Conservation Genet Resour
DOI 10.1007/s12686-014-0394-2

MICROSATELLITE LETTERS

Isolation and characterization of 16 microsatellite loci in the endemic *Viola cheiranthifolia* Humb. & Bonpl. (Violaceae) and their transferability to *Viola palmensis* Web & Berthel.

P. Rodríguez-Rodríguez · M. A. González-Pérez · T. M. Culley · E. Carqué · P. A. Sosa

Received: 10 November 2014 / Accepted: 23 November 2014

Abstract Sixteen nuclear microsatellite markers (SSR) were developed for *Viola cheiranthifolia* Humb. & Bonpl. (Violaceae), endemic to Tenerife Island (Canary Islands), and tested on the closely related *Viola palmensis* Web & Berthel., endemic to La Palma Island. All loci showed polymorphism and fifteen of them could be transferred to *V. palmensis*. They had an average of 5 and 3 alleles per locus in *V. cheiranthifolia* and *V. palmensis*, respectively. This set of markers will be useful for studying the population genetics of both species, helping to their conservation and management.

Keywords *Viola cheiranthifolia* · *Viola palmensis* · Microsatellites · Canary Islands · Genetic diversity · Conservation

The genus *Viola* (Violaceae) includes eight species in the Canary Islands, but only five belong to section *Melanium* (*V. arvensis* Murray, *V. cheiranthifolia* Humb. & Bonpl., *V. kitaibeliana* Schult. *in* Roem. & Schult., *V. palmensis* Webb & Berthel., and *V. tricolor* L.) Within this group, *V. cheiranthifolia* and *V. palmensis* are insular endemic species. *Viola cheiranthifolia* is endemic to Tenerife, growing in Teide National Park at 3,600 m a.s.l. *V. palmensis* is endemic to La Palma Island and is also found at high altitudes (1,800–2,400 m a.s.l). Both species are included as "vulnerable" by the Spanish Red List of Vascular Flora (Moreno et al. 2008).

Here, we describe the development of 16 microsatellite markers in *V. cheiranthifolia*, and their cross amplification on the related species *V. palmensis*, indicating their effectiveness in identifying patterns of genetic diversity.

Genomic DNA for the development of markers and subsequent surveys were extracted from leaf tissue using Dellaporta et al. (1983) protocol.

Microsatellite loci were developed by Savannah River Ecology Laboratory (University of Georgia) using a 454 sequencing. Extracted DNA was serially enriched twice for microsatellites using 3 probe mixes. 67 primers pairs were initially chosen, of which 16 amplified consistently and were used for initial screening. 33 natural individuals of Montaña Blanca population of *V. cheiranthifolia* and 32 of Pico de la Cruz population of *V. palmensis* were tested. Eight samples of each species of the genus in the archipelago and *V. paradoxa* section *Melanium* (Madeira) were included for cross amplification testing.

Each 25 μL PCR reaction contained approximately 20 ng of DNA, 10 pmol of each primer, as well as PCR Master Mix (Reddy-Mix, ABgene, Surrey, UK) that included 0.625 units of Taq DNA polymerase, 75 mM Tris–HCl, 20 mM $(NH4)_2SO_4$, 0.01 % Tween 20, and 0.2 mM of each dNTP. $MgCl_2$ concentrations are shown (Table 1). Forward primers were colour-labelled at the 5′-end with 6-FAM, PET, NED, VIC or TAMRA.

P. Rodríguez-Rodríguez (✉) · M. A. González-Pérez · P. A. Sosa
Departamento de Biología. Campus Universitario de Tafira, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de Gran Canaria, Canary Islands, Spain
e-mail: priscila.rodriguez102@alu.ulpgc.es

M. A. González-Pérez
Departamento de Biodiversidad Molecular y Banco de ADN, Jardín Botánico Canario "Viera y Clavijo"-Unidad Asociada CSIC, Cabildo de Gran Canaria, 35017 Las Palmas de Gran Canaria, Canary Islands, Spain

T. M. Culley
Department of Biological Sciences, University of Cincinnati, 614 Rieveschl Hall, Cincinnati, OH 45221-0006, USA

E. Carqué
TRAGSA, Las Palmas de Gran Canaria, Canary Islands, Spain

Published online: 29 November 2014

Table 1 Characteristics of sixteen microsatellite loci developed for *Viola cheiranthifolia* and transferred to *Viola palmensis*

Locus	Genebank ID	Motif	PCR primer sequence (5′ → 3′)	$MgCl_2$ (mM)	Size range (bp)	*V. cheiranthifolia*					*V. palmensis*				
						N	*A*	*Ho*	*He*	*F*	*N*	*A*	*Ho*	*He*	*F*
VIOdi-2	HE601734	$(AG)_9$	F: GGCGAGCAACCTATAATATC R: CACCACGTTCTTCCATACTC	2.0	136–141	33	2	0.333	0.416	0.202	30	2	1.000	0.508	−1.000[a]
VIOdi-6	HE601736	$(AG)_8$	F: CTTGATTGCTGGAGTGTGAC R: GGCGAATCACTACTGTTGTC	2.5	134–175	26	3	0.423	0.348	−0.222	26	3	0.269	0.446	0.400[b]
VIOdi-8	HE601737	$(AC)_9$	F: CACAGCTTCTCCATCACAAC R: TAGGAATGACTTGGCTTCTG	1.5	257–265	30	3	0.300	0.569	0.477[ab]	32	2	1.000	0.508	−1.000[a]
VIOdi-10	HG916757	$(AC)_{13}$	F: CTACTGATGGGTGTCGAATC R: GGAACGTGAAACTCTGTAGC	1.5	386–404	31	4	0.516	0.598	0.139	30	3	0.267	0.292	0.087
VIOdi-24	HG916762	$(AG)_{11}$	F: ACTTCTTGATTGAACGGAAC R: TCACATTCATCGGATCTTTC	2.5	271–279	32	4	0.156	0.391	0.605[ab]	28	3	0.286	0.447	0.365[b]
VIOtri-1	HE601738	$(AAC)_7$	F: CTTTCGCTGGAGGACTATAG R: TTAGCTGTGGTGGAGAAGTC	1.5	237–243	33	2	0.455	0.441	−0.032	32	2	0.094	0.091	−0.033
VIOtri-4	HE601739	$(ATC)_8$	F: GTGAGGATCGGAAACAATAG R: CTATGGCGGGTGTAGTAATC	2.0	149–177	33	6	0.455	0.545	0.167	31	2	0.129	0.123	−0.053
VIOtri-6	HE601740	$(AAC)_9$	F: ATGCACAGTCACAGCCTTAC R: GCTTCCGTGATTATTAGACC	2.0	257–269	29	3	0.069	0.194	0.648[ab]	32	–	–	–	–
VIOtri-7	HG916755	$(AAG)_8$	F: CTCGGTTCGGGATATATAAG R: ATGGAAAGTATGCGAGATTC	2.0	118–157	33	9	0.667	0.781	0.149	32	3	0.125	0.177	0.295
VIOtri-8	HE601741	$(ACT)_8$	F: TCGAAGGGTTCCATATAATC R: TTATCTCCGATCCTCAATTC	2.5	265–274	28	3	0.393	0.623	0.373[b]	29	2	0.034	0.034	−0.018
VIOtri-9	HG916756	$(ATC)_{10}$	F: TCCTTCAAATTCATGGTGAG R: CCACTCTTCAACAAGGAATG	2.5	174–204	33	8	0.697	0.809	0.141	30	3	0.567	0.595	0.048
VIOtri-13	HG916758	$(AAG)_7$	F: GAACTTTAAACCGCAGTGTC R: ATCAACCAAATCCATGAAAG	1.5	220–232	28	3	0.214	0.586	0.639[ab]	31	3	0.129	0.182	0.296
VIOtri-14	HG916759	$(ATC)_7$	F: CTTCCAGGTTTCAAAGACAG R: GTTATAGGCTGAAGGGTCAC	1.5	132–144	32	4	0.281	0.253	−0.114	30	2	0.133	0.127	−0.054
VIOtri-24	HG916761	$(ATC)_8$	F: ACTGAGAGCCAATCAAAGAG R: TCACTCCCAAATCAAGAAAC	2.5	263–284	32	5	0.625	0.689	0.094	25	2	0.200	0.301	0.341
VIOtet-8	HE601742	$(ACAT)_{13}$	F: AAACAGCCATCACCACTTAC R: ATTACAAACACGGGAAGTTG	2.0	218–304	31	16	0.774	0.870	0.112	39	11	0.688	0.791	0.132

Table 1 continued

Locus	Genebank ID	Motif	PCR primer sequence ($5' \rightarrow 3'$)	$MgCl_2$ (mM)	Size range (bp)	*V. cheiranthifolia*					*V. palmensis*				
						N	*A*	*Ho*	*He*	*F*	*N*	*A*	*Ho*	*He*	*F*
VIOtet-13	HG916763	$(AAAC)_8$	F: AGTCTAGTTTGCGCCTGTAG R: ATCTGCACAGGAGGTAAATG	2.5	156–172	33	5	0.242	0.276	0.123	33	4	0.296	0.710	0.587[ab]

N sample size, *A* allele number, *Ho* observed heterozygosity, *He* expected heterozygosity, *F* Fis inbreeding coefficient

[a] Significant deviation from Hardy–Weinberg equilibrium (α = 0.05)

[b] Frequency of null alleles >0.05

In general, amplifications were carried out using the following thermal cycling conditions: 3 min denaturation at 95 °C, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 60 °C (except Viodi-10 at 62.5 °C), and 72 °C for 1.5 min; followed by 5 min elongation at 72 °C. The products were detected with an ABI 3130XL, and fragment sizes were determined using GeneMapper 4.0 (Applied Biosystems, Inc.). We identified allele peak profiles at each locus and assigned a genotype to each individual.

The sixteen tested primer pairs amplified and were polymorphic in *V. cheiranthifolia* and fifteen could be transferred to *V. palmensis.* 10 of the markers also amplified in the other species belonging to section *Melanium,* not showing transferability to the section *Viola*.

Linkage disequilibrium and deviation from Hardy–Weinberg equilibrium were calculated using Genepop version 4.2 (Rousset 2008). Basic genetic diversity indices, mean number of alleles (A), and the observed (Ho), unbiased expected (He) heterozygosities for each locus were estimated with Cervus version 3.0.3 (Kalinowski et al. 2007). Estimation of null alleles was carried out with Microchecker 2.2.3 (Van Oosterhout et al. 2004; Table 1).

The sixteen microsatellites were polymorphic for *V. cheiranthifolia* with an average of 5 alleles per locus, the number of alleles ranging from 2 to 16. The expected heterozygosity (*He*) ranged from 0.194 (VIOtri-6) to 0.870 (VIOtet-8) with a mean value of 0.524 (Table 1). Fifteen loci could be transferred to *V. palmensis* succesfully, having an allele range from 2 to 10 with a mean of 3.07 alleles/locus, and expected heterozygosity (*He)* from 0.034 (Viotri-8) to 0.791 (Viotet-8) with a mean of 0.355. None of the loci showed to be linked after Bonferroni correction neither in *V. cheiranthifolia* nor *V. palmensis.* But four loci in *V. cheiranthifolia* and three in *V. palmensis* deviated from Hardy–Weinberg equilibrium after Bonferroni correction (Table 1) Markers with high frequency of null alleles are also indicated in Table 1.

Altogether, these are the first microsatellite markers developed for any *Viola* spp. in the Canary Islands. They are highly informative and can be useful for population genetics, evolutionary and conservation studies of the target species and others within the genus *Viola* section *Melanium*.

Acknowledgments This research was co-funded by the Transnational Cooperation Programme Madeira-Açores-Canary Islands 2007-2013 (MAC/01/C020) and "Organismo Autónomo de Parques Nacionales" (Project 255/2011). We also thank "Agencia Canaria de Investigación, Innovación y Sociedad de la Información" for the fellowship granted to Priscila Rodríguez.

References

Dellaporta S, Wood J, Hicks JB (1983) A Plant DNA Minipreparation: version II. Plant Mol Biol Report 1:19–21

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099–1106

Moreno JC, Bañares Á, Blanca G, Güemes J, Ortiz S (2008) Lista Roja 2008 de la flora vascular española. Dirección General de Medio Natural y Política Forestal, Madrid

Rousset F (2008) GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Res 8:103–106

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538