Establishment of an efficient genetic transformation system for the *Achnatherum inebrians-Epichloë* symbiosis

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The symbiosis between *Achnatherum inebrians* and endophytic fungi of the genus *Epichloë* (e.g., *E. gansuensis* and *E. inebrians*) can significantly improve the host plant's ability to tolerate salinity, drought and low nitrogen. A highly efficient genetic transformation system for *A. inebrians* was established to study mechanisms of the stress tolerance within this plant-fungal symbiosis. Mature seeds were surface disinfected before mechanical wounding of embryos to produce callus. The regeneration step was optimized based on the expression pattern of cell totipotent related genes, such as *ARF*s, *BBM*, and *WOX*s. The regeneration efficiency was around 70%. *Agrobacterium tumefaciens* strain EHA105 was utilized for stable integration of transgenes into callus-derived genomes through co-cultivation. By vacuum incubating the callus with EHA105 cells (OD_{600}=0.2) for 30 mins, the transformation efficiency stabilized at 12%-15%, with a rooting rate of up to 95%. Transformed plants were validated by PCR and sequencing, with a positive rate up to 75%. Our efficient transgenic system for *A. inebrians* provides a powerful tool for analyzing the molecular mechanism of *Epichloë*-grass interactions. Combined with gene editing techniques, such as CRISPR-Cas9, the generation of diverse mutant variants of *A. inebrians* will enable significant acceleration and profound enhancement in both the pace and depth of investigative progression.

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