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## Establishment and optimization of the ISSR-PCR reaction system in *Stipa krylovii*

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**Key words:** *Stipa krylovii*, ISSR, reaction system, optimization, molecular marker

**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 system.

**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°C pre-denaturation for 5 min, 35 cycles of 94°C for 45 sec, 55°C for 45 sec, 72°C for 1.5 min, and 5 min 72°C extension. The orthogonal design [L16 (4<sup>3</sup>)] 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°C-57°C, the high annealing temperature lead to failing linkage between primer and DNA template. There was not significant effect of DNA template concentration of 30~70 ng on ISSR-PCR products. Lower Mg<sup>2+</sup> 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<sup>2+</sup> 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 krylovii* was established, which is the 25 μL reaction mixture contains 50 ng template DNA, 1.5 mmol/L Mg<sup>2+</sup>, 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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