Dines 1995). Secondary branches arise subsequently in the axils of basal bracts, whereupon the appearance of the inflorescence may become dichasial. Development of the sympodial unit is normally continuous, but sometimes it can be interrupted such that the flower is not produced at a specific node. Fries (1911) and Danert (1958) described and illustrated the characteristic inflorescence branching of *Petunia* s.l. To distinguish *Petunia* and *Nicotiana*, Smith and Downs (1966) as well as Hunziker (1979) described *Petunia* with solitary flowers. However, the flowers of *Petunia* and *Calibrachoa* cannot be considered solitary, because they are arranged in a sequence of sympodial units that constitute an inflorescence. These sympodial units with two opposite leaf-like bracts are unique to *Petunia* and *Calibrachoa* in Petunioideae, and clearly constitute an apomorphic character of this group.

The calyx of most *Petunia* species is deeply lobed with linear or spatulate lobes, usually enlarged toward the apex. Calyx lobed to the middle appears only in flowers of *P. altiplana* T. Ando & Hashim. and coastal populations of *P. integrifolia*. In *Calibrachoa* the calyx is usually lobed to the middle, and the lobes are often acute and narrowed toward the apex. Exceptions can be found in *C. micrantha* (R.E. Fries) Stehmann & Semir and *C. pygmaea*, in which the calyx can be cleft up to 2/3 of its length. However, the lobes of the calyx of *C. micrantha* are ovate-lanceolate and narrowed at the apex, while in *C. pygmaea* they are linear and obtuse at the apex. Another useful characteristic of the calyx is the presence of marked ribs. In *Calibrachoa*, calyx ribs are prominent in most species, except in *C. pygmaea* and *C. parviflora*, which have a more fleshy calyx with less-evident ribs. Conversely, most *Petunia* species lack calyx ribs.

Fries (1911) and Smith and Downs (1966) used the degree of calyx partition as a diagnostic characteristic in *Petunia* s.l. Wijsman and de Jong (1985) observed that this distinguished most species belonging to the groups with different chromosome numbers. The deeply lobed calyx described in *P. parviflora* [= *C. parviflora*] was considered by them to be an artifact and treated as a pentapartite, not pentafid, calyx with lobes connected to a thin membrane. The halfway-lobed calyx of most *Calibrachoa* may reflect the higher level of fusion of the lobes and the lateral veins, forming ten thick ribs. These ribs are quite evident in the calyx of the other related genera, such as *Fabiana* (Barboza and Hunziker 1993) and *Nierembergia* (Millan 1946), in which the level of fusion is higher.

Studies on vasculature and structure of the calyx are important to the taxonomy of Solanaceae. D'Arcy (1986) described the solanaceous general calyx as a whorl of five lobes nerved by five primary traces that branch into the lobes, forming a pair of lateral veins with minor leaf-like venation. He pointed out a tendency of the calyx lobe and veins to fuse in different levels, giving rise to main traces or ribs in the

fused area. In *Lycianthes* the calyx nervation consists of primary traces with fused laterals leading to ten teeth in two series, and this pattern serves to distinguish it from *Solanum*, with which it shares poricidal anthers.

Calibrachoa and *Petunia* have different aestivation patterns, and this trait distinguishes the genera. *Petunia* species show imbricate aestivation (Fig. 1.1A), whereas most *Calibrachoa* species have reciprocative aestivation (Fig. 1.1B). This term was coined by Miers for instances in which the anterior induplicative lobe covers the four others, which are conduplicate (Hunziker 2001). Within *Calibrachoa*, only the *C. pygmaea* corolla seems to have an imbricate-reciprocative aestivation.

Aestivation was confirmed as an unambiguous characteristic that differentiates *Calibrachoa* and *Petunia* species, as Wijsman and Jong (1985) predicted. The *Calibrachoa* aestivation pattern is similar to the conduplicate pattern described for *Nicotiana* by Goodspeed (1954). However, in *Nicotiana* the conduplicate type is extremely variable in its degree of spirally. Within one species, *N. tomentosa* Ruiz & Pav., conduplication shifts via intermediate races into imbrication. In the Solanaceae, aestivation has long been used to distinguish subtribes (Baehni 1946) or tribes (Hunziker 1979). The distinct ontogenetic patterns of corolla development observed in buds of *Petunia* and *Calibrachoa* provide evidence that they are not

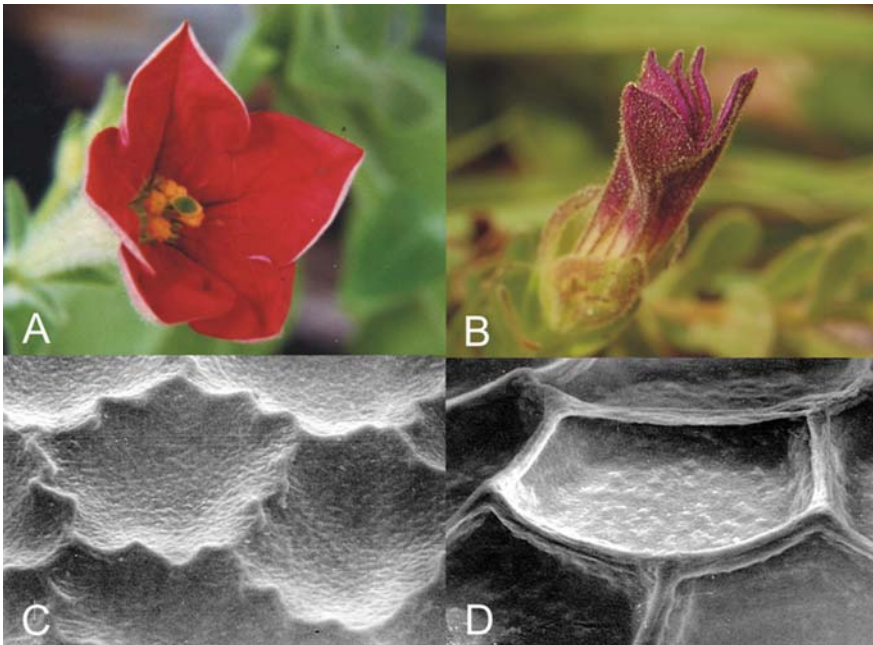


Fig. 1.1 Distinct patterns of corolla aestivation in *Petunia* and *Calibrachoa*. (A) *Petunia exserta* with imbricate aestivation. (B) *Calibrachoa sellowiana* with reciprocative aestivation. Seed coat of *Petunia* and *Calibrachoa* observed by SEM: (C) anticlinal walls are wavy in *Petunia* (*P. integrifolia*) and (D) straight in *Calibrachoa* (*C. dusenii*)

closely enough related to represent infrageneric taxa, for example, subgenera, but rather support their distinction at the generic level.

The general ontogenetic pattern of corolla aestivation is completely different in the two genera. After initiation of the corolla primordia, the lobes start the differentiation and growth process. The corolla lobes in *Petunia* become imbricate at the median stage of development, before elongation of the tube. At this same stage of bud development, corolla lobes in *Calibrachoa* become folded and aestivation shows an induplicate pattern. Later the one basal lobe turns upward, enfolding the four others. Anatomical studies are necessary to better describe these patterns.

The corolla color in *Calibrachoa* and *Petunia* species is usually purple, but both genera may also show whitish, reddish, or pinkish flowers. Species with a funnelform corolla may have a yellow corolla throat in *Calibrachoa*, but in *Petunia* yellow is never associated with this type of flower. It is also worthwhile to note the yellow color of the anthers in all *Calibrachoa*, but ranging from yellow to bluish or violet in *Petunia*. Ando et al. (1999) studied in detail the occurrence of floral anthocyanins in 20 taxa of *Petunia* and recognized four distinctive colors: white, purple (and red-purple), orange-red, and bright red.

The corolla shape typically found in *Calibrachoa* is funnelform. Only *C. pygmaea* shows a salverform, ventricose, and apically constricted corolla. *Petunia* shows various types of corolla, such as funnelform, campanulate, and salverform (Fig. 1.2). The limb is also quite diverse in *Petunia*, with lobes rounded, obtuse, retuse, or acute. In *P. exserta* Stehmann, a hummingbird-pollinated species, the corolla lobes become more cleft and reflexed with age.

Petunia and *Calibrachoa* have small foveolate-reticulated seeds (less than 1.4 mm), and seed coats observed under SEM show different patterning. Seed-coat anticlinal walls are wavy in *Petunia* (Fig. 1.1C) and straight in *Calibrachoa* (Fig. 1.1D). Bahadur, Venkateshwarlu and Swamy (1989) had previously described the different patterns of seed-coat morphology in *Petunia* s.l., but did not make any comments about the taxonomic implications of their discovery. Stehmann and Semir (1997) reported seed-coat epidermal cells as a fundamental diagnostic characteristic to distinguish the two genera. In order to check the assumption of those authors, Watanabe et al. (1999) studied seed coats in 45 taxa of the genus *Petunia* s.l. Three different seed-coat epidermal patterns were described: (1) wavy middle lamellae and anticlinal walls, (2) wavy middle lamellae embedded in straight anticlinal walls, and (3) straight middle lamellae and anticlinal walls. In fact, these three groups correspond to the two groups, one with wavy anticlinal walls (*Petunia* species) and the other with straight anticlinal walls (*Calibrachoa* species). The middle lamellae are not well characterized by SEM, and the results of this analysis must be treated with caution. The seed-coat epidermis, associated with aestivation pattern, clearly distinguishes *Petunia* from *Calibrachoa*.

There are few SEM descriptions of seed coats for related genera in Solanaceae. In *Nicotiana* seed-coat epidermal cells, there are variable types of anticlinal walls. Most *Nicotiana* species have wavy anticlinal walls, but straight anticlinal walls also occur in this genus (Goodspeed 1954). Features of the seed-coat epidermal wall

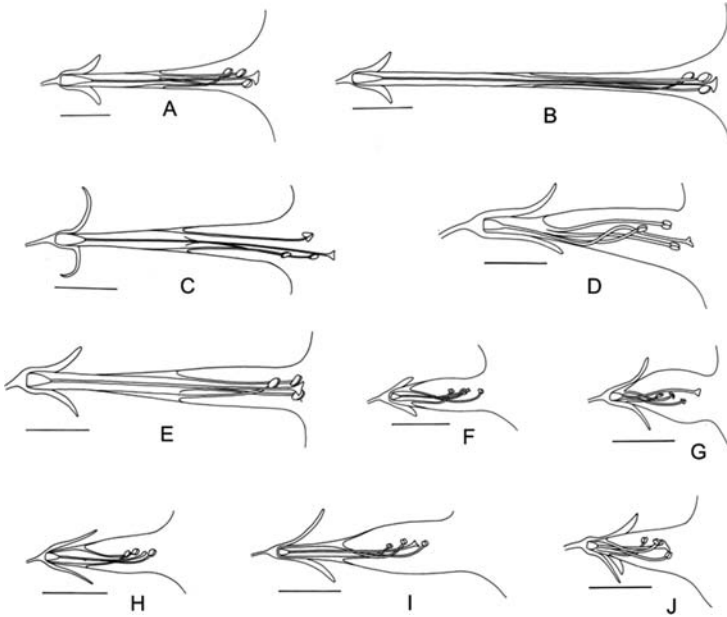


Fig. 1.2 Major patterns of *Petunia* flower arrangements. Salverform corolla: (A) *P. axillaris* subsp. *axillaris*; (B) *P. axillaris* subsp. *parodii*; (C) *P. exserta*; (D) *P. mantiqueirensis*; (E) *P. secreta*. Funnelform or campanulate corolla: (F) *P. altiplana*; (G) *P. bonjardinensis*; (H) *P. integrifolia*; (I) *P. reitzii*; (J) *P. scheideana*. Scale bar = 1 cm

are characteristics known to have taxonomic importance in *Physalis* (Axelius 1992) and *Schwenckia* (Carvalho, Machado and Bovini 1999), as well as in other families like Cactaceae (Barthlott and Voigt 1979) and Campanulaceae (Shetler and Morin 1986). Seed-coat attributes have been considered valuable characteristics to recognize species or genera, or even tribes and subtribes (Barthlott 1981).

1.3 Taxonomy

Petunia comprises annual or perennial herbs, up to 1 m tall, with erect, ascendant, decumbent, or procumbent stems, rarely rooting at the nodes. The leaves are sessile or petiolate, with blades elliptic, ovate or obovate, more rarely rounded or linear, membranaceous, somewhat juicy, flat, and usually without marked venation. Inflorescences are sympodial, with monochasial growth, whereby each flower is always associated with two opposite, leaf-like bracts. The calyx is green, deeply lobed, with a very short tube and long linear or spatulate lobes with usually inconspicuous ribs. The corolla is funnelform, campanulate, or salverform, with imbricate aestivation, tube 1.5–7 cm long, and purple, white, red (bright or orange), or pink limb. The five stamens are variously adnate to the corolla tube, usually included and arranged in

three levels: one short, two middle, and two longer (more rarely only in two levels). Anthers are ventrifixed, yellow or violaceous in color. The ovary is glabrous, surrounded by a lobed nectary, with filiform style, and disciform or lobed stigma. The stigma can be placed among the anthers of the didynamous pair of stamens, at the same level or above the longest pair, sometimes exerted to the corolla tube. The fruits are capsular, many seeded, with peduncle inflexed or deflexed at mature stage. Seeds range in size from 0.4 to 1.4 mm long and present seed coats with wavy anticlinal walls.

We recognize 14 species distributed in subtropical and temperate South America. *Petunia axillaris* and *P. integrifolia*, parents to the familiar garden hybrid, have the largest distribution. Several species are narrow endemics, for example, *P. bajeensis* T. Ando & Hashim., *P. bonjardinensis*, *P. exserta*, *P. mantiqueirensis* T. Ando & Hashim., *P. reitzii*, *P. saxicola* L.B. Sm. & Downs, and *P. secreta* Stehmann & Semir. Species of *Petunia* can be found in sunny, partially shaded, or completely shaded sites. *Petunia altiplana*, *P. axillaris*, *P. inflata*, and *P. integrifolia* are easily found in disturbed places such as roadside slopes, especially in rocky ground; *P. scheideana* and *P. mantiqueirensis* occur in partial shade at edges of *Araucaria* forests; and *P. exserta* grows in the shaded relief from shallow caves sculpted by the wind in sandstone.

The morphological circumscription of certain species is not easy, especially those related to *P. integrifolia*, characterized by purple and funnellform corolla, violaceous pollen, and stigma placed among the anthers of the didynamous pair of stamens. In a wide sense, this group corresponds to Wijsman's *P. integrifolia* complex (*P. integrifolia*, *P. inflata*, and *P. occidentalis* R.E. Fr.) and others to specific (*P. interior* T. Ando & Hashim., *P. riograndensis* T. Ando & Hashim., *P. littoralis* L.B. Sm. & Downs and *P. bajeensis*) and infraspecific (*P. integrifolia* subsp. *depauperata* (R.E.Fr.) Stehmann) taxa. This complex group of taxa called "integrifolia" comprises at least four distinct genetic lineages (Lorenz-Lemke, unpublished data).

A key to recognizing the species of *Petunia* is presented below, together with comments about morphology, nomenclature, geographic distribution, and habitat.

1.3.1 Key to the Native Species of *Petunia*

- 1a. Corolla salverform (tube cylindrical or slightly enlarged toward the apex).
- 2a. Corolla white. **2. *P. axillaris***
- 2b. Corolla purple or reddish.
- 3a. Corolla purple; anthers and stigma included in the corolla tube; heliophilous plants.
- 4a. Plant erect; corolla tube and throat purple; filaments adnate nearly to middle of corolla tube; pollen yellow. **14. *P. secreta***
- 4b. Plant procumbent, ascendant or climbing; corolla tube and throat whitish and purple reticulate-veined; filaments adnate below the middle of tube; pollen bluish or violet. **9. *P. mantiqueirensis***

- 3b. Corolla reddish; anthers and stigma exerted from corolla tube; sciophilous plants. **5. *P. exserta***
- 1b. Corolla funnellform or campanulate (tube clearly enlarged toward the apex).
- 5a. Corolla pink to bright red.
- 6a. Filaments adnated >9 mm to corolla tube base; stigma slightly exerted above the anthers of longest pair of stamens. **12. *P. saxicola***
- 6b. Filaments adnated up to 8 mm to corolla tube base; stigma located below the anthers of the longest pair of stamens. **11. *P. reitzii***
- 5b. Corolla purple.
- 7a. Stigma exerted above anthers of the longest stamens. **4. *P. bonjardinensis***
- 7b. Stigma located at the same level or below the anthers of the longest pair of stamens.
- 8a. Stigma located at the same level to the anthers of the longest pair of stamens; corolla throat pale purple to whitish with contrasting deep-purple reticulation; stigma >1.5 mm long, vertically two lobed. **13. *P. scheideana***
- 8b. Stigma located below the anthers of the longest pair of stamens; corolla throat purple with dark purple stripes or reticulation; stigma <1.2 mm long, not two lobed (except *P. occidentalis*).
- 9a. Plant repent, rooting at the nodes; leaves widely obovate or orbicular, usually rounded to the base, more rarely attenuate or short attenuate; calyx halfway lobed. **1. *P. altiplana***
- 9b. Plant erect, ascendant or decumbent, not rooting at the nodes; leaves ovate, elliptic, oblanceolate, or obovate, rarely orbicular, with attenuate or long-attenuate base; calyx deeply lobed (less so in coastal populations) (*Petunia integrifolia* complex).
- 10a. Anthers with channeled lobes at dehiscence. **8. *P. interior***
- 10b. Anthers with lobes flat at dehiscence.
- 11a. Plant viscid; leaves with prominent venation; corolla mouth reniform in frontal view, with intruded throat. **3. *P. bajeensis***
- 11b. Plant not obviously viscid; leaves with obscure venation; corolla mouth elliptic in frontal view, with flat throat.
- 12a. Stems decumbent; capsule subglobose with peduncle deflexed. **7. *P. integrifolia***
- 12b. Stems usually erect or ascendant; capsule ovoid with peduncle inflexed or weakly deflexed.
- 13a. Corolla limb 25–40 mm in diameter, filaments adnated <5 mm to the corolla tube base. **6. *P. inflata***
- 13b. Corolla limb 20–25 mm in diameter, filaments adnated >7 mm to the corolla tube base. **10. *P. occidentalis***

1. *Petunia altiplana* T. Ando & Hashim. (Figs. 1.2F and 1.3A–B) – This species is easily recognized by its rooting stems, usually broadly spatulate leaves, purple corolla, and stigma located below the anthers of the longest pair of stamens. Its repent habit of usually forming a round mat is unique to the genus. The radial growth pattern plus the massive blooming permit the use of the species for ornamental



Fig. 1.3 *Petunia* species. (A) and (B) *P. atiplana*; (C) *P. axillaris*; (D) *P. bajeensis*; (E) *P. bonjardinensis*; (F) *P. exserta*

purposes. This species is distributed in the highlands of Santa Catarina and Rio Grande do Sul, Brazil, in altitudes from 800 to 1200 m, and grows in outcrops or exposed roadside slopes (Ando and Hashimoto 1993).

2. *Petunia axillaris* (Lam.) Britton et al. (Figs. 1.2A–B and 1.3C) – It can be readily identified by its erect habit, salverform white corolla, yellow pollen, and inflexed pedicel in fruit stage. The flowers emit a scent at dusk and are visited by sphingid hawkmoths (Galeto and Bernardello 1993; Ando et al. 2001;

see Chapter 2). It exhibits the largest geographic distribution in the genus and is known to occur in Brazil (Rio Grande do Sul), Argentina, Uruguay, Paraguay, and Bolivia. Three allopatric subspecies have been accepted based on corolla tube length and stamen arrangement (Ando 1996; Kokubun et al. 2006). Individuals of *P. axillaris* are heliophilous and inhabit rocky sites, but can also be found along roadsides.

3. *Petunia bajeensis* T. Ando & Hashim. (Fig. 1.3D) – The species is characterized by its viscid vestiture, foliose stems forming a cushion-like structure, ovate, elliptic, or oblong leaves with prominent primary and secondary veins (showing the marked brochidromous venation), purple funnellform corolla, stamens adnated more than 7 mm to the base of the corolla tube, stigma located below the anthers of the longest pair of stamens, and deflexed pedicel in fruit stage. Vegetatively, the individuals of this species roughly resemble more robust plants of *P. bonjardinensis*, but the morphology of the flowers does not differ from that of *P. integrifolia* except for the larger size of the floral parts. To date found only in the extreme southern region of Rio Grande do Sul, Brazil, in the municipalities of Bajé, Canguçu, and Lavras do Sul, it can be found growing along roadside slopes (Ando and Hashimoto 1998).

4. *Petunia bonjardinensis* T. Ando & Hashim. (Figs. 1.2G and 1.3E) – The decumbent habit, with very fragile stems, villose vestiture, campanulate and purple corolla, stigma positioned above the anthers of the longest pair of stamens, and deflexed peduncles in fruit stage are characters permitting clear identification of the species. *Petunia bonjardinensis* is endemic to a small area near to the border of the southern Brazilian plateau, in the municipality of Bom Jardim da Serra, Santa Catarina (Ando and Hashimoto 1993), where it is not difficult to find individuals growing on roadside slopes.

5. *Petunia exserta* Stehmann (Figs. 1.2C and 1.3F) – *Petunia exserta* is unique in the genus, showing red (red-orange) corolla and distinct exerted stamens and stigma, attributes associated with hummingbird pollination (Stehmann 1987; Lorenz-Lemke et al. 2006; see Chapter 2). It shares the erect habit, salverform corolla, yellow pollen, and inflexed stalk with *P. axillaris* and *P. secreta*. This strictly endemic species is known only from the “guaritas” and adjacent areas, at the municipality of Caçapava do Sul, Rio Grande do Sul, Brazil, growing in shallow caves sculpted by the wind in sandstone towers.

6. *Petunia inflata* R.E.Fr. – *Petunia inflata* can be recognized by its ascendant habit, purple corolla with slightly constricted tube, and capsule with usually inflexed fruit-stalk. This species was originally described as differing from *P. violacea* [= *P. integrifolia*] because of its inflexed pedicel in the fruiting state and disjunct geographic distribution (Fries 1911). Smith and Downs (1966) considered *P. inflata* a synonym of *P. integrifolia*, but Wijsman (1982) resurrected the taxon at the sub-specific level under *P. integrifolia*, a widespread species in southern South America with three geographic subspecies: *P. integrifolia* subsp. *integrifolia*, *P. integrifolia* subsp. *inflata* (R.E.Fr.) Wijsman, and *P. integrifolia* subsp. *occidentalis* (R.E.Fr.) Wijsman. In a recent study based on morphometric analysis of cultivated material, Ando et al. (2005a) accepted *P. inflata* as distinct from *P. integrifolia* and pointed