ONLINE RESOURCES

Development and characterization of chloroplast microsatellite markers in *Pseudoroegneria* and *Leymus* (Poaceae: Triticeae)

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Pseudoroegneria (Nevski) Á. Löve and *Leymus* Hochst. are important genera of forage and turf grasses in temperate regions. Many of the members are valuable sources of germplasm, and are used for introducing desirable characters like salt tolerance and pathogen resistance into the small grain crops. As a diploid outbreeder, *Pseudoroegneria* can easily hybridize with other allogamous *Agropyron* Gaertner, *Australopyrum* (Tavelev) Á. Löve, and *Hordeum* L. species to form *Elymus* L. and *Douglasdeweya* C. Yen, J. L. Yang and B. R. Baum after polyploidization (Dewey 1984; Lu 1994; Yen 2005). Because of their importance, they have been the focus of numerous phylogenetic and evolutionary studies considering morphological, cytogenetic (Dewey 1984; Löve 1984), and molecular characteristics (Hsiao *et al*. 1995; Liu *et al*. 2006, 2008). However, there is a lack of chloroplast DNA markers capable of detecting high levels of polymorphism in *Pseudoroegneria, Leymus,* and their closely related genera.

By contrast, chloroplast simple sequence repeat (cpSSR) markers have been used in other taxa for various purposes, including the assessment of paternal versus maternal plastid inheritance (Cato and Richardson 1996), the detection of hybridization and introgression (Bucci *et al*. 1998), and the analysis of the genetic structure and phylogeography of plant populations (Palmé and Vendramin 2002; Grivet and Petit 2003). These studies indicated that the corresponding SSR regions in related species could be amplified by PCR and that chloroplast microsatellites had the ability to investigate phylogenetic relationships.

As sequence information is lacking in perennial Triticeae species, cpSSR markers of *Pseudoroegneria* and *Leymus* may be developed from the cpSSRs pools of the related species. Previous studies have shown that microsatellites are transferable across grass species for example, 24 cpSSRs were identified to evaluate genetic diversity of *Triticum* and *Aegilops* species (Ishii *et al*. 2001) based on the entire chloroplast DNA sequence of common wheat (*T. aestivum* cv. Chinese Spring). Also five cpSSRs suitable to success amplification in grasses were developed from the complete chloroplast genome sequences of *Oryza sativa* (rice), *Zea mays* (maize) and *Triticum aestivum* (wheat) (Provan *et al*. 2004). In the present study, cpSSR markers from *Lolium perenne, Hordeum,* and various Poaceae were tested to determine their potential for the phylogenetic and population studies of *Pseudoroegneria* and *Leymus.*

A total of 139 taxa were used in this study (table 1). All seeds were collected from the field or kindly provided by American National Plant Germplasm System (Pullman, Washington, USA) and Sichuan Agriculture University, P. R. China. Total DNA was extracted from leaf tissue using a CTAB method as previously described by Liu *et al*. (2006) and kept at −20°C for long term storage or 4°C for immediate use. A set of 18 chloroplast microsatellite primer pairs (Provan *et al*. 1999, 2004; Mcgrath *et al*. 2006) was selected according to their ability to be transferable to the studied accessions and their potential polymorphism. Among the selected chloroplast microsatellite primer pairs, nine primers (TeaSSR1-9) were selected from *Lolium perenne* primers; seven primers (psbK, psbA, rpoA, rps12, trnS1, trnS2, and trnLF) were cited from *Hordeum* primers; two primers (trnK and atpB) were selected from Poaceae primers. Detailed information about the primer pairs is given in table 2.

Amplification of DNA was carried out in 20 μL reaction mixture containing 30 ng template DNA, 0.2 μM of each primer, 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (dATP, dCTP, dGTP, dTTP), 1 unit of *Taq* DNA polymerase

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Table 1. List of *Pseudoroegneria*, *Leymus*, and closely related genera used in this study.

No.	Taxa	Accession no.	Origin
Pseudoroegneria (Nevski) Á. Löve			
1	*P. alashanica*	Z2006	Ningxia, China
2	*P. elytrigioides*	Z2005	Tibet, China
3	*P. geniculata*	PI565009	Russian Federation
4		PI632554	Uzbekistan
5		PI634248	Russian Federation
6	*P. geniculata* subsp. *pruinifera*	PI547374	Russian Federation
7	*P. geniculata* subsp. *scythica*	PI283272	Russian Federation
8		PI502271	Russian Federation
9	*P. gracillima*	PI440000	Stavropol, Russian Federation
10		PI420842	Stavropol, Russian Federation
11	*P. kosaninii*	PI237636	Turkey
12	*P. libanotia*	PI228389	Iran
13		PI228390	Iran
14		PI228391	Iran
15		PI228392	Iran
16		PI229583	Iran
17		PI330687	Iran
18		PI330688	Iran
19		PI330689	Iran
20		PI330690	Iran
21		PI343188	Iran
22		PI401274	Iran
23	*P. spicata*	PI232123	United States
24		PI232134	United States
25		PI232139	United States
26		PI506259	United States
27		PI563867	United States
28		PI563870	United States
29	*P. stipifolia*	PI314058	Stavropol, Russian Federation
30		PI325181	Stavropol, Russian Federation
31		PI440095	Russian Federation
32		PI531751	Ukraine
33		PI636641	Ukraine
34	*P. strigosa*	PI499637	Xinjiang, China
35		PI499638	Xinjiang, China
36		PI531752	Estonia
37		PI531753	Estonia
38		W614049	Russian Federation
39	*P. strigosa* subsp. *aegilopoides*	PI531754	Xinjiang, China
40		PI531755	Xinjiang, China
41		PI565082	Xinjiang, China
42		PI595164	Xinjiang, China
43		PI595172	Xinjiang, China
44		PI636577	Mongolia
45		W613089	Xinjiang, China
46		W619723	Mongolia
47	*P. tauri*	PI380644	Iran
48		PI380648	Iran
49		PI380649	Iran
50		PI380650	Iran
51		PI380651	Iran
52		PI380652	Iran
53		PI401319	Iran

Table 1 *(contd)*

No.	Taxa	Accession no.	Origin
54		PI401320	Iran
55		PI 401321	Iran
56		PI 401322	Iran
57		PI401323	Iran
58		PI401324	Iran
59		PI401325	Iran
60		PI401326	Iran
61		PI401327	Iran
62		PI401331	Iran
63		PI401333	Iran
64		PI401334	Iran
65		PI401336	Iran
66		PI401338	Iran
67		PI401339	Iran
Douglasdeweya C. Yen, J. L. Yang & B. R. Baum			
68	*D. wangii*	PI401328	Iran
69		PI401329	Iran
70		PI380645	Iran
71		PI380646	Iran
72		PI380647	Iran
Elymus L.			
73	*E. dolichatherus*	YN3110	Yunnan, China
74	*E. kamoji*	SH-003	Shanghai, China
75	*E. melantherus*	ZY3146	Sichun, China
76	*E. rectisetus*	H3152	Lake Lyndon, Australia
77	*E. rigidulus*	ZY3113	Gansu, China
78	*E. tsukushiensis*	H3302	Xinjiang, China
Leymus L.			
79	*L. akmolinensis*	PI440306	Russian Federation
80		PI531794	Germany
81	*L. alaicus*	PI499505	China
82	*L .alaicus.*subsp. *karataviensis*	PI314667	Russian Federation
83		PI314677	Russian Federation
84	*L. ambiguuus*	PI531795	United States
85		PI547331	United States
86	*L. angustus*	PI314673	Russian Federation
87		PI429795	Russian Federation
88		PI499508	China
89		PI499509	China
90	*L. arenarius*	PI272126	Kazakhstan
91		PI531801	Norway
92	*L.chinensis*	PI499516	China
93		PI499518	Nei Monggol, China
94		PI633715	Mongolia
95	*L. cinereus*	PI537332	United States
96		PI537336	United States
97		PI537337	United States
98		PI537340	United States
99	*L. condensatus*	PI442483	Belgium
100	*L. hybrid*	PI537362	United States
101		PI537363	United States
102	*L. innovatus*	PI236818	Canada
103	*L. karelinii*	PI595143	Xinjiang, China
104		PI598541	Xinjiang, China
105		PI598551	Xinjiang, China
106	*L. multicaulis*	PI314663	Former Soviet Union

Table 1 *(contd)*

No.	Taxa	Accession no.	Origin
107		PI440320	Former Soviet Union
108		PI499520	China
109	*L. paboanus*	PI272135	Kazakhstan
110		PI316234	Former Soviet Union
111		PI422250	Former Soviet Union
112	*L. pseudoracemosus*	PI531810	China
113	*L. racemosus*	PI313965	Former Soviet Union
114		PI314928	Former Soviet Union
115		PI598752	Kazakhstan
116		PI598753	Kazakhstan
117	*L. racemosus* subsp. *sabulosus*	PI531813	Estonia
118		PI531814	Estonia
119		PI598727	Argentina
120	*L. ramosus*	PI440333	Former Soviet Union
121		PI499654	China
122	*L. salinus*	PI531817	United States
123		PI565038	United States
124		PI58748	United States
125	*L. salinus* subsp. *mojavensis*	PI531818	United States
126	*L. salinus* subsp. *salinus*	PI636574	Mongolia
127	*L. secalinus*	PI499531	China
128		PI598754	Kazakhstan
129		PI598756	Kazakhstan
130		PI598757	Kazakhstan
131		CG2007	Gansu, China
132	*L. secalinus* subsp. *secalinus*	PI639770	Mongolia
133	*L. tianschanicus*	PI636647	Xinjiang, China
134		PI639827	Xinjiang, China
135	*L. triticoides*	PI578749	United States
136		PI610977	United States
Psathyrostachys Nevski			
137	*P. juncea*	PI595142	Xinjiang, China
138		PI595168	Xinjiang, China
139		PI595177	Xinjiang, China

(TaKaRa, Dalian, China) and distilled water to the final volume. The mixture was amplified using the Biorad Mycycler Thermal Cycler (Biorad, California, USA) PCR condition was as follows: one cycle of 4 min at 94°C; 35 cycles of 94°C for 1 min, 50–60°C for 1 min (depending on the annealing temperature of the primers), 72°C for 1 min; followed by 10 min at 72°C for the finial extension. The PCR products were separated on 6% polyacrylamide denaturing gels and silver stained as described by Song *et al*. (2003). The molecular size of amplified alleles for each SSR locus was determined based on its migration relative to the DL2000 DNA marker (TaKaRa, Dalian, China).

Cytological and molecular data suggested that *Pseudoroegneria* was the maternal donor of *Elymus* and *Douglasdeweya* (Lu 1994; Yen 2005; Liu *et al*. 2006; Baum and Johnson 2008), and *Psathyrostachys* was the maternal donor of *Leymus* (Löve 1984; Liu *et al*. 2008). Thus, cpSSRs of *Pseudoroegneria, Elymus, Douglasdeweya, Leymus,* and *Psathyrostachys* were developed in the present study. Of the 18 tested cpSSR primer pairs, 15 (7, *Lolium perenne*; 6, *Hordeum*; 2, Poaceae) primer pairs were amplified in almost all of the wild taxa (table 3). Our results revealed high marker transferability among *Lolium, Hordeum, Pseudoroegneria, Leymus,* and closely related genera. Several investigators (Provan *et al*. 2004; Mcgrath *et al*. 2006) have also shown that cpSSR primer pairs can amplify DNA of close relatives. In addition, the transferability of cpSSR markers varied, i.e. of nine tested *Lolium perenne* cpSSR markers, seven markers (78%) gave amplification products in almost all the used taxa; of seven tested *Hordeum* markers, six markers (86%) were amplified from almost all the accessions; of two tested Poaceae markers, they all (100%) gave reproducible amplification products from almost all the accessions (table 3). It should be noted that the Poaceae cpSSR primer pair atpB got amplified products from all the accessions in the present study, although the primer pair showed intraspecific variation among 25 accessions of *Anthoxanthum odoratum* (Avenae)

Table 2. Characteristics of cpSSRs used in the present study. This table was modified, based on Mcgrath *et al*. (2006), and Provan *et al*. (1999, 2004).

Locus	Location (gene)	Repeat motif	Primer sequence (5′–3′)	Expected size (bp)	Annealing temperature (° C)
cpSSRs cited from *Lolium perenne* (Mcgrath *et al*. 2006)					
TeaSSR1	atpB-*rbcL* intergenic spacer region	$(AAC)_3$	ATTGATTTGGGTTGCGCTAT TCATTAAAGAAAATTGAGGGCATA	229[a]	60
TeaSSR2	*trn-L* intron and *trn-F* intergenic spacer region $(CT)_4$		GGTTTGGGGATAAAGGGACT TCCATTCCAATTGAATATTTTGT	196[a]	45
TeaSSR3	*trn-L* intron and *trn-F* intergenic spacer region	A_9	AGGGACTTGAACCCTCACAA GCAAACGATTAATCATGGAACC	311[a], 312[a]	60
TeaSSR4	*23S-5S* internal transcribed sapcer	A_8	ACGAACGAACGATTTGAACC TGAAGCCCCAATTCTTGACT	185[a], 186[a], 188[a]	60
TeaSSR5	Herbicide binding protein D1*(psbA)*	$(CTT)_3$	TGAAGCCCCAATTCTTGACT GCTATGCATGGTTCCTTGGT	209[a]	60
TeaSSR6	*trnV* gene for valine transfer RNA (GAC)	T_{12}	CGGATTCTAACCGTAGACCTTC TCAAAGCCAGGAAGCAATCT	188[a]	45
TeaSSR7	*rpbl2* gene for ribosomal protein L2 and *trnH* gene for Histine transfer RNA (CAC)	$(ATT)_5$	GGAATTTGCAATAATGCGATG –	60	
TeaSSR8	*rpoA* gene for RNA polymerase α sub-unit	T_{11}	TCGATCGAGGTATGGAGGTC TTGACAGTTTTCGTATGGAAGA	178[a]	45
TeaSSR9	*trn-L* intron and *trn-F* intergenic spacer region	G_{12}	GATTGTGCCAAAGATGCAAA TCCTGACCTTTTCTTGTGCAT AACCCGGTTTTTCGGTTTAT	–	45
cpSSRs cited from *Hordeum* (Provan *et al*. 1999)					
psbK	*psbK/ORF174* intergenic spacer region	$(A)_{11}$	TAGCCTTTGTTTGGCAAGCT TAAAACTTCTCGGCTTTTACCC	121[c]	60
psbA	Downstream of *psbA*	$(T)_8$	AATGGATAAGGTTTTTCTG CTGAATAGAAAGATTAAGAAGA	146[c]	50
rpoA	Downstream of *rpoA*	$(T)_8(CTT)_3$	CTCTCGTTTTAAATCCATTGCA TGATCCATTTCGCGAAAATA	122[c]	60
rps12	*rpS12* intron I	$(T)_8$	AAGAAAGGGCTCCGGTGTAT CCACGATTTTTTATTCCACTCC	148[c]	60
trnS1	Downstream of *trnS*	$(A)_7CGC(T)_{13}$	CTTTAGCGGGCATTTCCATA ATGGTGGATTTGATAAGAACCC	128[c]	60
trnS2	Downstream of *trnS*	$(T)_{10}$	CAACTCCTTTGCGCTACACA ACCCCTTTTTTCCCATTCC	115[c]	60
trnLF	*trnL/trnF* intergenic region	$(C)_9$	GAGTATCGGCAAGAAATCTTGG TCAAAATTTGAAAGGGGGG	101[c]	60
cpSSRs cited from Poaceae (Provan *et al*. 2004)					
trnK		$(A)_{10}G(A)_6$b	ATACAGTCTCTTTATCAATATACTG GACGTTAAAAATAGATTAGTGCC	172	58
atpB		$(T)_7$ and $(A)_8$b	GATTGGTTCTCATAATTATCAC TATTGAATTAACTAATTCATTTCC	149	59

[a]Expected size in *Bromus erectus*; [b]repeat motif in Triticum; [c]expected size in *Hordeum bogdanii.*

Table 3. Amplification products obtained from 139 accessions. The taxon no. is identical to the taxon no. in table 1.

	Allele size of cpSSR primers														
Taxon no.	TeaSS R1	TeaSS R3	TeaSS R4	TeaSS R5	TeaSS R6	TeaSS R7	TeaSS R8	psbK	rpoA	rps12	trns1	trns2	trnLF	trnK	atpB
Pseudoroegneria (Nevski) Á. Löve															
1	238	302	220	220	211	198	192	132	115	121	151	119	99	203	156
2	238	302	219	220	211	198	192	132	115	121	150	120	99	203	156
3	238	302	218	220	211	198	190	133	114	121	149	120	99	203	156
4	238	302	219	220	211	198	190	132	114	121	151	120	99	203	156
5	238	302	219	220	211	198	190	132	114	121	149	120	99	203	156
6	238	302	219	220	211	198	190	132	114	121	150	120	99	200	156
7	238	302	219	220	211	198	190	132	114	121	151	118	99	204	156
8	238	302	219	220	211	198	190	132	114	121	150	118	99	202	156
9	238	302	219	220	211	198	192	132	122	121	149	120	99	202	156
10	238	302	219	220	211	198	192	132	115	121	150	120	99	204	156
11	238	302	219	220	211	198	190	132	115	121	150	120	99	203	156
12	238	302	219	228	211	198	202	132	114	121	152	120	99	202	157
13	238	302	219	228	211	198	202	132	114	121	151	118	99	202	157
14	238	302	219	228	211	198	202	132	114	121	152	118	99	203	157
15	238	302	219	228	211	198	202	132	114	121	152	118	99	203	157
16	238	302	219	228	211	198	202	132	114	121	159	118	99	202	157
17	238	302	219	228	211	198	202	132	114	121	152	118	99	203	157
18	238	302	219	228	211	198	202	132	115	121	159	119	99	200	156
19	238	302	219	228	211	198	202	132	114	121	159	118	99	202	157
20	238	302	219	228	211	198	202	132	114	121	153	118	99	202	157
21	238	302	219	228	211	198	202	132	114	121	152	118	99	202	157
22	238	302	219	229	211	198	202	132	114	121	152	120	99	202	157
23	238	302	220	229	211	198	202	131	122	175	149	121	99	199	157
24	238	302	220	229	211	198	202	132	114	175	150	118	99	199	157
25	238	302	220	229	211	198	202	132	114	175	152	119	99	199	157
26	238	302	220	229	211	198	202	132	114	175	150	119	99	199	157
27	238	302	220	229	211	198	202	132	114	175	151	120	99	200	156
28	238	302	220	229	211	198	203	132	114	175	149	120	99	198	157
29	238	302	219	220	211	198	192	132	115	121	149	120	99	203	156
30	238	302	218	220	211	198	192	132	115	121	149	120	99	203	156
31	238	302	219	220	211	198	192	132	122	121	150	120	99	203	156
32	238	302	219	220	211	198	192	132	115	121	150	120	99	203	156
33	238	302	219	220	211	198	192	132	115	121	150	119	99	203	156
34	238	302	220	229	211	198	196	132	110	175	150	120	99	200	156
35	238	302	220	229	211	198	202	132	114	175	152	121	99	200	158
36	238	302	220	229	211	198	202	132	114	175	150	120	99	200	156
37	238	302	220	229	211	198	202	132	122	175	150	118	99	199	156
38	238	302	220	229	211	198	202	132	115	175	150	120	99	200	156
39	238	302	220	229	211	198	196	132	103	175	150	120	99	202	156
40	238	302	220	229	211	198	196	132	103	175	151	120	99	200	156
41	238	302	220	229	211	198	196	132	103	175	151	120	99	200	156
42	238	302	220	229	211	198	202	132	114	175	150	121	99	199	156
43	238	302	220	229	211	198	202	132	114	175	150	121	99	199	156

Table 3 *(contd)*

Taxon no.	Allele size of cpSSR primers														
	TeaSS R1	TeaSS R3	TeaSS R4	TeaSS R5	TeaSS R6	TeaSS R7	TeaSS R8	psbK	rpoA	rps12	trns1	trns2	trnLF	trnK	atpB
44	238	302	220	229	211	198	202	133	114	175	150	121	99	199	158
45	238	302	220	229	211	198	196	132	103	175	150	120	99	199	156
46	238	302	220	229	211	198	202	132	114	175	150	120	99	200	156
47	238	302	219	228	211	198	201	132	114	121	159	120	99	203	155
48	238	302	219	228	211	198	202	132	114	121	152	120	99	203	157
49	238	302	219	–[a]	211	198	202	132	114	121	152	120	99	202	157
50	238	302	219	228	211	198	202	132	114	121	152	120	99	203	157
51	238	302	219	228	211	198	201	132	114	121	152	120	99	203	157
52	238	302	219	228	211	198	201	132	114	121	150	120	99	203	157
53	238	302	219	228	211	198	202	132	114	121	150	120	99	203	157
54	238	302	219	228	211	198	202	132	114	121	152	120	99	203	157
55	238	302	219	228	211	198	202	132	114	121	159	120	99	203	157
56	238	302	219	228	211	198	202	132	114	121	159	120	99	203	155
57	238	302	219	228	211	198	201	132	114	121	159	120	99	203	157
58	238	302	219	228	211	198	202	133	114	121	153	120	99	203	157
59	238	302	219	228	211	198	202	131	114	121	152	118	99	202	157
60	238	302	219	–[a]	211	198	202	132	114	121	152	118	99	202	157
61	238	302	219	228	211	198	202	131	114	121	152	118	99	202	157
62	238	302	219	228	211	198	202	132	114	121	153	118	99	202	157
63	238	302	219	228	211	198	203	132	114	121	153	118	99	202	157
64	238	302	220	229	211	198	202	132	114	175	152	120	99	199	157
65	238	302	219	228	211	198	202	132	114	121	152	121	99	203	157
66	238	302	219	228	211	198	202	132	114	121	152	120	99	203	157
67	238	302	219	228	211	198	202	132	114	121	152	120	99	202	157
Douglasdeweya C. Yen, J. L. Yang and B. R. Baum															
68	238	302	219	228	211	198	202	132	122	121	152	118	99	202	157
69	238	302	219	228	211	198	202	132	122	121	153	118	99	202	157
70	238	302	219	228	211	198	200	132	114	121	150	120	99	202	157
71	238	302	219	228	211	198	200	132	114	121	150	–[a]	99	202	157
72	238	302	219	228	211	198	201	132	114	121	152	120	99	202	157
Elymus L.															
73	238	302	220	220	211	198	190	132	115	175	150	120	99	200	156
74	224	302	220	220	211	198	190	132	115	175	151	118	99	200	156
75	238	302	220	220	211	198	190	132	117	174	151	118	99	200	156
76	238	302	220	220	211	198	190	132	115	175	150	119	99	199	156
77	238	302	220	220	211	198	190	132	115	175	152	119	99	200	156
78	238	302	220	220	211	198	190	132	115	175	151	119	99	200	156
Leymus L.															
79	238	302	220	229	211	198	203	132	116	176	130	125	101	203	157
80	238	302	220	229	211	198	202	131	116	176	130	125	101	203	157
81	238	302	220	229	211	198	201	132	114	175	116	124	101	203	155
82	238	302	219	228	211	198	–[a]	132	115	176	110	125	101	204	157
83	238	302	219	228	211	198	204	132	116	176	127	126	101	202	157
84	240	302	220	220	211	198	203	132	115	176	116	118	101	199	156

Table 3 *(contd)*

Taxon no.	Allele size of cpSSR primers														
	TeaSS R1	TeaSS R3	TeaSS R4	TeaSS R5	TeaSS R6	TeaSS R7	TeaSS R8	psbK	rpoA	rps12	trns1	trns2	trnLF	trnK	atpB
85	242	302	220	220	211	198	202	132	115	176	116	118	101	199	156
86	238	302	220	229	211	198	202	132	116	176	127	125	101	203	157
87	238	302	220	229	211	198	202	132	116	176	127	125	101	203	157
88	238	302	220	229	211	198	202	132	116	176	127	125	101	203	157
89	238	302	220	229	211	198	202	132	116	176	127	125	101	202	157
90	238	302	220	228	139	198	202	132	116	176	127	125	101	203	157
91	238	302	220	228	139	198	202	132	116	176	127	125	101	203	157
92	238	302	220	229	211	198	202	132	116	176	132	125	101	203	157
93	238	302	220	229	211	198	202	132	116	176	132	125	101	203	157
94	238	302	219	229	211	198	202	133	115	175	126	117	101	202	157
95	242	302	220	220	211	198	202	132	115	176	117	117	101	199	156
96	240	302	220	220	211	198	202	132	115	176	117	117	101	199	156
97	242	302	220	220	211	198	202	132	115	176	117	117	101	199	156
98	240	302	220	220	211	198	202	132	115	176	117	117	101	199	156
99	240	302	220	220	211	198	202	132	115	176	115	118	101	199	156
100	242	–[a]	220	220	211	198	–[a]	132	128	176	117	117	101	202	156
101	240	–[a]	220	220	211	198	202	132	115	176	117	117	101	202	156
102	240	302	219	220	211	198	203	132	116	176	117	117	101	200	158
103	238	302	219	229	211	198	202	132	116	176	127	125	101	203	157
104	238	302	219	229	211	198	202	132	116	176	127	125	101	203	157
105	238	302	219	229	211	198	202	132	116	176	127	125	101	203	155
106	238	302	220	229	211	198	202	132	116	176	148	125	101	203	157
107	238	302	220	229	211	198	202	132	116	176	126	125	101	203	157
108	238	302	220	229	211	198	202	132	116	176	126	125	101	203	157
109	238	302	219	228	211	198	202	132	116	158	127	125	101	202	157
110	238	302	219	228	211	198	202	132	116	176	127	126	101	203	157
111	238	302	219	228	211	198	202	132	116	158	127	125	101	203	157
112	238	302	220	229	211	198	202	132	116	176	132	125	101	203	157
113	238	302	220	228	211	198	202	132	116	176	127	125	101	203	157
114	238	302	220	228	211	198	202	132	116	176	127	125	101	203	157
115	238	302	220	228	211	198	202	132	116	176	127	125	101	203	157
116	238	302	220	228	211	198	202	132	116	176	127	125	101	203	157
117	238	302	220	228	211	198	201	132	115	176	127	125	101	203	157
118	238	302	220	228	211	198	202	132	115	176	127	125	101	203	157
119	238	302	220	228	211	198	202	132	117	176	127	125	101	203	157
120	238	302	220	228	211	198	202	132	117	176	127	125	101	203	157
121	238	302	220	230	211	198	203	132	117	176	127	125	101	203	157
122	240	302	219	229	139	198	201	132	116	176	117	118	101	200	156
123	242	302	219	229	139	198	202	132	116	176	117	118	101	202	156
124	238	302	219	229	139	198	202	132	117	178	127	125	101	200	156
125	242	302	219	229	211	198	202	132	117	176	117	123	101	199	156
126	237	302	219	229	211	198	202	132	117	176	127	125	101	200	156
127	238	302	219	228	139	198	202	132	117	176	132	125	101	203	157
128	239	302	219	228	139	198	202	132	117	176	132	125	101	203	157

Table 3 *(contd)*

Taxon no.	Allele size of cpSSR primers														
	TeaSS R1	TeaSS R3	TeaSS R4	TeaSS R5	TeaSS R6	TeaSS R7	TeaSS R8	psbK	rpoA	rps12	trns1	trns2	trnLF	trnK	atpB
129	238	302	220	228	139	198	202	133	117	176	132	125	101	203	157
130	238	302	219	228	139	198	200	–[a]	116	176	132	125	101	203	157
131	238	302	219	228	139	198	202	132	116	175	132	125	101	203	157
132	238	302	219	228	139	198	201	132	115	175	115	124	101	203	155
133	238	302	220	229	139	198	202	132	116	175	127	125	101	203	157
134	238	302	220	229	139	198	201	132	116	175	127	125	101	203	157
135	238	302	220	229	211	198	202	132	116	175	127	125	101	203	157
136	238	302	220	229	211	198	202	133	128	175	117	118	101	203	157
Psathyrostachys Nevski															
137	238	302	220	229	211	198	202	132	116	177	127	125	101	203	157
138	238	302	220	229	211	198	202	133	116	177	127	125	101	203	157
139	238	302	220	229	211	198	202	133	116	177	127	125	101	203	157

–[a]represents no amplified product.

(Provan *et al*. 2004). The high rate of transferability is most likely due to the conservation of the priming sites within the flanking sequences to enable amplification, and on the maintenance of repeat arrays long enough to promote polymorphism as suggested by Fitzsimmons *et al*. (1995). Primer sequence conservation has been tested in a variety of plant species (Ishii and McCouch 2000; Vogel *et al*. 2003; Provan *et al*. 2004; Mcgrath *et al*. 2006). For example, Ishii and McCouch (2000) tested the transferability of *Oryza sativa* cv. Nipponbare cpSSRs within the genus and within eight other Gramineae species. They found that amplified products were obtained in nine out of ten chloroplast microsatellite loci for all samples.

Among the developed cpSSR markers that worked in *Pseudoroegneria, Elymus,* and *Douglasdeweya,* five cpSSR markers (TeaSSR1, TeaSSR3, TeaSSR6, TeaSSR7, and trnLF) showed monomorphism, 10 primer pairs showed polymorphism. The number of allele produced by primers of trnS1, trnK, and rpoA was the highest with six alleles, respectively. The number of allele amplified from TeaSSR8 primers was five. The number of allele produced by trnS2 and atpB was four, respectively. Three alleles were found at each of the locus of TeaSSR4, TeaSSR5, psbK, and rps12. The number of alleles detected by each primer excluding five monomorphic primers is listed in table 4.

Among the developed cpSSR markers that worked in *Psathyrostachys* and *Leymus*, three cpSSRs (TeaSSR3, TeaSSR7, and trnLF) showed monomorphism, 12 primer pairs showed polymorphism. The number of alleles produced by primers of trnS1 was the highest with nine alleles. The number of alleles amplified from primers TeaSSR1, TeaSSR8, trnK, trnS2, and rpoA were five. Four alleles were found for atpB and rpbs12, three alleles for TeaSSR5 and psbK, and two alleles for TeaSSR4. The number of alleles detected by each primer excluding three monomorphic primers is listed in table 5.

Thus, the present study has shown that 78% of *Lolium perenne,* 86% of *Hordeum*, and 100% of Poaceae cpSSR primer pairs generated reproducible amplification product from *Pseudoroegneria, Leymus, Elymus, Douglasdeweya,* and *Psathyrostachys* species. The amplification success rates of cpSSR primer pairs used in this study are very similar to those of Ishii and McCouch (2000), who were successful in amplifying 90% of *Oryza sativa* cv Nipponbare cpSSR primer pairs tested in *Oryza* and eight other Gramineae species. The high levels of chloroplast microsatellite found by Ishii and McCouch (2000) and in this study are not surprising, considering that comparative maps suggest that grass genomes retain extensive regions of colinearity (Gaut 2002). In addition, results from the present study suggest that *Lolium perenne, Hordeum,* and other Poaceae cpSSR primer pairs are a valuable source of polymorphic markers for analysing the relatively unknown *Pseudoroegneria, Leymus, Elymus, Douglasdeweya,* and *Psathyrostachys* gene pool, for genetic mapping and for alignment of genetic maps with other Gramineae species.

Table 4. Number of alleles produced by 10 polymorphic cpSSRs within *Pseudoroegneria, Douglasdeweya* and *Elymus* species.

	Number of alleles produced									
Taxon	TeaSSR 4	TeaSSR 5	TeaSSR 8	atpB	trnK	psbK	rps12	trns1	trns2	rpoA
Pseudoroegneria (Nevski) Á. Löve										
P. alashanica	1	1	1	1	1	1	1	1	1	1
P. elytrigioides	1	1	1	1	1	1	1	1	1	1
P. geniculata	2	1	1	1	1	2	1	2	1	1
P. geniculata subsp. *pruinifera*	1	1	1	1	1	1	1	1	1	1
P. geniculata subsp. *scythica*	1	1	1	1	2	1	1	2	1	1
P. gracillima	1	1	1	1	2	1	1	2	1	2
P. kosaninii	1	1	1	1	1	1	1	1	1	1
P. libanotia	1	2	1	2	3	1	1	4	3	2
P. spicata	1	1	2	2	3	2	1	4	4	2
P. stipifolia	2	1	1	1	1	1	1	2	2	2
P. strigosa	1	1	2	2	2	1	1	2	3	4
P. strigosa subsp. *aegilopoides*	1	1	2	2	3	2	1	2	2	2
P. tauri	2	1	3	2	3	3	2	4	3	1
Douglasdeweya C. Yen, J. L. Yang and B. R. Baum										
D. wangii	1	1	3	1	1	1	1	3	2	2
Elymus L.										
E. dolichatherus	1	1	1	1	1	1	1	1	1	1
E. kamoji	1	1	1	1	1	1	1	1	1	1
E. melantherus	1	1	1	1	1	1	1	1	1	1
E. rectisetus	1	1	1	1	1	1	1	1	1	1
E. rigidulus	1	1	1	1	1	1	1	1	1	1
E. tsukushiensis	1	1	1	1	1	1	1	1	1	1

Table 5. Number of alleles produced by 12 polymorphic cpSSRs within *Leymus* and *Psathyrostachys* species.

	Number of alleles produced											
Taxon	TeaSSR 1	TeaSSR 4	TeaSSR 5	TeaSSR 6	TeaSSR 8	atpB	trnK	psbK	rps12	trns1	trns2	rpoA
Leymus L.												
L. akmolinensis	1	1	1	1	2	1	1	2	1	1	1	1
L. alaicus	1	1	1	1	1	1	1	1	1	1	1	1
*L. alaicus.*subsp. *karataviensis*	1	1	1	1	1	1	2	1	1	2	2	2
L. ambiguuus	2	1	1	1	2	1	1	1	1	1	1	1
L. angustus	1	1	1	1	1	1	2	1	1	1	1	1
L. arenarius	1	1	1	1	1	1	1	1	1	1	1	1
L. chinensis	1	2	1	1	1	1	2	2	2	2	2	2
L. cinereus	2	1	1	1	1	1	1	1	1	1	1	1
L. condensatus	1	1	1	1	1	1	1	1	1	1	1	1
L. hybrid	2	1	1	1	1	1	1	1	1	1	1	2

Table 5 *(contd)*

Taxon	Number of alleles produced											
	TeaSSR 1	TeaSSR 4	TeaSSR 5	TeaSSR 6	TeaSSR 8	atpB	trnK	psbK	rps12	trns1	trns2	rpoA
L. innovatus	1	1	1	1	1	1	1	1	1	1	1	1
L. karelinii	1	1	1	1	1	2	1	1	1	1	1	1
L. multicaulis	1	1	1	1	1	1	1	1	1	2	1	1
L. paboanus	1	1	1	1	1	1	2	1	2	1	2	1
L. pseudoracemosus	1	1	1	1	1	1	1	1	1	1	1	1
L. racemosus	1	1	1	1	1	1	1	1	1	1	1	1
L. racemosus subsp. *sabulosus*	1	1	1	1	2	1	1	1	1	1	1	2
L. ramosus	1	1	2	1	2	1	1	1	1	1	1	1
L. salinus	3	1	1	1	2	1	2	1	2	2	2	2
L. salinus subsp. *mojavensis*	1	1	1	1	1	1	1	1	1	1	1	1
L. salinus subsp. *salinus*	1	1	1	1	1	1	1	1	1	1	1	1
L. secalinus 2	2	1	1	2	1	1	2	2	1	1	2	
L. secalinus subsp. *secalinus*	1	1	1	1	1	1	1	1	1	1	1	1
L. tianschanicus	1	1	1	1	2	1	1	1	1	1	1	1
L. triticoides	1	1	1	1	1	1	1	2	1	2	2	2
Psathyrostachys Nevski												
P. juncea	1	1	1	1	1	1	1	2	1	1	1	1

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