Establishment and Optimization of the ISSR-PCR Reaction System in *Stipa krylovii*

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Introduction Species of *Stipa L* . of family gramineae is one of the most important forages on the grassland in north China . *Stipa L* . as the constructive species is dominant in the typical steppe zone of plant communities in Eurasian . *Stipa krylovii* as a perennial grass of *Stipa L* . ¯ not only form the unique plant formation in steppe of middle Asia but also is dominant species in the main vegetation of Inner Mongolia plateau around Beijing (WANG Yanfang *et al* . , 2006) . Many studies of *Stipa L* . have mainly focused on their ecobiological characteristics and the impact of different ecological factors (eg . grazing gradient , fire , cutting) on the population (AN Yuan *et al* . , 2002) . but there have been relatively few studies on genetic variability , particularly no papers on genetics *Stipa krylovii* using ISSR molecular marker has not been published in China so far . The purpose of the paper is to establish and optimize ISSR-PCR amplification sysytem .

Materials and methods Samples of *Stipa krylovii* were collected on grassland near National Grassland Ecosystem Station of GuYuan , Hebei Province (115°41′ E , 41°46′ N) in Aug 2006 . Leaves of plants were sealed in plastic bag with silica gel , and the genome DNA was extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray M G *et al* . , 1980) . Primer (UBC857) of ISSR offered by University of Columbia sequenced as 5′ ACACACACACACACACYG3′ . The PCR amplification program was 94℃ pre-denaturation for 5 min , 35 cycles of 94℃ for 45 sec , 55℃ for 45 sec , 72℃ for 1 .5 min , and 5 min 72℃ extension . The orthogonal design [L16 (4^5)] combined with single factor experiment was applied to optimize ISSR-PCR amplification system of *Stipa krylovii* .

Results Experiments showed that the suitable annealing temperature of primer UBC857 was in the range of 55℃-57℃ , the high annealing temperature lead to failing linkage between primer and DNA template . There was not siginificant effect of DNA template concentration of 30～70 ng on ISSR-PCR products . Lower Mg^{2+} concentration (＜1 mmol/L) got less products of amplification by reducing the enzyme activity , and nonspecific products of amplification was produced when the concentration of Mg^{2+} beyond 2 .5 mmol/L level . The electrophoresis results showed that the concentration of dNTP was optimal at 0.2 mmol/L in eight concentration gradients .

Conclusions The optimal ISSR-PCR reaction system for *Stipa kryloviii* was established , which is the 25 μL reaction mixture containes 50 ng template DNA , 1 .5 mmol/L Mg^{2+} , 0 .2 mmol/L dNTP , 0 .8 μmol/L primer , 1 U Taq DNA polymerase and 10× buffer 2 .5 μL . This reaction system will provide the basis for the analysis of diversity , map construction and gene localization of *Stipa krylovii* in further studies using ISSR molecular marker .

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