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Optimization of SCoT-PCR reaction system in *Dactylis glomerata* by orthogonal design

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ABSTRACT. The effects of 5 factors (template DNA, Mg^{2+}, dNTPs, *Taq* DNA polymerase, and primer) on the polymerase chain reaction (PCR) were investigated to optimize the start codon targeted polymorphism (SCoT)-PCR system of *Dactylis glomerata* L., using an orthogonal design L_{16} (4^5). A suitable SCoT-PCR system for *D. glomerata* was established; the 20 µL reaction volume contained 3.0 mM Mg^{2+}, 0.2 mM dNTPs, 1.0 U *Taq* DNA polymerase, 0.2 µM primer, 20 ng template DNA, and 2 µL 10X buffer. Each factor had a different effect on the amplification reaction, and the concentration of dNTPs had the largest effect on the SCoT-PCR system. We tested 10 orchardgrass samples to determine and verify the stability of the reaction system. The results showed that amplified bands from diverse materials were clear, stable,

and rich in polymorphisms, indicating that the optimized system was very stable.

Key words: *Dactylis glomerata* L.; Reaction system; Optimization; Start codon target polymorphism-polymerase chain reaction; Orchardgrass; Orthogonal design

