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## Application of ISSR-PCR to identification two different ecotypes of *Lespedeza hedysaroides*

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**Key words:** *Lespedeza hedysaroides*, ISSR-PCR, molecular marker, identification

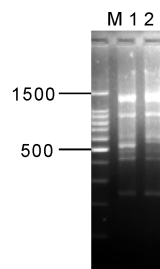
**Introduction** Inter-simple sequence repeats-polymerase chain reaction (ISSR-PCR) is a new type of molecular marker technology in recent years (Zietkiewicz, 1994). Universal primers were designed according to the genome of microsatellite sequences for the genomic DNA PCR amplification. ISSR-PCR has been widely applied variety identification, fingerprint mapping and genetic diversity analysis. The objective of this research was to identify two different ecotypes of *L. hedysaroides* (the light green leaf and the dark green leaf) by Zhao Yang etc. (Zhao Yang, 2006) *Lespedeza* spp. ISSR-PCR reaction system optimized.

**Materials and methods** Materials came from the test area in Linxi county of Inner Mongolia. Collected barley leaves stored in liquid nitrogen, then stored at -20°C. The genomic DNA extracted by the extraction kit. In 20μL reaction mixture are as follows: 40 ng genomic DNA, 0.2 μmol L<sup>-1</sup> ISSR primer, 10 μL 2×Taq PCR MasterMix (0.1 U μL<sup>-1</sup> Taq Polymerase, 500 μM dNTP each, 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 3 mM MgCl<sub>2</sub>). The PCR procedure is one preliminary denaturation at 94°C for 5 min; 35 cycles each involved denaturation at 94°C for 30 s, anneal at 50~56°C (decided by primer) for 45 s, extended at 72°C for 1 min 30 s and a final extension at 72°C for 7 min, then keep the temperature at 4°C. The PCR products were examined by electrophoresis with 2.0% agarose gel.

**Results** Among the eight primers (Table 1), the primer UBC-880 was able to distinguish the examined two different ecotypes of *L. hedysaroides* (Figure 1). There is a clear and obvious band in 500bp.

**Table 1** Used ISSR primer sequences.

primer	sequence
UBC-812	(GA) <sub>8</sub> A
UBC-809	(AG) <sub>8</sub> G
UBC-811	(GA) <sub>8</sub> C
UBC-825	(AC) <sub>8</sub> T
UBC-827	(AC) <sub>8</sub> G
UBC-880	(GGAGA) <sub>3</sub>
UBC-842	(GA) <sub>8</sub> YG
UBC-824	(TC) <sub>8</sub> G



**Figure 1** ISSR-PCR profiles of two different ecotypes of *L. hedysaroides*. M indicates DNA standard molecular weight. 1-the dark green leaf; 2-the light green leaf.

**Conclusion** ISSR-PCR provides a quick, reliable molecular marker technique for identification of two different ecotypes of *L. hedysaroides*. This method can be used in intraspecific identification of forage.

### References

- Zietkiewicz E, Rafalski A and Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.
- Zhao yang, Chen Xiao-yang & Li Tong-sen etc. 2006. Establishment and Optimization of ISSR Reaction System for *Lespedeza* spp. *Journal of Southwest Forestry College* 26(2): 6-9.