Application of ISSR-PCR to Identification Two Different Ecotypes of *Lespedeza hedysaroides*

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Introduction Inter-simple sequence repeats-polymerase chain reaction (ISSR-PCR) is a new type of molecular marker technology in recent years (Zietkiewiez , 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 form the test area in Linxi county of Inner Mongolia . Collected barley leaves stored in liquid nitrogen , then stored at -20℃ . The genomic DNA extracted by the extraction kit . In 20μL reaction mixture are as follows : 40 ng genomic DNA , 0.2μmol L^{-1} ISSR primer ,10μL 2×Taq PCR MasterMix (0.1UμL^{-1} Taq Polymerase , 500μM dNTP each ,20mM Tris-HCL(PH8.3) ,100 mM KCL , 3 mM MgCl$_2$) . The PCR procedure is one preliminary denaturation at 94℃ for 5 min ; 35 cycles each involved denaturation at 94℃ for 30 S ,anneal at 50～56℃ (decided by primer) for 45 S , extended at 72℃ for 1 min 30 S ,and a final extension at 72℃ for 7 min , then keep the temperature at 4℃ . 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)$_8$ A
UBC-809	(AG)$_8$ G
UBC-811	(GA)$_8$ C
UBC-825	(AC)$_8$ T
UBC-827	(AC)$_8$ G
UBC-880	(GGAGA)$_3$
UBC-842	(GA)$_8$ YG
UBC-824	(TC)$_8$ G
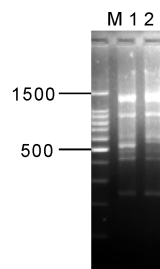
Figure 1 _ISSR-PCR profiles of two different ecotypes of L . hedysaroides M indicates DNA standard molecular weight .1-the dark green leaf ;2- the light green leaf ._

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
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