

No.	Group	Region	Locality	Longitude	Latitude	Elevation (m)	Aspect
66	a	Shanting District	Northwest Mountain	117°36'29"	35°03'40"	280	Southeast
68	a	Shanting District	Northwest Mountain	117°36'31"	35°03'43"	280	East
70	a	Shanting District	Northwest Mountain	117°36'38"	35°03'22"	280	South
72	a	Shanting District	Northwest Mountain	117°36'29"	35°03'40"	290	Southeast
74	a	Shanting District	Northwest Mountain	117°36'28"	35°03'40"	240	South
76	a	Shanting District	Northwest Mountain	117°36'27"	35°03'39"	280	Southwest
78	a	Shanting District	Northwest Mountain	117°36'40"	35°03'09"	280	South
80	a	Shanting District	Northwest Mountain	117°36'25"	35°03'38"	270	South
81	a	Shanting District	Yanggang Mountain	117°36'30"	35°02'55"	230	East
83	a	Shanting District	Yanggang Mountain	117°36'28"	35°02'57"	280	Southeast
85	a	Shanting District	Yanggang Mountain	117°36'10"	35°03'21"	300	Southwest
89	a	Shanting District	Yanggang Mountain	117°36'29"	35°02'56"	290	Southeast
96	a	Shanting District	Shengshan'an Pass	117°34'08"	35°00'19"	180	North
98	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'38"	290	Northwest
99	a	Shanting District	Yanggang Mountain	117°36'00"	35°03'22"	280	South
100	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'38"	216	Southwest
102	a	Shanting District	Shengshan'an Pass	117°34'10"	34°59'39"	213	Southwest
103	a	Shanting District	Jiguan Gu	117°36'57"	34°58'15"	270	Southwest
107	a	Shanting District	Jiguan Gu	117°37'09"	34°58'09"	260	Southwest
109	a	Shanting District	Jiguan Gu	117°36'46"	34°58'37"	300	Southwest
113	a	Shanting District	Baodu Gu	117°42'54"	34°59'11"	330	Northwest
115	a	Shanting District	Baodu Gu	117°42'55"	34°59'08"	340	Southwest
118	b	Yicheng District	Qingtian Temple	117°34'23"	34°57'39"	410	East
135	c	Shizhong District	Guishan Forest Farm	117°40'51"	34°46'43"	200	Northeast
151	d	Tengzhou City	East Mountain	117°23'13"	34°55'51"	120	East
155	d	Tengzhou City	Hutou Mountain	117°16'44"	34°53'28"	120	Northwest
166	d	Tengzhou City	Mushi Forest Farm	117°16'59"	34°58'08"	100	South
183	d	Tengzhou City	Hulutao	117°16'34"	34°52'58"	80	Northeast
200	a	Shanting District	Beiyu	117°25'53"	35°08'46"	360	Northeast

3.2 Experimental methods

3.2.1 Extraction and quality assessment of total plant DNA

Genomic DNA was extracted from the leaves of 100 *Platycladus orientalis* samples using a modified cetyltrimethylammonium bromide (CTAB) method. After the extracted DNA was completely dissolved, its quality was assessed by electrophoresis on 2% agarose gels.

3.2.2 Primer screening

In this study, SSR primers of *Platycladus orientalis* were developed based on the identification of SSR loci and primer screening from *P. orientalis* transcriptome sequences, and a total of 45 pairs of SSR primers were initially selected. Prior to large-scale PCR amplification and sequencing of all individuals, eight individuals were randomly selected for preliminary screening of the 45 microsatellite primers (Figure 5). Using the annealing temperature of 58 °C reported in the literature as a reference, PCR products were examined by 3% agarose gel electrophoresis and capillary sequencing. Primers with poor amplification efficiency or unclear banding patterns were discarded. Ultimately, seven pairs of SSR primers that produced clear gel electrophoresis profiles, could be stably amplified in all populations, showed satisfactory performance, and were easy to score and statistically analyze were selected for subsequent analyses.

3.2.3 SSR analysis

In this study, seven pairs of polymorphic primers with clear and well-resolved DNA bands were selected (Table 6). Detailed information on the PCR reaction system (Table 7) and amplification program is provided below.