HortScience 58(9):1055–1056. 2023. https://doi.org/10.21273/HORTSCI17333-23

Buddleja Cultivar Identification Using Microsatellite Markers

Cara Still, Jacob Snelling, Shawn Mehlenbacher, and Ryan Contreras
Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA

Keywords. butterfly bush, DNA fingerprinting, hybrids, simple sequence repeat, SSR

Abstract. Buddleja **(butterfly bush) is a genus of common landscape plants in temperate and subtropical gardens. Substantial breeding has led to a wealth of diverse cultivars with varied pedigrees. Molecular markers would be useful tools for breeders and others studying butterfly bush to identify cultivars. We evaluated SSR markers developed in *Buddleja* to fingerprint 11 cultivars to determine whether they were useful in cultivar identification. Markers Bud_03, Bud_10, and Bud_13 were polymorphic across all genotypes in the study and capable of accurate cultivar identification. These markers may be useful to breeders for intellectual property protection and to identify cultivars in instances of mislabeling.**

Butterfly bush (*Buddleja davidii*) is a popular ornamental plant for its ease in culture, fragrant and colorful flowers, and pollinator attraction. Its popularity has led breeders to generate an array of clonally propagated cultivars, including the 121 listed by Dirr (2009). Molecular markers may be useful in identification of cultivars where morphological identification falls short (Laurentin 2009). Fingerprinting specific cultivars could also provide some protection to breeders seeking plant patents for their new cultivars where morphological identification alone is inadequate (Campi and Córdoba 2018).

Microsatellite markers may be transferrable between closely related species or genera (Kalia et al. 2011). Studies of cross-transferability of markers can be useful in taxa that have not been widely studied at the genomic level and lack molecular tools. Markers for *B. davidii* have been published along with some exploration of their interspecific cross-transferability (Schreiter et al. 2011). The objective of this study was to determine the usefulness of published microsatellite markers for *Buddleja* cultivar identification.

Materials and Methods

Plant material. A collection of cultivars including two *B. davidii* and nine interspecific hybrids was assembled during Fall 2019 (Table 1). Plants were shipped from three nurseries: Ball Horticultural Company—Star Roses (Sultana, CA, USA), Bailey Nurseries (St. Paul, MN, USA), and Spring Meadow (Grand Haven, MI, USA). There was some confusion surrounding proper identification of some plants due to worker error at the time of potting. In Spring 2021, new shipments were requested from the original sources or collected at a local retail nursery (Garland Nursery, Corvallis, OR, USA) for confirmation of cultivar identity (Table 1).

Marker selection and screening. Markers were selected from those found transferrable across several species of *Buddleja* (Schreiter et al. 2011). All eight markers from Schreiter et al. (2011) went through preliminary screening. Fresh, newly expanded leaves were collected either the night before or morning of extraction and stored in a refrigerator at 4 °C. The extraction protocol was a fresh leaf grinding method outlined in Lunde et al. (2000), skipping the RNAase step. Double-stranded DNA suspended in TE buffer was quantified on a Synergy 2 plate reader using a Take 3 microplate and operated with Gen5 version 2.00.18 (Biotek Instruments, Winooski, VT, USA). Samples were diluted to 10 ng·μL^{-1} before polymerase chain reaction (PCR) steps.

Primers were tested in 10-μL reactions including 4.35 μL sterile deionized water, 0.8 μL 25 mM $MgC1_2$, 0.2 μL 10 mM dNTP mix, 2 μL 5× buffer (GoTaq™ Flexi, Promega Corporation, Madison, WI, USA), 0.3 μL each of 10 mM forward and reverse primer, 0.05 μL 5 U·μL^{-1} Taq DNA polymerase (GoTaq™ G2 polymerase) and 2 μL of 10 ng·μL^{-1} DNA sample. The following PCR thermocycler program was used: 95 °C for 2 min (once), 35 cycles of 95 °C for 15 s, 50 °C for 40 s (annealing stage), 75 °C for 25 s, then 72 °C for 5 min (once), and samples held at 4 °C until samples were retrieved from thermocycler. Our PCR program was modified from published methods. Annealing temperatures from Schreiter et al. (2011) matched the annealing temperature of 50 °C we used.

The first screening was used to identify markers that were polymorphic for the 11 genotypes. Confirmation of acceptable amplification was carried out on 2% agarose gels, soaked in an ethidium bromide bath, rinsed with water, and then visualized using an ultraviolet imager (BioDoc-It®; UVP, Upland, CA, USA). Amplification resulting in one to four bands per sample was considered appropriately polymorphic as all test plants were tetraploid. Only markers Bud_03, Bud_10, Bud_12, and Bud_13 from Schreiter et al. (2011) amplified all samples. Forward primers of these four with fluorescent tags were ordered for the cultivar identification step. Bud_03 and Bud_10 were tagged with 5′6-FAM and Bud_12 and Bud_13 were tagged with 5′ HEX (Integrated DNA Technologies, Coralville, IA, USA).

Cultivar identification. Samples were amplified with fluorescent primers to test cultivar identification by comparing allele sizes for original and confirmation samples, which were prepared using the PCR protocol described earlier. PCR products were diluted (1:100 PCR product: deionized sterile water) and duplexed, combining Bud_03 5′6-FAM with Bud_12 5′HEX and Bud_10 5′6-FAM with Bud_13 5′HEX. Samples were sent to the Center for Quantitative Life Sciences at Oregon State University, Corvallis, OR for fragment analysis on an AB3730 Capillary DNA sequencer using GS500 ROX as the size standard. Data were analyzed and allele sizes manually called using GeneMapper® (Life Technologies, Carlsbad, CA, USA) and recorded for the matching step. Each cultivar was checked to ensure that duplicate samples had identical allele sizes between study samples and confirmation samples.

Results and Discussion

Three primers of Schreiter et al. (2011) amplified all 11 cultivars and allele sizes matched original and confirmation samples, which confirmed identity (Table 2). Markers Bud_03, Bud_10, and Bud_13 amplified all genotypes in the study, although Bud_13 had more stuttering of peaks than Bud_10 or Bud_03. This is likely due to the structure of the repeat motif in Bud_13, which is a simple dinucleotide repeat, where Bud_10 and Bud_03 were both more complex.

The study population yielded allele sizes from 77 to 169 bp across the three analyzed markers with 7 to 10 alleles at each locus (Table 2). Individuals had up to four alleles per locus. Every genotype was heterozygous at every locus, except for ‘Blue Chip Jr.’ at the Bud_13 locus.

Although some genotypes matched others at a single locus, these primers generated a distinct fingerprint that could be used for identification of all individuals. All 11 confirmation samples matched the samples from the plants originally received, confirming the identity of all cultivars as originally labeled.

This study confirmed the usefulness of three SSR primers to identify a diverse collection of butterfly bush cultivars with varying pedigrees. These markers may be useful

Received for publication 30 Jun 2023. Accepted for publication 30 Jun 2023.
Published online 18 Aug 2023.
We gratefully acknowledge Tyler Hoskins, Ivory Knutson, and many undergraduate students for maintaining the source material. This research was funded, in part by the Horticultural Research Institute under projects 577645 and 2021-111.
C.S. and J.S. are faculty research assistants.
S.M. and R.C. are professors.
R.C. is the corresponding author. E-mail: ryan.contreras@oregonstate.edu.

Table 1. Buddleja cultivars including trade names, reported parent species, and sources of original study plants and confirmation samples.

Taxon	Parent species reported in patent or Dirr (2009)[i]	Source[ii]	Confirmation source[ii]
B. davidii 'PIBD-I' USPP26,305 Groovy Grape™	*B. davidii*	Bailey	Bailey
B. davidii 'Black Knight'	*B. davidii*	Bailey	Bailey
B. 'PIIBD-II' USPP26,278 Funky Fuchsia™	*B. davidii, B. fallowiana, B. globosa*	Bailey	Bailey
B. 'Miss Molly' USPP23,425	*B. davidii, B. fallowiana, B. globosa*	Spring	Garland
B. 'PIIBD-III' USPP26,306 Psychedelic Sky™	*B. davidii, B. fallowiana, B. globosa*	Bailey	Bailey
B. 'Blue Chip Jr.' USPP26,581	*B. davidii, B. globosa, B. lindleyana*	Spring	Garland
B. 'Blue Chip' USPP19,991	*B. davidii, B. globosa, B. lindleyana*	Spring	Garland
B. 'Pink Microchip' USPP26,547	*B. davidii, B. globosa, B. lindleyana*	Spring	Spring
B. 'Purple Haze' USPP24,514	*B. davidii, B. globosa, B. lindleyana*	Spring	Garland
B. 'Ice Chip' USPP24,015	*B. davidii, B. fallowiana, B. globosa, B. lindleyana*	Spring	Spring
B. 'Miss Violet' USPP28,448	*B. davidii, B. fallowiana, B. globosa, B. lindleyana*	Spring	Garland

[i] Parent species either reported in patents, or if cultivar was used in pedigree, patent for parent cultivar was also investigated and reported here.
[ii] Nursery abbreviations as follows: Ball = Ball Horticultural Company; Bailey = Bailey Nursery; Spring = Spring Meadow; Garland = Garland Nursery.

Table 2. Primers and allele sizes generated from three SSR markers to identify 11 *Buddleja* cultivars including original plants received for which labeling was thought to have been confused (program sample) and the confirmation samples received with correct identification.

Locus	Primer sequence (5′-3′)	Fluorescent tag	Taxon	Program sample alleles	Confirmation sample alleles
Bud_03	F: GCATGCGCTGACATTTTTC.	5′6-FAM	Black Knight	96, 100, 104	96, 100, 104
	R: GTCTTCTCGACCCATGTGC		Blue Chip	89, 96, 100	89, 96, 100
			Blue Chip Jr.	89, 96	89, 96
			Funky Fuchsia™	77, 116	77, 116
			Groovy Grape™	100, 104	100, 104
			Ice Chip	96, 100, 114	96, 100, 114
			Miss Molly	91, 96, 116	91, 96, 116
			Miss Violet	92, 100	92, 100
			Pink Microchip	89, 91, 96	89, 91, 96
			Psychadelic Sky™	77, 100, 104	77, 100, 104
			Purple Haze	110, 116	110, 116
Bud_10	F: TCCCTCTCATATTGGGATAACA	5′6-FAM	Black Knight	134[i], 153	133[i], 153
	R: GCATTTGGAACCGTTAAAGC		Blue Chip	134, 140, 146	134, 140, 146
			Blue Chip Jr.	134, 140, 169	134, 140, 169
			Funky Fuchsia™	134, 169	134, 169
			Groovy Grape™	134, 140, 153	134, 140, 153
			Ice Chip	134, 140, 146, 155	134, 140, 146, 155
			Miss Molly	134, 169	134, 169
			Miss Violet	97, 134, 146	97, 134, 146
			Pink Microchip	134, 140, 169	134, 140, 169
			Psychadelic Sky™	134, 140, 169	134, 140, 169
			Purple Haze	134, 155, 169	134, 155, 169
Bud_13	F: CCTAACTGCGAATTGTATAGTTTCC	5′HEX	Black Knight	109, 120, 124, 150	109, 120, 124, 150
	R: TCTGATGCAGTCAGGTTTGC		Blue Chip	120, 136, 150	120, 136, 150
			Blue Chip Jr.	136	136
			Funky Fuchsia™	120, 126, 136	120, 126, 136
			Groovy Grape™	109, 119, 121	109, 119, 121
			Ice Chip	109, 136	109, 136
			Miss Molly	120, 122, 136	120, 122, 136
			Miss Violet	120, 136, 150	120, 136, 150
			Pink Microchip	120, 122, 136	120, 122, 136
			Psychadelic Sky™	120, 121, 136	120, 121, 136
			Purple Haze	124, 128, 136	124, 128, 136

[i] 'Black Knight' at locus Bud_10 varies slightly with rounding. Original samples round up to 134 (raw data 133.55), the confirmation samples round down to 133 (raw data 133.4).

for breeders as part of intellectual property protection and confirming identity where plants may have been mislabeled.

References Cited

Campi M, Córdoba A. 2018. The co-evolution of science and law in plant breeding: Incentives to innovate and access to biological resources. J Intellectual Property Rights. 23:198–210.

Dirr MA. 2009. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 6th ed. Stipes Publishing, Champaign, IL.

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK. 2011. Microsatellite markers: An overview of the recent progress in plants. Euphytica. 177:309–334. https://doi.org/10.1007/s10681-010-0286-9.

Laurentin H. 2009. Data analysis for molecular characterization of plant genetic resources. Genet Resources Crop Evol. 56:277–292. https://doi.org/10.1007/s10722-008-9397-8.

Lunde F, Mehlenbacher SA, Smith DC. 2000. Survey of hazelnut cultivars for response to eastern filbert blight inoculation. HortScience. 35(4):729–731. https://doi.org/10.21273/HORTSCI.35.4.729.

Schreiter S, Ebeling SK, Durka W. 2011. Polymorphic microsatellite markers in the invasive shrub *Buddleja davidii* (Scrophulariaceae). Am J Bot. 98(2):e39–e40. https://doi.org/10.3732/ajb.1000417.