Establishment and Orthogonal Optimization of ISSR-PCR Reaction System of Conference pear (*Pyrus communis* L.)

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Abstract The purpose of this study was to establish and optimize the ISSR reaction system and amplification procedure for the Western Europe pear *Pyrus communis* L.cv 'Conference'. The orthogonal and single factor experimental design methods were used to optimize the effects of dNTPs, Taq DNA polymerase, primers, template DNA and Mg^{2+} on the ISSR-PCR reaction system of 'Conference'. A stable and reproducible ISSR-PCR amplification reaction system was established. The test results were as follows: in a 50 μL reaction system, the dNTPs concentration was 0.15 mmol/L, the Taq DNA polymerase amount was 6.25 U, the primer concentration was 0.4 μmol/L, the Mg^{2+} concentration was 2.0 mmol/L, and the DNA concentration was 25 ng. The amplification procedure was as follows: pre-denaturation for 3 min at 94℃; denaturation for 30 s at 94℃, annealing for 30 s at 59.4℃, extension for 30 s at 72℃, 38 cycles of the above three steps, and extension for 10 min at 72℃. The establishment of this optimization system could be helpful for further research on the genetic diversity of 'Conference'.

Keywords *Pyrus communis*, ISSR-PCR, Orthogonal test