An efficient protoplast transient expression system developed for *Achnatherum inebrians*

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With climate change intensifying and extreme weather patterns becoming more prevalent worldwide, there is growing urgency to cultivate crop varieties that demonstrate both high productivity and environmental resilience. *Achnatherum inebrians*, a cool-season grass, often coexists with the mutualistic endophytic fungi *Epichloë gansuensis* or *Epichloë inebrians*. Many studies have shown that the *Epichloë* symbiont confers excellent resistance to biotic and abiotic stresses over plants that lack this fungal association. *A. inebrians* is a diploid self-pollinating grass species and its genome was assembled by the College of Pastoral Agriculture Science and Technology (Lanzhou University, China). Polyethylene glycol (PEG)-mediated protoplast transient expression system is a convenient and valid method for supporting investigations into the biological and biochemical functions of plant genes. To better understand the molecular mechanisms of symbiosis and stress resistance in *Epichloë-A. inebrians* symbiosis, we developed a highly efficient protoplast transient expression system. In detail, as the leaves of *A. inebrians* seedlings are narrow and contain a high fibre content, we wounded the leaves along veins instead of cutting into segments, which significantly increased the protoplast yield. These 10-15-day old leaves were then subjected to a 5-hour enzymatic treatment using an enzymatic solution containing 1.5% cellulase, 0.4% isolase, and 0.5-0.6 M mannitol, that yielded 2×10^7 protoplast cells per gram of fresh weight with 85% cell viability. Relative to reported protocols for protoplast isolation for wheat, our method achieved a twofold increase in yield, substantially reducing digestion time, and enhanced cell viability. The transient protein expression system developed here can rapidly evaluate the expression of exogenous genes in *A. inebrians* by transferring the target genes into *A. inebrians* protoplasts. This system will be used in future research to support understanding of the functions and interactions of the plant genes. This knowledge will further support investigations into the molecular mechanisms of key biological processes such as endophytic fungal symbiosis and stress tolerance in *A. inebrians*.

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online) https://doi.org/10.33584/rps.18.2025.3822