Molecular discrimination among taxa of *Petunia axillaris* complex and *P. integrifolia* complex based on *PolA1* sequence analysis

Xinwang Zhang¹⁾, Hiroko Takahashi¹⁾, Ikuo Nakamura^{*2)} and Masahiro Mii²⁾

¹⁾ Graduate School of Science and Technology, Chiba University, 1-33 Yayoi, Inage, Chiba, Chiba 263-8522, Japan

²⁾ Faculty of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan

Petunia axillaris complex and *P. integrifolia* complex consist of three subspecies (ssp. *axillaris*, ssp. *parodii* and ssp. *subandina*) and two species (*P. integrifolia* and *P. inflata*), respectively. Since these taxa within each complex can only be distinguished by the flower morphology, it would be useful to discriminate them by DNA markers. In this study, we sequenced the 19th intron and the 20th exon of the *PolA1* gene, a single gene encoding the largest subunit of RNA polymerase I, and showed clear differences between *P. axillaris* complex and *P. integrifolia* complex, and also among all the taxa within each complex. These sequence variations will be promising to develop PCR-based markers to discriminate taxa within *P. axillaris* complex and *P. integrifolia* complex.

Key Words: *PolA1*, *Petunia*, Solanaceae, DNA marker, discrimination, sequence analysis.

Introduction

The genus *Petunia* (Solanaceae) was originally established by Jussieu (1803) and occurs primarily in subtropical and temperate South America. Later, Wijsman (1990) divided the genus into two genera, *Petunia* ($2n = 14$) and *Calibrachoa* ($2n = 18$). The genus *Petunia* is considered to comprise 19 taxa (Watanabe *et al.* 1999, Mishiba *et al.* 2000), which were classified into four groups (A, B, C and D) by Ando *et al.* (1999) according to their constituents and contents of major anthocyanins and their difference in pigment biosynthesis patterns with respect to the presence of blocking mechanisms or inhibitors for hydroxylation, glucosylation and acylation reactions.

Cultivars of garden petunias (*Petunia hybrida* Vilm.) have been bred since the early 1830s (Paxton 1836). The progenitors of garden petunias have been presumed to be of two species groups, *P. axillaris* complex with a white flower, and *P. integrifolia* complex with a purple flower (Ferguson and Ottley 1932, Sink 1975), which were classified into A group and D group, respectively by Ando *et al.* (1999). However, there is no clear evidence to show which taxa were actually used as genetic donors for garden petunias. After a long controversial history of classification of *P. axillaris* (Steere 1931, Ferguson and Ottley 1932, Cabrera 1977, Wijsman 1982), Ando (1996) revealed that the *P. axillaris* complex consists of three subspecies, ssp. *axillaris*, ssp. *parodii* and ssp. *subandina*, which are morphologically similar to each other except for the flower characters: ssp.

axillaris having didynamous stamens, two long, two medium and one short, a short corolla tube, and rounded corolla lobes; ssp. *subandina* having the same didynamous stamens as ssp. *axillaris*, a long corolla tube, and acute corolla lobes; ssp. *parodii* having four long stamens of equal length and a short one, a long slender corolla tube, and acute corolla lobes. Since these three subspecies are morphologically similar to each other except for the flower characters, it is difficult to identify subspecies of *P. axillaris* before blooming, which has caused a long history of confusion in recognizing infraspecific taxa in *P. axillaris* complex (Steere 1931, Ferguson and Ottley 1932, Cabrera 1977, Wijsman 1982, Ando 1996).

The classification of *P. integrifolia* complex has also been controversial (Fires 1911, Smith and Downs 1966, Wijsman 1982). Fires (1911) noted that one of the main characters distinguishing *P. integrifolia* and *P. inflata* is the pedicel condition in the fruiting state: the pedicels are deflexed in *P. integrifolia*, whereas inflexed in *P. inflata*. However, Wijsman (1982) discovered that both flower size and pedicel condition were correlated with geographical distribution, and concluded that *P. integrifolia* and *P. inflata* were not distinct species, but were subspecies of a single, broadly defined species. In contrast, Ando *et al.* (2005) recognized that *P. integrifolia* and *P. inflata* are allopatric species based on their geographically distinct distributions and the statistically significant gaps between morphological measurements in these two taxa. However, because of their close similarity in morphological characters, there have been no distinct qualitative indicators to distinguish them until now.

In the genus *Petunia*, several molecular studies have been reported to clarify the relationships among the several taxa, such as RFLP analysis of ribosomal DNA (Kabbaj *et*

Communicated by H. Yamagishi

Received October 15, 2007. Accepted January 16, 2008.

*Corresponding author (e-mail: inakamur@faculty.chiba-u.jp)

al. 1995), DNA amplification fingerprinting (Cerny *et al.* 1996), sequence polymorphism in a chalcone synthase intron (Griesbach *et al.* 2000), and RFLP analysis of chloroplast DNA (Ando *et al.* 2005). Kulcheski *et al.* (2006) attempted to analyze 11 taxa of *Petunia* using combined data of nuclear ribosomal DNA sequences and plastid DNA sequences. However, none of these molecular studies was designed to provide molecular evidence for distinguishing the taxa within *P. axillaris* and *P. integrifolia* complexes.

One of the most useful methods for investigating taxonomic and phylogenetic relationships among living organisms of all types is the comparison of particular DNA sequences. Sang (2002) clearly stated that sequences of single or low-copy nuclear genes are particularly helpful to understand inter- and intra-specific relationships of various plant groups. Recently, we found that DNA sequences of the 19th intron and 20th exon of the *PolA1* gene showed species-specific variation in the genus *Oryza* (Takahashi and Nakamura 2005). The *PolA1* gene encodes the largest subunit of RNA polymerases I and presents as a single copy per haploid genome in almost all plants. In the present study, we focused on sequence variability in the *PolA1* 19th intron and 20th exon to distinguish closely related taxa in *P. axillaris* and *P. integrifolia* complexes, and provide evidence for the usefulness of the *PolA1* gene as a nuclear marker to discriminate these taxa.

Materials and Methods

Plant materials

Seeds of 5 wild *Petunia* taxa with 2 accessions per taxon were collected from geographically isolated wild localities (Table 1). Plants raised from seeds were grown in a greenhouse, following standard horticultural practices for garden petunias, and used for DNA analysis. As out-group material, a vegetative cultivar 'Million Bell' (MB) of the genus *Calibrachoa*, which is a closely related genus to *Petunia*, was obtained from Suntory Flowers Ltd., Japan and cultivated under the same conditions as petunias.

Genomic DNA isolation and PCR amplification

Genomic DNA was extracted from *ca.* 0.5 g of young leaves of every accession using a modified CTAB method (Lassner *et al.* 1989). The forward primer, designated 19ex5P (5'-CTCGCTGGACGGGGTGAGATGAATG-3'), and the reverse primer, designated 21ex3P (5'-ATACTTTC TTTGCAGCTTTTGGG-3'), were designed based on the *PolA1* gene (GenBank accession No. NM_125397) of *Arabidopsis thaliana* and EST (GenBank accession No. BI179110) of potato, respectively. DNA fragments containing 19th intron and 20th exon sequences of the *PolA1* gene were amplified by PCR using a pair of 19ex5P and 21ex3P primers (Fig. 1).

The reaction mixture of 25 µl contained 10–50 ng of genomic DNA, 1 unit of *Ex Taq* DNA polymerase (TaKaRa Co., Japan), 2.5 µl of 10× buffer (100 mM Tris-Cl, 500 mM KCl, and 15 mM MgCl₂, pH 8.0), 2 µl of 2.5 mM dNTPs, 1 µl

Table 1. Collecting localities and specimen codes of *Petunia* taxa used in this study

Taxon	Code	Location (Country, area)	DDBJ ^a
<i>P. axillaris</i> ssp. <i>axillaris</i>	U1	URUGUAY, Canelones	AB369403
	U157	URUGUAY, Montevideo	AB369404
<i>P. axillaris</i> ssp. <i>parodii</i>	U27	URUGUAY, Artigas	AB369408
	U298	URUGUAY, Cerro Largo	AB369407
<i>P. axillaris</i> ssp. <i>subandina</i>	A100	ARGENTINA, Salta	AB369405
	A203	ARGENTINA, Salta	AB369406
<i>P. integrifolia</i>	B294	BRAZIL, Rio Grande do Sul	AB369411
	U106	URUGUAY, Rio Negro	AB369412
<i>P. inflata</i>	A7	ARGENTINA, Misiones	AB369409
	A9	ARGENTINA, Misiones	AB369410
<i>Calibrachoa</i>	'Million Bell'	Suntory Flowers Ltd.	AB369413

^a Accession No. of DDBJ (DNA databank of Japan).

Fig. 1. Schematic representation showing the 3'-region (Exon 19–21) of *Petunia PolA1* gene. Primers 19ex5P and 21ex3P (solid arrow) were used to amplify DNA fragment that contained 19th intron (207–214 bp) and 20th exon (772–775 bp). Primers 20ex5P and 20ex3P were used for direct sequencing of the amplified fragments. The sequences indicated by two bidirectional arrows were determined.

of 2.0 μ M each primer (19ex5P and 21ex3P), and 17.5 μ l of distilled water. PCR was performed with a condition of 35 cycles of 94°C for 1 min denaturation, 58°C for 1 min annealing, and 72°C for 2 min elongation in a PTC200 Thermocycler (MJ Research Co., USA).

Direct Sequencing of PCR products

The amplified PCR products were subjected to 1.2% agarose gel electrophoresis, purified with QIAquick PCR Purification Kit (Qiagen Co., USA), and directly sequenced with 19ex5P or 21ex3P primer used for PCR amplification by an ABI3100 Automated DNA Sequencer with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems Co., USA), and 20ex3P (5'-ACCATCCCCATAATCCATCTCATC-3') or 20ex5P (5'-TCAAGACAAGCTCGGAATCAGTGG-3') primer was used as an internal sequencing primer. The two internal primers were designed based on the partially determined sequences of *PolA1* 20th exon of *Petunia* in this study.

The determined sequences of the 19th intron and the 20th exon of the *PolA1* gene were analyzed using a NCBI web-based Blast server (Altschul *et al.* 1990) and aligned using CLUSTAL W (Thompson *et al.* 1994) with manual refinement. The aligned sequences were subjected to phylogenetic analysis using Maximum Likelihood software, with bootstrap analysis using 1000 replicates, from the PHYLML package (Guindon and Gascuel 2003) of the Pasteur Institute, France (<http://bioweb.pasteur.fr/intro-uk.html#phylo>). The *Calibrachoa* cultivar MB was used as an out-group sample.

The DNA sequences described in this paper have been deposited in the DDBJ DNA database (accession nos. AB369403-AB369413).

Results

Amplified DNA fragments

DNA fragments (*ca.* 1.3 kb in length) containing the 19th intron and 20th exon of the *PolA1* gene were clearly amplified by PCR using extracted total DNA as templates in 10 accessions of five taxa. Although faint shorter bands (*ca.* 500 bp) were also observed, they did not affect the direct sequencing of PCR products. From the length of fragments amplified, it was difficult to discriminate all the taxa examined in the present study by 1.2% (w/v) agarose gel electrophoresis (Fig. 2).

Polymorphisms in the sequences of *PolA1* 19th intron

The *PolA1* 19th intron sequences of *P. axillaris* complex, *P. integrifolia* complex and *Calibrachoa* MB were 207 bp, 214 bp and 212 bp in size, respectively. The nucleotide substitution (G/A) at the 19th position and a 7 bp insertion/deletion between 52nd and 58th were uniquely shared by all six accessions of *P. axillaris* (Table 2). Two accessions of *P. axillaris* ssp. *parodii* showed a nucleotide substitution A/G at the 50th position. *P. inflata* A9 accession

Fig. 2. Electrophoresis pattern of amplified PCR products on a 1.2% agarose gel. White arrow denotes amplified DNA fragments by PCR with 19ex5P and 21ex3P primers. Size markers (ϕ X174/*Hae*III) are shown on the left (lane M). Lane 1, *P. axillaris* ssp. *axillaris* (U1); lane 2, *P. axillaris* ssp. *parodii* (U27); lane 3, *P. axillaris* ssp. *subandina* (A100); lane 4, *P. integrifolia* (U106); lane 5, *P. inflata* (A7); lane 6, *P. axillaris* ssp. *axillaris* (U157); lane 7, *P. axillaris* ssp. *parodii* (B298); lane 8, *P. axillaris* ssp. *subandina* (A203); lane 9, *P. integrifolia* (B294); lane 10, *P. inflata* (A9).

showed an additional heterologous site (A and T) at the 81st position (Table 2).

Polymorphisms in the sequences of the *PolA1* 20th exon

The *PolA1* 20th exon sequences of *P. axillaris* complex, *P. integrifolia* complex and *Calibrachoa* MB were 775 bp, 772 bp and 757 bp in size, respectively. All accessions of the *P. axillaris* complex had a 3 bp longer exon with a 3 base duplication (AAG) than *P. integrifolia* complex at the 612–613th position (Table 2). Moreover, five independent nucleotide substitutions (positions at 298th, 370th, 475th, 479th and 663rd) were found in common in the *P. axillaris* complex. Within the *P. axillaris* complex, ssp. *subandina* was clearly discriminated from the other two subspecies by two nucleotide substitutions, A vs. G (44th and 514th). *P. integrifolia* complex shared one common nucleotide substitution (A/G) at the 627th position. Within the *P. integrifolia* complex, *P. integrifolia* showed two specific nucleotide substitutions (T and A at 241st and 754th, respectively), while *P. inflata* showed one specific nucleotide substitution (T at 629th).

Phylogenetic tree using *PolA1* 19th intron and 20th exon sequence variations

In each three taxa of *P. axillaris* complex, each two accessions showed identical sequences in the 19th intron and 20th exon of the *PolA1* gene. The 11 aligned sequences of the gene were analyzed using DNA Maximum Likelihood analysis of PHYLML. The majority consensus tree was calculated and drawn by drawtree software (Fig. 3). The obtained phylogenetic tree clearly showed that *P. integrifolia* and *P. axillaris* complexes formed separated clades. A similar tree with the same topology was also obtained by DNA distance and Neighbor-Joining analyses (data not shown). In the *P. integrifolia* complex, two species, *inflata* and *integrifolia*, were clearly separated into each group. In the *P. axillaris* complexes, three branches corresponding to each taxon, ssp. *parodii*, ssp. *subandina* and ssp. *axillaris*, were clearly separated, respectively. The *Calibrachoa* MB

Table 2. Comparison of variable sites in *PolA1* 19th intron and 20th exon sequences among species in *P. integrifolia* complex and *P. axillaris* complex

Taxon ^a	Name	Positions in 19th intron ^b				Positions in 20th exon ^b												
		19	50	52-58	81	20	44	241	298	370	475	479	514	612_613	627	629	663	754
<i>Calibrachoa</i>	MB	G	A	TGACTTT	A	T	G	C	C	C	G	A	-	---	A	G	A	T
<i>P. inflata</i>	A7	•	•	••••••	•	C/T	•	•	•	•	•	•	G	---	G	T	•	•
	A9	•	•	••••••	A/T	C/T	•	•	•	•	•	•	G	---	G	T	•	•
<i>P. integrifolia</i>	B294	•	•	••••••	•	•	•	T	•	•	•	•	G	---	G	•	•	A
	U106	•	•	••••••	•	•	•	T	•	•	T	•	G	---	G	•	•	A
<i>P. axillaris</i> ssp. <i>axillaris</i>	U1	A	•	-----	•	•	•	•	T	T	A	G	G	AAG	•	•	C	•
	U157	A	•	-----	•	•	•	•	T	T	A	G	G	AAG	•	•	C	•
<i>P. axillaris</i> ssp. <i>parodii</i>	U27	A	G	-----	•	•	•	•	T	T	A	G	G	AAG	•	•	C	•
	U298	A	G	-----	•	•	•	•	T	T	A	G	G	AAG	•	•	C	•
<i>P. axillaris</i> ssp. <i>subandina</i>	A100	A	•	-----	•	•	A	•	T	T	A	G	A	AAG	•	•	C	•
	A203	A	•	-----	•	•	A	•	T	T	A	G	A	AAG	•	•	C	•

^a *PolA1* 19th intron and 20th exon sequences of *Calibrachoa* ‘Million Bell’ (MB) were used as an out-group. The sequences of *P. integrifolia* complex and *P. axillaris* complex were compared with those of *Calibrachoa* MB and only unique sites of *Petunia* were shown in this Table.

^b Number indicates position of the unique site based on the sequence of 19th intron (214bp) or 20th exon (772bp) in *P. inflata* A7. Dot indicates the same base with *Calibrachoa* MB. Dash indicates deletion and underbar between 612 and 613 shows insertion site.

formed a different clade from *P. integrifolia* and *P. axillaris* complexes.

Discussion

In the present study, we successfully sequenced the 19th intron and 20th exon of the *PolA1* gene of 5 taxa (2 accessions per taxon) in the genus *Petunia*; i.e. 3 subspecies in *P. axillaris* complex and 2 species in *P. integrifolia* complex, respectively. Sequence variations clearly identified the two complexes as separate large groups with eight differences (Table 2 and Fig. 3), showing large disparity between them in accordance with A and D groups classified on the basis of the analysis of flower color (Ando *et al.* 1999). Each corresponding taxon within these complexes was also identified with 1–3 differences. Between two species of the *P. integrifolia* complex, *P. integrifolia* and *P. inflata*, three unique nucleotide substitutions were found in *PolA1* 20th exon. On the other hand, within the *P. axillaris* complex, ssp. *parodii* has 1 unique nucleotide substitution in *PolA1* 19th intron, whereas ssp. *subandina* has 2 unique nucleotide substitutions in *PolA1* 20th exon (Table 2). Thus, the present study clearly showed the usefulness of the sequence of the 19th intron and 20th exon of the *PolA1* gene.

The variations found in this study would be useful to develop DNA markers such as single nucleotide polymorphisms (SNPs) or sequence-characterized amplified region (SCAR) markers for early discrimination of the taxa before blooming, phylogenetic analysis of the genus *Petunia*, and to resolve the breeding history of garden petunias (*Petunia hybrida*). We would also be interested in the phylogenetic relationships within all species of the genus *Petunia* if the sequences of the *PolA1* gene could be analyzed on the other 14 taxa.

Fig. 3. Phylogenetic tree was constructed using combined sequences of 19th intron and 20th exon in *PolA1* gene by DNA maximum likelihood analysis of PHYML. *P. inflata* (A7, A9), *P. integrifolia* (U106, B294), *P. axillaris* subsp. *subandina* (A100, A203), *P. axillaris* subsp. *axillaris* (U1, U157), *P. axillaris* subsp. *parodii* (U27, A298), *Calibrachoa* ‘Million Bell’ (MB).

Acknowledgements

We would like to express our sincere thanks to Professor T. Ando (Faculty of Horticulture, Chiba University, Chiba, Japan) for providing original plant materials, and to Associate Professor H. Kokubun (Faculty of Horticulture, Chiba University, Chiba, Japan) for his kind assistance during the course of this research.

Literature Cited

- Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman (1990) Basic local alignment search tool. *J. Mol. Bio.* 215: 403–410.
- Ando, T. (1996) Distribution of *Petunia axillaris* (Solanaceae) and its new subspecies in Argentina and Bolivia. *Acta Phytotax. Geobot.* 47: 19–30.
- Ando, T., N. Saito, F. Tatsuzawa, T. Kakefuda, K. Yamakage, E. Ohtani, M. Koshi-ishi, Y. Matsusake, H. Kokubun, H. Watanabe, T. Tsukamoto, Y. Ueda, G. Hashimoto, E. Marchesi, K. Asakura, R. Hara and H. Seki (1999) Floral anthocyanins in wild taxa of *Petunia* (Solanaceae). *Biochem. Syst. Ecol.* 27: 623–650.
- Ando, T., N. Ishikawa, H. Watanabe, H. Kokubun, Y. Yanagisawa, G. Hashimoto, E. Marchesi and E. Suarez (2005) A morphological study of the *Petunia integrifolia* complex (Solanaceae). *Ann. Bot.* 96: 887–900.
- Cabrera, A.L. (1977) Novedades en las Solanaceas de Entre Rios. *Hickenia* 1: 79–81.
- Cerny, T.A., G. Caetano-Anolles, R.N. Trigiano and T.W. Starman (1996) Molecular phylogeny and DNA amplification fingerprinting of *Petunia* taxa. *Theor. Appl. Genet.* 92: 1009–1016.
- Ferguson, M.C. and A.M. Ottley (1932) Studies in *Petunia*. III. A re-description and additional discussion of certain species of *Petunia*. *Am. J. Bot.* 19: 385–403.
- Fries, R.E. (1911) Die Arten der Gattung *Petunia*. *Kungl. Svenska Vetenskapsakad. Handl.* 46: 1–72.
- Griesbach, R.J. and R.M. Beck (2000) Molecular heterogeneity of the chalcone synthase intron in *Petunia*. *HortScience* 35: 1347–1349.
- Guindon, S. and O. Gascuel (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Sys. Bio.* 52: 696–704.
- Jussieu, A.L. (1803) Sur le *Petunia*, genre nouveau de la famille des plantes solanees. *Annales du Museum National d'Histoire Naturelle* 2: 214–216.
- Kabbaj, A., F. Zeboudj, D. Peltier, A. Tagmount, M. Tersac, H. Delieu and A. Berbille (1995) Variation and phylogeny of the ribosomal DNA unit types and 5S DNA in *Petunia* Jussieu. *Gen. Res. Crop Evol.* 42: 311–325.
- Kulcheski, F.R., V.C. Muschner, A.P. Lorenz-Lemke, J.R. Stehmann, S.L. Bonatto, F.M. Salzano and L.B. Freitas (2006) Molecular phylogenetic analysis of *Petunia* Juss. (Solanaceae). *Genetica* 126: 3–14.
- Lassner, M.W., P. Peterson and J.I. Yoder (1989) Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. *Plant Mol. Biol. Rep.* 7: 116–128.
- Mishiba, K., T. Ando, M. Mii, H. Watanabe, H. Kokubun, G. Hashimoto and E. Marchesi (2000) Nuclear DNA content as an index character discriminating taxa in the genus *Petunia sensu* Jussieu (Solanaceae). *Ann. Bot.* 85: 665–673.
- Paxton, J. (1836) *Petunia nyctaginiflora violacea*. *Paxton's Magazine of Botany* 2: 173.
- Sang, T. (2002) Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.
- Sink, K.C. (1975) Inheritance of three genes for morphological characters in *Petunia hybrida* in crosses with four *Petunia* species. *Can. J. Genet. Cytol.* 17: 67–74.
- Smith, L.B. and R.J. Downs (1966) *Petunia*. In Reitz, P.R. (ed.) *Flora Illustrata Catarinense. Solanaceas. Herbário 'Barbosa Rodrigues'*, Itajai, Santa Catarina, Brazil. pp. 261–291.
- Steere, W.C. (1931) *Petunia parodii*, a new species of the subgenus *Pseudonicotiana* from Argentina. *Pap. Michigan Acad. Sci. Arts Let.* 13: 213–215.
- Takahashi, H. and I. Nakamura (2005) Phylogenetic relationships of the genus *Oryza* on the analysis of *PolA1* 20th exon. *Breed. Res.* 8 (Suppl. 2): 226 (in Japanese).
- Thompson, J.D., D.G. Higgins and T.J. Gibson (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nuc. Aci. Res.* 22: 4673–4680.
- Watanabe, H., T. Ando, E. Nishino, H. Kokubun, T. Tsukamoto, G. Hashimoto and E. Marchesi (1999) Three groups of species in *Petunia sensu* Jussieu (Solanaceae) inferred from the intact seed morphology. *Amer. J. Bot.* 86: 302–305.
- Wijsman, H.J.W. (1982) On the inter-relationship of certain species of *Petunia* I. Taxonomic notes on the parental species of *Petunia hybrida*. *Acta Bot. Neerl.* 31: 477–490.
- Wijsman, H.J.W. (1990) On the inter-relationship of certain species of *Petunia* VI. New names for the species of *Calibrachoa* formerly included into *Petunia* (Solanaceae). *Acta Bot. Neerl.* 39: 101–102.