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Source: Applications in Plant Sciences, 4(11)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1600068

Applications in Plant Sciences 2016 4(11): 1600068

PRIMER NOTE

DEVELOPMENT OF SINGLE-NUCLEOTIDE POLYMORPHISM MARKERS FOR *BROMUS TECTORUM* (POACEAE) FROM A PARTIALLY SEQUENCED TRANSCRIPTOME[1]

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- *Premise of the study: Bromus tectorum* (Poaceae) is an annual grass species that is invasive in many areas of the world but most especially in the U.S. Intermountain West. Single-nucleotide polymorphism (SNP) markers were developed for use in investigating the geospatial and ecological diversity of *B. tectorum* in the Intermountain West to better understand the mechanisms behind its successful invasion.
- *Methods and Results:* Normalized cDNA libraries from six diverse *B. tectorum* individuals were pooled and sequenced using 454 sequencing. Ninety-five SNP assays were developed for use on 96.96 arrays with the Fluidigm EP1 genotyping platform. Verification of the 95 SNPs by genotyping 251 individuals from 12 populations is reported, along with amplification data from four related *Bromus* species.
- *Conclusions:* These SNP markers are polymorphic across populations of *B. tectorum*, are optimized for high-throughput applications, and may be applicable to other, related *Bromus* species.

Key words: *Bromus tectorum*; cheatgrass; invasive; Poaceae; single-nucleotide polymorphism (SNP) development.

Bromus tectorum L. (Poaceae) is an annual grass species that is extremely successful at invading shrubland habitats in the U.S. Intermountain West (IMW). Over the past 40 years, its range has expanded into both desert and montane habitats previously considered resistant to invasion pressure. Population and ecological genetic research on *B. tectorum* in the North American invaded range has relied on either six allozyme loci, with between two and four alleles per locus (Novak et al., 1991; Valliant et al., 2007; Schachner et al., 2008), or seven microsatellite (simple sequence repeat [SSR]) markers (Ramakrishnan et al., 2002, 2004), although some studies used only four of the seven markers (Leger et al., 2009; Merrill et al., 2012). Use of these marker systems has revealed populations throughout the invaded region that are largely homogeneous, dominated by one or a few common genotypes, with very few heterozygous individuals. Debate exists over the relative role of outcrossing in the success of *B. tectorum*, especially in adapting to novel or stringent habitats (Ramakrishnan et al., 2006; Ashley and Longland, 2007; Valliant et al., 2007; Leger et al., 2009). It is possible that outcrossing rates in *B. tectorum* have been underestimated in homogeneous populations because the small number of markers and the low level of polymorphism may not provide enough resolution to observe recombinant genotypes (Meyer et al., 2013). To provide a larger genetic marker set, we report here the development of single-nucleotide polymorphism (SNP) marker assays for the population genetic study of *B. tectorum*. SNPs are ideal for examining the role of outcrossing in the *B. tectorum* invasion and recent range expansion, as well as quantifying variation in these invasive populations, because of the ease of assaying numerous polymorphic loci simultaneously.

METHODS AND RESULTS

For cDNA library construction, we used inflorescences and whole seedlings of six individuals with diverse SSR genotypes commonly found in multiple habitats within the IMW. Inflorescence tissue was collected and combined from three individuals with SSR genotypes IEBB, DCBB, and FEDD at SSR loci BT05, BT26, BT30, and BT33 and, likewise, for whole seedlings collected from three individuals with SSR genotypes EZBY, DABB, and KCBB (Merrill et al., 2012). RNA was extracted from each tissue sample using the ZR Plant RNA MiniPrep Kit (Zymo Research, Irvine, California, USA). A SMART approach cDNA synthesis was performed separately with RNA from the two tissue types (Zhu et al., 2001). After synthesis, cDNA was combined and normalized by treatment with a duplex-specific nuclease (Zhulidov et al., 2004).

The normalized cDNA was sequenced on a single run using a Roche 454 GS FLX instrument and Titanium reagents (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) without DNA fragmentation at the Brigham Young University DNA Sequencing Center (Provo, Utah, USA). Newbler (version 2.0.01; 454 Life Sciences, a Roche Company) was used to assemble, de novo, 1,258,041 DNA reads into 65,486 contigs. For assembly, the minimum

[1]Manuscript received 2 June 2016; revision accepted 17 August 2016.

The authors thank S. Harrison for his help with tissue collection and preparation. This research was funded in part through grants from the Joint Fire Sciences Program (2007-1-3-10) and the Cooperative State Research, Education, and Extension Service (CSREES) National Resource Initiative Biology of Weedy and Invasive Species Program (2008-35320-18677), and through support from the University of Nevada, Reno.

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doi:10.3732/apps.1600068

TABLE 1. *Bromus tectorum* SNP primers used in the KASP SNP genotyping assays.

SNP ID	GenBank accession no.	SNP position[a]	Allele-specific primers (5′–3′)[b]	Common primer (5′–3′)[c]
BTEC.0001	GELF01054254	231	ATCCTTTCGAATTCAGATTTATCTGC(A/G)	ACGTTCCTCTGGTTTTTTGAATTTTCCATT
BTEC.0004	GELF01005168	71	CCCTTTGGTAAGAAATAAAGACGC(G/A)	AGTGTCAAACGCCAATCAAAATACTGGTA
BTEC.0007	GELF01054606	264	GTCGATGAAGCGCAAGCTGT(T/C)	GCCTTCCAAGTTACCAGGTCCTTT
BTEC.0009	GELF01000627	159	CACTCCTTGATCCATGAGATAAC(G/A)	CCCTCGTGCCATTGTTTATTCTGCAA
BTEC.0013	GELF01018846	1035	CTGCAGCTGCTCATATGAAATTG(C/T)	TGGCTTCTATGTTCCCTGCCTCAAT
BTEC.0014	GELF01051811	434	GTGTTGCGTGGCGGCCT(G/A)	AACATGAGGAATCATCGCCTGAAACAAA
BTEC.0019	GELF01010834	307	GATCACTGCACACCAACCTCAA(T/C)	CAAGCTTCAGAGCGTCACACTTGAA
BTEC.0082	GELF01018777	284	GTAAAAGTTAGGTTGTTTTTGTTGGAG(T/C)	GCTAAATGAAAATAGGTTGAGAACAACCTT
BTEC.0129	GELF01007707	1215	GGCGCTCTTCCATGGTGC(C/T)	CAAGGTACTTCTTCGTTCTTCAAGACAT
BTEC.0175	GELF01010057	1079	AATGGGTTTTGGATAATGCCCTG(G/A)	GTCCTGAGGATGCTGTAGGCTCAT
BTEC.0229	GELF01028688	297	AATGCAGTGTCAATGTAGAGTCAAT(C/A)	GAGTTCGACAGCAGCAATCCCATAT
BTEC.0232	GELF01050427	59	AATAGACCATCTCGAGATGAACC(A/G)	TGCGAGGGACAACATTTACATTGTGAAAA
BTEC.0402	GELF01017057	1337	GCCTTCTTCTTGCTGCCAGG(T/A)	TGCTGGCAGCTCCCACACCATT
BTEC.0433	GELF01002345	817	GTGACCGGTCTCACGTGAGT(A/G)	GGTCTCTGAGTGCACAACGATACTT
BTEC.0448	GELF01014161	1080	GGATGTCTGATATTCCGCTGG(T/C)	GCTGTTTCTCTACCCCTCTGTTGTA
BTEC.0449	GELF01014584	65	GAGATTGCTGTAATGCAGTATCG(T/C)	CGCAAACCCTTCTCCTCAAGAGATT
BTEC.0468	GELF01052618	538	CTATCCCCATCACGATGAAATTTC(T/C)	CGCTACACTGATGTATTCAACGAGTATAT
BTEC.0505	GELF01013189	1094	GAAGTTCAAGAACAACAGATTTCGC(C/T)	ATCCAGTTCTGGAATAAGAACAAGTCCAT
BTEC.0583	GELF01015310	1024	ACCAGGGCGCCCTTATTACTG(A/T)	GAGATCTTCCTCTTGCCATCTCCTT
BTEC.0601	GELF01023207	395	GACAGTGACACCGAGATTGAG(G/T)	ATCTTCTCGTCCAAGTCCTCAGCAA
BTEC.0605	GELF01029244	874	AGCGTCTTCTTTGTTATCGTCA(G/A)	TCATACAGTATTTTGTAGCTTCGCCTCAA
BTEC.0637	GELF01004375	952	GTTGGATAAGCAAGGCTGCATG(T/G)	ATTAAGGGAGCATACATAAGCCAAAAACAA
BTEC.0657	GELF01009986	233	CAATGATGCACTCATGGGAATTCT(T/C)	GTAAGGCGGACTGCACAAATTACGAA
BTEC.0663	GELF01012084	401	GAAAACTGATGAAGCTACATGCCAT(G/C)	CCGTGGGGGAAGCTGGCAATAT
BTEC.0696	GELF01021947	714	GTACATCATCCATTATTCCTCCTTG(A/G)	GAAGCCTATTCGTACCTGATCTGACAA
BTEC.0697	GELF01022416	433	CTGCAGATCTCCCGTATCCT(T/C)	CCCTGGATCTTTCTACCTACTCCTT
BTEC.0718	GELF01050160	1124	AAGGTCCCAGGGAAGGCG(C/T)	CAACCTCCCCAGTCTCCAAAGAAAA
BTEC.0751	GELF01005830	1123	AAGTCAAGTTCTGTTAATTTTCCTCC(A/G)	CGACTCGATTGGCTCCAACATTTGAA
BTEC.0775	GELF01017138	777	GGACAAGCTCTAAATTTTTGGTTCTG(T/A)	GCTACTCTAAACAACGGGAGCAAGTA
BTEC.0790	GELF01026632	341	CCTGGATATATTATGTTCTGTAATATTCTA(G/A)	GGTTTACAATGTGAGGTAAAGGAAGGAAA
BTEC.0818	GELF01001943	125	GTAGTTGGCTCACCTGTACCTA(T/G)	CCATTCATAGTGAAGGAGTATGCTACAAA
BTEC.0853	GELF01009140	810	CTGACTGTTCTCCTGAGGTGT(A/C)	CGGACATTGCTGAATACTTTTCTCGTT
BTEC.0854	GELF01009368	538	ATGCAACAGCAATCTTCAGACC(C/T)	GGGGGGCTCTGGAACTCATCAT
BTEC.0874	GELF01015193	95	CACCCACGTACCAGTGGAC(G/A)	CACAAGTTTGACCTCATGTACGCCAA
BTEC.0882	GELF01017525	452	CGCCATCGAGAACTTCAGCTT(C/T)	CCAGGGCGATTTTGTTTCGTCGAA
BTEC.0887	GELF01018801	791	CAGTACTAGCCCCCAGATGA(T/C)	GGTGGCAGATGGTTCCTGTGGAA
BTEC.0904	GELF01024980	474	GCAGCCACTGAGCAATGTTAC(G/A)	TTAAGCAGAGTCGGATCCACAGGAA
BTEC.0973	GELF01004564	197	AGAACAATTGTAGTATGGTTATTGTCTA(A/C)	GATGTGACAAAGTGATATTTTCCGGTGTA
BTEC.0992	GELF01009440	193	AGTACTAGAGGTATGCCTGGC(G/T)	GCAGCTAATAACAGTGTAGTATCTTGGTA
BTEC.0997	GELF01010059	331	CAGAAAGAACCGACTTGCCG(C/T)	CAGAAGATACTGCATGTTCCAGAGGTT
BTEC.1013	GELF01014723	978	CATCACTGAAGCTTCTCAAGG(C/T)	CAAGAGCAAATCCTCAACAGAAGGATATA
BTEC.1058	GELF01024676	911	AGGCCTCGATTGATTGATTTCAG(C/T)	GATAACAGTCCTTCTAGGGTTCAAGAAT
BTEC.1064	GELF01026664	300	GAGGCGTCGGCCATCTC(C/T)	CTTCGTCTTGTGGACCACGGGTT
BTEC.1125	GELF01004063	529	GTAGTGAAAATTATCCATAGCCTGAT(T/C)	CACAAAAAAACATTAAGAGGGGATAGCAAA
BTEC.1203	GELF01017713	1086	ATACCCCCAGCAAGCTTATATACA(G/A)	CTTGGATTTATGATTCATGGTACCACTATT
BTEC.1204	GELF01017791	682	GTCATGACTTCAGGATCCCTTAA(C/T)	CATAGTTAATGTGCTGCGTCGGCAA
BTEC.1211	GELF01019599	169	ATAGAAACATGAACCTTCTGCGTG(G/A)	AACGTAAACAGGAGGGCGTAAATAATCTT
BTEC.1270	GELF01051217	739	ATGTCCTGACTGAGGTGCCTT(A/G)	CAAGGTAAGACATCTCAGGCAGGTA
BTEC.1352	GELF01007634	1909	ACAGGTTCAACGTTCCATGGAA(T/C)	TTATCTTAACGGGGCACACCTCACT
BTEC.1383	GELF01011814	596	CCTCAGTACCATCACAAAGATC(G/A)	GAATACTATGCACAAGTCGGTAACTGTAT
BTEC.1388	GELF01012246	1343	GGAGAGCAAACAGCTGCGCA(A/G)	TGCTGTGGCCTACATGTACCCAAT
BTEC.1398	GELF01012763	530	AAATGGCAGCAGCTCTGGTGT(T/C)	GTTCAGTGCTGCTCCCGTTTCTTTT
BTEC.1400	GELF01012857	1753	CGAGGTAGAGCTTTATCCAAC(A/G)	TTATCGCTCAATCCGTAACTTCTTGCTTT
BTEC.1407	GELF01014240	790	AAAAGAGATCCGGGCCATCG(G/A)	GTACACCTTTTGGGGATATGGGTCAT
BTEC.1413	GELF01015084	869	CCATACGCGTCTCCACTGGA(C/T)	GAAGGAGATGAGAGGCGCGCTA
BTEC.1438	GELF01018665	1266	AATTGTGTTTCTCAAATCAGTGGAG(A/G)	TTTTTTTACGAGCAAAAAAGAATCATCCAT
BTEC.1450	GELF01020563	958	CATCAGGTCATCAGAAGCTAATGC(A/T)	TGTAAATCTGATCTTGAGACCGCAGTA
BTEC.1489	GELF01027878	561	GCATCATCGGAGCACTGGC(C/G)	GTGCTGCTACTTTTGTTGCTGTCCTT
BTEC.1507	GELF01049408	106	ATTTTAAGCGGTACAAAATTTAAGACCA(A/G)	GAGGTGGGAGAGGGAGACTGAA
BTEC.1586	GELF01002187	770	CCCTGCACGCGTCGGTTT(A/G)	ATGGAGCGGCAGCAAGGATAACAAA
BTEC.1635	GELF01007503	274	GTGTTGAGAAGTTCATCGTGCAA(G/A)	TGAGCGTCCGTGGGTGTCTGTT
BTEC.1640	GELF01008175	568	ACCGACTAGCGACTCTGAAGA(A/G)	CCATTTCACCAGGTAGAGGTTCCAT
BTEC.1647	GELF01008776	986	CATCAATGGGCTCAGTGTGAG(A/G)	CGTCTACTGGCAGTTTCCGCCAT
BTEC.1652	GELF01008927	1096	AAATCGGGATTCTGGAGCTGAT(A/C)	TCCAATTTCATGAGACCAATACGGTTCAA
BTEC.1654	GELF01009235	888	CCAATATCAAAGGAGTTTGCTTGTAT(A/G)	CAAACTCCTACTGTGAACACCTATACTTT
BTEC.1724	GELF01016487	282	GGGAAGCATCGGTGCATTCT(T/G)	ATTACGCTACTGGTTCCTCGCCTTT
BTEC.1794	GELF01027724	449	GTTTCCTTGTGTCACTAGTGTATAG(C/T)	CAACATAGAGCAACCGGAAGCCAAA
BTEC.1818	GELF01049436	173	AGGGTGTTGGAACAATATCCCTT(G/C)	ATTCCATCAGAAGAGAGACTAGGAACTAA
BTEC.1832	GELF01050418	98	GCGCGAGAACATGGTCTGA(C/A)	GGTCCTAGGTGTCTGTCGGACTA
BTEC.1873	GELF01053648	678	GAGGTGTATCCTTAATGTCATGTG(C/T)	GAAGAACATTATCTCAAAGCTTGGACCAT

Table 1. Continued.

SNP ID	GenBank accession no.	SNP position[a]	Allele-specific primers (5′–3′)[b]	Common primer (5′–3′)[c]
BTEC.1907	GELF01000259	337	GACAGCCTCAACTATTCCATTATC(G/A)	GGAAGGCAGTGTTCAACTATGATTTTGAT
BTEC.1930	GELF01003039	619	CGTACAACCCTTCCATCGTCT(C/T)	TAGGGAGAATCCCAATCCGCATCAA
BTEC.2120	GELF01021228	171	TTGGCCGAGGCATGGATGA(C/T)	AAACCTCTCCTTAATTCACACGCAGAAAT
BTEC.2141	GELF01024898	1271	CATCTGGAATTTTTCATGTACATTCTC(G/A)	GTGTTTGAACTCAGCCTTATATCTGGAAT
BTEC.2142	GELF01025393	35	AGGGTCAATCGTGATAAGGCATT(G/A)	GCAGAGTGGTTGTGGTTTCAGGATA
BTEC.2148	GELF01026366	44	GGAGCCACTGTATGAAGATTTCA(C/T)	TTTCAAGAAATAGCAAACAGGCGAGCAT
BTEC.2166	GELF01030989	710	GTAGCTCTAGTTTGACAGCGCA(A/G)	GGTCACTGAACAAAAGAATACAAAGTATAA
BTEC.2399	GELF01003355	403	AGCATTCCAAGAGCAGCCAC(A/G)	GCCTTGCCGCGATGTATGGTGTT
BTEC.2409	GELF01003846	146	CACTACACTGCTAGAGCAGGT(C/T)	ACATACGCCGGATCTGAACTCTCTT
BTEC.2436	GELF01005070	247	AACCAATTGATTTTCTTCTCCTTATAAG(T/G)	GACATTGTGTAGTTGTGTGACTAGTCAAA
BTEC.2521	GELF01008215	1041	CATTGAATTTCATAGACACATTACCTCT(A/G)	CCGATAAATAGCCTGAGTGGATCCAA
BTEC.2704	GELF01014969	49	AAATCGCTGTCAGAAGGCCCA(A/G)	TTCTATCTATCGCTCCCCTGGCTT
BTEC.2773	GELF01018572	131	CAGAAAATGATTCATTATCCTCTACATC(A/G)	TAACTTCACTTCTGCAGTTTCCACCAT
BTEC.2795	GELF01019366	1033	GTTTCCCAATACCACTAGGCAC(T/C)	TGGTTCTGGAAGACCTCGCCATATA
BTEC.2807	GELF01019660	178	GCATATGACACATGTGCCATCATTT(G/A)	CAAGACAGTACAAGACTAACACGAAGTAT
BTEC.2834	GELF01020921	158	AATCAGGTTCGCTCCTGACAG(C/T)	CAAGATAGGACCCGATGTAGGTCAT
BTEC.2850	GELF01021363	198	AACATGCTGTATACTAGTGGCCA(T/G)	TTGTGTCTCTGTGGTACATAATGGGAAT
BTEC.2869	GELF01022307	405	CCCTAACGAGTTTACGAGACTC(G/A)	GGCCTATGTGCCGCCGGAGAT
BTEC.2877	GELF01022686	436	GGAGACCTACAGCTTTGGATTC(C/A)	CCATTGATGTGTGAATATCAGAAGTTCATA
BTEC.2918	GELF01024948	398	GAATTGCAATCCAATTAAAGTCGTC(G/A)	AGGGCGACCAAGTAGATGACCTTTA
BTEC.2919	GELF01024958	395	CGTACACCGGAGATGAAGAC(T/C)	CCCCACTCGCGGCGGAAGAA
BTEC.3025	GELF01035468	119	CAACATCGAGACCAGGCATTC(G/T)	GGCAGAAGAGCAGGCACAGAGAA
BTEC.3049	GELF01042054	144	AATCATGTCCCTTTCTTGTTTCTATC(A/G)	GTTAAGCATGATGGGAAGGACTGCAA
BTEC.3142	GELF01051573	612	CCCCCTAGTAACTCAAACGGC(T/A)	CGCCTGAGGCACTTGAGGTACTT
BTEC.3285	GELF01054303	450	GGATATGTTTTTTATTCTTGCTCATGTT(G/A)	GGGCGCCATACAAAGATAATTTTGTTGTA

[a] The SNP position is the number of the variable nucleotide within the contig counting from the 5′ end of the sequence as deposited in GenBank.

[b] Allele-specific primers differ at the 3′ terminal nucleotide and each includes a distinctive sequence of nucleotides at the 5′ end that are complementary to separate universal FRET cassettes contained in the KASP reagent.

[c] The common primer is used during amplification of products containing both SNPs.

Table 2. Information for the 95 *Bromus tectorum* SNPs used to genotype 251 individuals from 12 populations.

				Population-level major allele frequencies[c,d]											
SNP ID	Major allele[a]	Minor allele	Major freq.[b]	ALB	BN1	BN2	BER	GA1	GA2	GRA	LOS	MIL	PR1	PR2	SAN
BTEC.0001	G	A	0.876	0.679	0.957	1.000	0.846	0.760	0.948	0.885	0.950	0.981	0.955	1.000	0.613
BTEC.0004	C	T	0.754	0.808	0.909	1.000	0.192	0.160	0.897	0.846	0.550	0.963	0.818	0.667	0.935
BTEC.0007	G	A	0.512	0.679	0.952	0.000	0.786	0.440	0.897	0.333	0.474	0.593	1.000	0.250	0.000
BTEC.0009	T	C	0.948	1.000	1.000	1.000	1.000	0.750	0.897	0.923	1.000	0.926	1.000	0.917	1.000
BTEC.0013	C	T	0.911	0.786	1.000	1.000	0.857	0.640	0.897	0.885	1.000	0.889	1.000	0.917	1.000
BTEC.0014	T	C	0.663	0.821	0.932	0.947	0.214	0.380	0.759	0.788	0.325	0.444	0.500	0.583	0.935
BTEC.0019	G	A	0.932	1.000	1.000	1.000	0.929	0.800	0.897	0.962	1.000	0.815	1.000	0.833	1.000
BTEC.0082	G	A	0.890	0.769	0.957	1.000	0.846	0.660	0.879	0.740	0.900	0.926	1.000	0.917	0.964
BTEC.0129	C	T	0.757	0.643	0.864	1.000	0.214	0.600	0.862	0.692	0.725	0.926	0.818	0.917	0.517
BTEC.0175	T	C	0.820	0.393	0.909	0.947	0.071	0.667	0.897	0.846	0.800	0.926	1.000	0.917	1.000
BTEC.0229	G	T	0.564	0.286	0.043	1.000	0.107	0.560	0.241	0.827	0.550	0.889	0.091	0.917	0.871
BTEC.0232	G	A	0.865	0.786	0.957	1.000	0.286	0.680	0.879	0.904	0.850	0.889	1.000	0.917	1.000
BTEC.0402	A	T	0.906	0.786	1.000	1.000	0.929	0.660	0.879	0.904	1.000	0.926	1.000	0.917	0.935
BTEC.0433	A	G	0.654	0.750	1.000	0.000	0.750	0.840	0.828	0.308	0.800	0.630	1.000	0.500	0.419
BTEC.0448	G	A	0.859	0.857	1.000	0.947	0.286	0.640	0.862	0.923	0.750	0.926	1.000	0.917	0.968
BTEC.0449	G	A	0.942	1.000	1.000	1.000	0.857	0.840	0.897	1.000	1.000	0.000	1.000	0.500	1.000
BTEC.0468	A	G	0.909	0.769	1.000	1.000	0.857	0.640	0.862	0.904	1.000	0.889	1.000	0.917	1.000
BTEC.0505	T	C	0.843	0.692	0.909	1.000	0.250	0.640	0.879	0.923	0.800	0.926	1.000	0.917	0.968
BTEC.0583	A	T	0.882	1.000	0.955	1.000	0.214	0.800	0.828	0.923	0.850	0.926	1.000	0.917	0.967
BTEC.0601	T	G	0.950	0.786	1.000	1.000	0.929	0.800	0.983	0.885	1.000	1.000	1.000	1.000	1.000
BTEC.0605	T	C	0.861	0.857	0.889	1.000	0.357	0.778	0.400	1.000	1.000	0.926	1.000	0.917	0.613
BTEC.0637	A	C	0.974	1.000	1.000	1.000	0.929	0.920	0.981	1.000	0.950	1.000	1.000	1.000	0.935
BTEC.0657	A	G	0.851	0.786	0.957	1.000	0.286	0.640	0.862	0.885	0.800	0.926	1.000	0.917	0.935
BTEC.0663	C	G	0.808	0.893	1.000	0.944	0.250	0.947	0.852	0.760	0.900	0.444	0.455	0.917	1.000
BTEC.0696	G	A	0.862	0.786	0.957	0.947	0.250	0.680	0.897	0.923	0.800	0.926	1.000	0.917	1.000
BTEC.0697	A	G	0.816	0.964	0.952	1.000	0.929	0.957	0.828	0.619	1.000	0.481	0.100	0.917	0.857
BTEC.0718	T	G	0.950	1.000	0.957	1.000	0.885	0.820	0.897	0.981	1.000	0.926	1.000	0.917	1.000
BTEC.0751	A	G	0.542	0.179	0.043	0.947	0.214	0.560	0.362	0.615	0.700	0.926	0.545	0.917	0.452
BTEC.0775	A	G	0.910	0.786	1.000	1.000	0.893	0.660	0.862	0.904	1.000	0.926	1.000	0.917	1.000
BTEC.0790	T	C	0.804	0.714	0.870	1.000	0.179	0.640	0.879	0.800	0.650	0.926	0.900	0.917	0.821
BTEC0.818	G	A	0.912	0.786	1.000	1.000	0.929	0.660	0.879	0.923	1.000	0.926	1.000	0.917	0.968
BTEC.0853	C	A	0.522	0.786	0.935	0.000	0.929	0.640	0.776	0.200	1.000	0.074	0.091	0.417	0.419

Table 2. Continued.

SNP ID	Major allele[a]	Minor allele	Major freq.[b]	Population-level major allele frequencies[c,d]											
				ALB	BN1	BN2	BER	GA1	GA2	GRA	LOS	MIL	PR1	PR2	SAN
BTEC.0854	T	C	0.643	0.643	—	—	0.643	—	—	—	—	—	—	—	—
BTEC.0874	C	T	0.780	0.571	0.913	1.000	0.286	0.660	0.810	0.885	0.750	0.907	0.909	0.917	0.581
BTEC.0882	T	C	0.845	1.000	1.000	0.947	0.929	0.760	0.879	0.942	1.000	0.389	0.091	0.917	1.000
BTEC.0887	G	A	0.808	0.929	0.600	1.000	0.286	1.000	1.000	1.000	1.000	1.000	0.667	1.000	0.800
BTEC.0904	C	T	0.907	0.786	0.932	1.000	0.929	0.640	0.897	0.818	1.000	0.926	1.000	0.917	0.967
BTEC.0973	C	A	0.584	0.286	0.130	1.000	0.857	0.420	0.293	0.846	0.250	0.963	0.909	0.583	0.629
BTEC.0992	T	C	0.856	0.964	1.000	1.000	0.750	0.880	0.828	0.896	1.000	0.481	0.300	0.917	1.000
BTEC.0997	T	C	0.766	1.000	0.737	1.000	0.893	0.880	0.828	0.667	0.778	0.481	0.000	0.917	0.667
BTEC.1013	T	C	0.920	0.786	0.957	1.000	1.000	0.680	0.897	0.885	1.000	0.926	1.000	0.917	1.000
BTEC.1058	T	C	0.894	1.000	1.000	1.000	1.000	1.000	0.862	0.885	1.000	0.500	0.545	0.917	1.000
BTEC.1064	T	C	0.978	1.000	1.000	1.000	0.821	0.960	0.931	0.962	1.000	1.000	1.000	0.917	1.000
BTEC.1125	G	A	0.900	0.750	0.957	0.947	0.923	0.660	0.845	0.904	1.000	0.944	1.000	0.917	1.000
BTEC.1203	T	C	0.932	0.714	0.957	1.000	0.786	0.840	0.966	0.923	1.000	0.963	0.909	1.000	0.968
BTEC.1204	T	C	0.863	0.786	0.957	1.000	0.250	0.660	0.897	0.904	0.850	0.926	1.000	0.917	1.000
BTEC.1211	T	C	0.912	0.786	1.000	1.000	0.857	0.660	0.862	0.904	1.000	0.889	1.000	0.917	1.000
BTEC.1270	G	A	0.736	0.500	0.935	0.974	0.214	0.660	0.724	0.827	0.775	0.926	0.636	0.833	0.548
BTEC.1352	G	A	0.905	0.536	0.957	0.895	0.750	0.760	0.931	0.885	0.900	1.000	0.273	0.917	0.774
BTEC.1383	G	T	0.502	0.231	0.619	0.474	0.750	0.600	0.931	0.458	0.550	0.074	0.636	0.083	0.400
BTEC.1388	A	G	0.910	0.786	1.000	1.000	0.857	0.640	0.879	0.923	1.000	0.926	1.000	0.917	1.000
BTEC.1398	G	A	0.931	0.962	1.000	0.947	0.821	0.875	0.793	0.952	1.000	0.963	0.273	1.000	1.000
BTEC.1400	G	A	0.866	0.786	0.722	1.000	0.929	0.750	0.786	0.708	0.857	1.000	0.450	1.000	0.952
BTEC.1407	T	C	0.521	0.885	0.364	0.526	0.643	0.609	0.172	0.391	0.600	0.481	0.091	0.917	0.393
BTEC.1413	C	T	0.918	0.786	1.000	1.000	0.964	0.680	0.897	0.885	1.000	0.926	1.000	0.917	1.000
BTEC.1438	G	A	0.554	0.286	0.043	1.000	0.679	0.400	0.138	1.000	0.200	0.963	1.000	0.583	0.548
BTEC.1450	T	A	0.857	1.000	0.957	1.000	0.821	1.000	0.828	0.920	1.000	0.481	0.100	0.833	0.968
BTEC.1489	G	C	0.953	—	0.889	1.000	—	0.944	0.800	1.000	1.000	1.000	1.000	1.000	0.947
BTEC.1507	A	G	0.841	0.714	0.957	0.947	0.893	0.960	0.828	0.962	0.800	0.444	0.455	0.917	0.935
BTEC.1586	A	G	0.768	0.786	1.000	1.000	0.231	0.500	0.200	0.824	0.889	0.889	0.750	0.917	0.895
BTEC.1635	T	C	0.857	0.786	0.957	0.944	0.321	0.640	0.879	0.923	0.850	0.907	0.909	0.917	1.000
BTEC.1640	G	A	0.715	0.692	0.870	1.000	0.250	0.640	0.759	0.640	0.500	0.926	0.000	0.917	0.806
BTEC.1647	G	A	0.928	1.000	1.000	1.000	0.893	0.820	0.879	0.981	1.000	0.000	1.000	0.500	0.935
BTEC.1652	C	A	0.833	0.786	0.957	1.000	0.821	0.375	0.948	0.827	0.550	0.963	1.000	0.667	0.968
BTEC.1654	A	G	0.843	0.000	1.000	1.000	0.107	0.857	1.000	1.000	1.000	—	1.000	0.000	1.000
BTEC.1724	C	A	0.784	0.429	0.978	1.000	0.286	0.840	0.672	0.923	0.579	1.000	0.955	0.917	0.629
BTEC.1794	G	A	0.938	0.923	1.000	1.000	0.714	0.820	0.879	1.000	1.000	0.944	1.000	0.917	0.968
BTEC.1818	C	G	0.833	0.786	0.938	1.000	0.357	0.640	0.875	0.706	0.750	0.889	1.000	0.917	0.964
BTEC.1832	T	G	0.706	0.500	0.087	0.947	0.964	0.960	0.241	0.615	0.650	1.000	1.000	0.833	0.903
BTEC.1873	C	T	0.773	0.929	0.826	0.639	0.857	0.500	0.845	0.769	0.500	0.942	0.909	0.727	0.935
BTEC.1907	T	C	0.934	0.893	1.000	1.000	0.821	0.820	0.879	0.942	0.950	0.926	1.000	0.917	1.000
BTEC.1930	T	C	0.579	0.462	1.000	0.900	0.286	1.000	1.000	0.538	0.250	0.444	0.750	0.917	0.176
BTEC.2120	T	C	0.571	0.643	1.000	1.000	0.143	0.500	0.200	0.441	0.778	0.000	0.250	0.500	0.895
BTEC.2141	C	T	0.562	0.286	0.667	0.500	0.214	1.000	1.000	0.733	0.333	0.407	0.500	0.667	0.615
BTEC.2142	C	T	0.819	1.000	1.000	0.947	0.286	0.920	0.862	0.885	0.900	0.481	0.273	0.917	0.968
BTEC.2148	T	C	0.882	0.643	1.000	1.000	0.821	0.640	0.793	0.923	0.950	0.926	1.000	0.917	0.935
BTEC.2166	G	A	0.837	0.929	1.000	1.000	0.821	0.960	0.931	0.731	1.000	0.481	0.000	0.917	0.935
BTEC.2399	G	A	0.932	0.571	1.000	1.000	0.714	0.833	0.966	0.923	1.000	1.000	1.000	1.000	1.000
BTEC.2409	C	T	0.704	0.769	0.739	0.947	0.214	0.640	0.862	0.423	0.700	0.926	0.818	0.917	0.548
BTEC.2436	C	A	0.729	0.923	0.682	0.947	0.875	0.920	0.966	0.500	0.650	0.519	0.636	0.833	0.484
BTEC.2521	G	A	0.640	0.500	0.087	1.000	0.464	1.000	0.362	0.615	0.900	0.481	0.300	0.917	0.871
BTEC.2704	C	T	0.820	0.231	0.957	1.000	0.143	0.640	0.862	0.885	1.000	0.926	1.000	0.917	0.903
BTEC.2773	A	G	0.885	0.929	0.957	0.947	0.857	0.920	0.966	0.923	1.000	0.481	0.545	1.000	1.000
BTEC.2795	A	G	0.714	0.571	0.130	0.947	0.929	1.000	0.345	0.731	0.800	0.462	1.000	0.917	1.000
BTEC.2807	C	T	0.500	0.393	0.043	0.947	0.286	0.880	0.172	0.820	0.450	0.444	0.136	0.833	0.484
BTEC.2834	T	C	0.952	1.000	1.000	1.000	1.000	0.800	0.897	0.962	0.950	0.926	1.000	0.917	1.000
BTEC.2850	A	C	0.946	1.000	1.000	1.000	0.893	0.820	0.879	0.981	1.000	0.907	1.000	0.917	1.000
BTEC.2869	T	C	0.902	0.679	1.000	1.000	0.857	0.640	0.862	0.923	1.000	0.926	1.000	0.917	1.000
BTEC.2877	G	T	0.893	0.857	0.957	1.000	0.821	0.820	0.724	1.000	0.800	0.907	1.000	0.917	0.871
BTEC.2918	T	C	0.766	0.536	0.913	1.000	0.769	0.160	0.828	0.846	0.550	0.889	1.000	0.583	1.000
BTEC.2919	G	A	0.695	0.500	0.286	0.842	0.893	0.960	0.310	0.788	0.850	0.463	0.300	0.917	0.935
BTEC.3025	T	G	0.907	0.786	1.000	1.000	0.893	0.660	0.875	0.865	0.947	0.923	1.000	0.917	1.000
BTEC.3049	G	A	0.902	1.000	1.000	1.000	0.250	0.840	0.897	1.000	0.800	0.926	1.000	0.917	1.000
BTEC.3142	T	A	0.653	0.929	0.952	0.000	0.714	0.920	0.793	0.273	0.895	0.630	1.000	0.417	0.423
BTEC.3285	T	C	0.896	0.857	1.000	0.947	0.714	0.640	0.862	0.885	1.000	0.889	1.000	0.917	1.000

[a] The major allele is the nucleotide that occurs at the highest frequency among the 251 individuals sampled.
[b] Frequency of the major allele across all 12 populations.
[c] Dash marks within a population indicate that no lines amplified successfully at that locus.
[d] Population abbreviations are defined in Appendix 1.

Table 3. Cross-amplification for the 95 *Bromus tectorum* SNPs within related *Bromus* species.

SNP ID	*B. rubens* (*N* = 5)	*B. diandrus* (*N* = 5)	*B. sterilis* (*N* = 5)	*B. arvensis* (*N* = 5)
BTEC.0001	+	+	+	—
BTEC.0004	+	+	+	+
BTEC.0007	+	+	+	+
BTEC.0009	+	—	—	—
BTEC.0013	+	+	+	+
BTEC.0014	+	+	+	+
BTEC.0019	+	+	+	+
BTEC.0082	+	+	+	+
BTEC.0129	+	+	+	+
BTEC.0175	+	+	+	+
BTEC.0229	+	+	+	—
BTEC.0232	+	+	+	+
BTEC.0402	+	+	+	+
BTEC.0433	+	+	+	—
BTEC.0448	+	+	+	+
BTEC.0449	+	+	+	+
BTEC.0468	+	+	+	+
BTEC.0505	+	+	+	+
BTEC.0583	+	+	+	—
BTEC.0601	+	+	+	—
BTEC.0605	+	+	+	+
BTEC.0637	+	+	+	+
BTEC.0657	+	+	+	+
BTEC.0663	+	+	+	+
BTEC.0696	+	+	+	+
BTEC.0697	+	+	+	+
BTEC.0718	+	—	+	—
BTEC.0751	+	+	+	+
BTEC.0775	+	+	+	+
BTEC.0790	+	+	+	—
BTEC.0818	+	+	+	—
BTEC.0853	+	+	+	+
BTEC.0854	—	—	—	—
BTEC.0874	+	+	+	+
BTEC.0882	+	+	+	+
BTEC.0887	+	+	+	+
BTEC.0904	+	+	+	+
BTEC.0973	+	+	+	—
BTEC.0992	+	+	+	—
BTEC.0997	+	+	+	+
BTEC.1013	+	+	+	+
BTEC.1058	+	+	—	+
BTEC.1064	+	+	+	+
BTEC.1125	+	+	—	+
BTEC.1203	+	+	+	+
BTEC.1204	+	+	+	+
BTEC.1211	+	+	+	—
BTEC.1270	+	+	+	+
BTEC.1352	—	—	—	—
BTEC.1383	+	+	+	+
BTEC.1388	+	+	+	+
BTEC.1398	+	+	+	+
BTEC.1400	+	+	+	+
BTEC.1407	+	+	+	+
BTEC.1413	+	+	+	+
BTEC.1438	+	+	—	—
BTEC.1450	+	+	+	+
BTEC.1489	+	+	+	+
BTEC.1507	+	+	+	—
BTEC.1586	+	+	+	+
BTEC.1635	+	+	+	+
BTEC.1640	+	+	+	+
BTEC.1647	+	+	+	+
BTEC.1652	+	+	+	+
BTEC.1654	+	+	+	+
BTEC.1724	+	+	+	+
BTEC.1794	+	+	+	+
BTEC.1818	+	+	+	—
BTEC.1832	+	+	+	+
BTEC.1873	+	+	+	+
BTEC.1907	+	+	+	+
BTEC.1930	+	+	+	+
BTEC.2120	+	+	+	+
BTEC.2141	+	+	+	+
BTEC.2142	+	+	—	—
BTEC.2148	+	+	+	+
BTEC.2166	+	+	+	+
BTEC.2399	+	+	+	+
BTEC.2409	+	+	+	+
BTEC.2436	+	+	+	—
BTEC.2521	+	+	+	—
BTEC.2704	+	+	+	+
BTEC.2773	+	+	+	+
BTEC.2795	+	+	+	+
BTEC.2807	+	+	+	—
BTEC.2834	+	+	+	—
BTEC.2850	+	+	+	+
BTEC.2869	+	+	+	—
BTEC.2877	+	+	+	+
BTEC.2918	+	+	+	+
BTEC.2919	+	+	+	+
BTEC.3025	+	+	—	—
BTEC.3049	+	+	—	+
BTEC.3142	+	+	+	+
BTEC.3285	+	+	—	—

Note: + = successful amplification; — = unsuccessful amplification; *N* = number of individuals sampled.

overlap length was set to 50 bp and the minimum overlap identity to 95%. This Transcriptome Shotgun Assembly project has been deposited at the DDBJ/ENA/GenBank International Nucleotide Sequence Database (INSD; a collaboration between the DNA Data Bank of Japan [DDBJ], the European Nucleotide Archive [ENA], and GenBank) under the accession GELF00000000. The version described in this paper is the first version, GELF01000000. A total of 3333 putative SNPs were identified using a SNP finder tool within the BamBam genome sequence analysis package (Page et al., 2014), employing the following criteria: (1) sequence coverage depth at the SNP must be ≥10, (2) the minor allele must represent at least 30% of the alleles observed, and (3) only SNPs that did not have another SNP within 50 bp of either side were considered for possible assay development.

A diverse panel of 23 individuals was created for validating SNP assays by selecting a wide range of SSR genotypes using the four *B. tectorum* SSR loci BT05, BT26, BT30, and BT33 (Merrill et al., 2012). These 23 individuals were full siblings of previously genotyped individuals. Due to the inbred nature of *B. tectorum*, high levels of homozygosity are commonly observed and seeds from the same maternal plant are expected to be near-isogenic. Seeds collected from individual plants in the field were sown in the greenhouse, and leaf tissue DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Germantown, Maryland, USA) or a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Fulton et al., 1995). A set of 101 SNPs were validated for KASP genotyping (LGC Genomics, Beverly, Massachusetts, USA) following the KASP Genotyping Manual (version 3.0) using a PHERAstar Plus Microplate Reader (BMG Labtech, Ortenberg, Germany), and the data were analyzed using KlusterCaller (version 2.15, LGC Genomics). Primers for each assay were designed using PrimerPicker Lite for KASPar (version 0.26, LGC Genomics). This software designs two forward allele–specific primers (one for each allele) and two potential common reverse primers, only one of which is used in the actual assay (Table 1). The two allele-specific primers differ at the terminal, 3′ nucleotide, which defines the SNP. Each assay was tested on the panel of 23 individuals, with one nontemplate control per assay, in a 384-well plate. Data displays from successful assays had two (or three, in the case of heterozygotes) distinct clusters with good separation. If initial separation was poor, samples were amplified for an additional five, 10, or 15 cycles, as needed. For assays that failed using the first common reverse primer, the second common reverse primer was substituted and validated.

KASP assays for 95 polymorphic SNPs were converted for use on the Fluidigm EP1 SNP Genotyping System (Fluidigm Corporation, San Francisco, California, USA) with the 96.96 Dynamic Array IFC. The assays were tested on 95 individuals according to the Fluidigm SNP Genotyping Advanced Development Protocol. As positive controls, four individuals were included that had already been genotyped using all 95 SNPs on the PHERAStar during the marker development process. These four individuals collectively represented both alleles for each SNP assayed. Genotypes of these individuals as determined on the Fluidigm platform were 100% identical to the genotypes generated by the PHERAStar method. At least one nontemplate control was used for each 96.96 array. Data were analyzed using the Fluidigm SNP Genotyping Analysis Software (version 3.0.2).

Verification of the 95 SNPs is demonstrated for 251 individuals collected from 12 populations located in New Mexico, USA (Table 2, Appendix 1). Genotyping of 10 of the 12 populations is described in Lara (2013). All of the 95 SNP markers are polymorphic across the 12 populations but not necessarily within populations. It is assumed that none of the populations are in Hardy–Weinberg equilibrium, with respect to these markers, because *B. tectorum* is cleistogamous and heterozygosity is very low within any given population (Meyer et al., 2013). The 95 SNP assays were performed on five individuals each from four related species—*B. rubens* L., *B. diandrus* Roth, *B. sterilis* L., and *B. arvensis* L.—with 93, 91, 85, and 70 loci amplifying, respectively (Table 3).

CONCLUSIONS

Using *B. tectorum* from the IMW, we successfully developed 95 polymorphic SNP assays for studying its population and ecological genetics and found they have potential use in related *Bromus* species. SNPs were optimized for use with both the KASP and Fluidigm EP1 SNP genotyping platforms.

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Appendix 1. Information for 12 New Mexico (USA) populations of *Bromus tectorum* included in the survey of SNP polymorphisms.

Population name[a]	No. sampled	Latitude	Longitude	Elevation (m)
Albuquerque (ALB)	14	34.906900	−106.671700	1500
Belen 1 (BN1)	23	34.675814	−106.771692	1465
Belen 2 (BN2)	19	34.654545	−106.778418	1466
Bernalillo (BER)	14	35.301900	−106.536600	1566
Gallup 1 (GA1)	25	35.528598	−108.667243	2017
Gallup 2 (GA2)	29	35.527506	−108.722211	2004
Grants (GRA)	26	35.143402	−107.838666	1972
Los Lunas (LOS)	20	34.811533	−106.753898	1480
Milan (MIL)	27	35.189228	−107.900339	1999
Prewitt 1 (PR1)	11	35.363436	−108.046764	2088
Prewitt 2 (PR2)	12	35.365321	−108.053825	2093
San Fidel (SAN)	31	35.076028	−107.555127	2003

[a] Seeds collected from individual plants are deposited in the laboratory of S. E. Meyer.

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