

# Identification of a domain of unknown function family gene involved in mediating the drought tolerance of *Achnatherum inebrians* colonised by an *Epichloë* endophyte

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The increasing frequencies of severe global droughts highlights the importance of cultivating high-yielding, drought-resistant crops. The *Epichloë-Achnatherum inebrians* symbiosis exhibits remarkable drought tolerance and investigating the molecular mechanism responsible for this trait would provide important information for drought tolerance in graminaceous crops. The transcriptome and proteome of *A. inebrians* and *Epichloë-A. inebrians* symbiosis was compared under the conditions of 100% and 40% soil moisture saturation, respectively. The expression of numerous genes exhibited differential patterns even at 100% soil moisture saturation, with the divergence intensifying significantly at 40% saturation. To detect key drought responsive genes, the top 20 genes exhibiting the most significant differences in expression levels were cloned. Utilizing a *Agrobacterium*-mediated transient expression system in *Nicotiana benthamiana*

and a luciferase (*LUC*) gene drove by the promoter of a drought response marker gene as a reporter, the functions of these candidate genes in regulating drought response were detected. A DUF (Domain of Unknown Function) family gene, named *AiDR6*, significantly induced *LUC* expression under both of the control and PEG6000 treatment conditions. Intriguingly, subcellular localization analysis revealed that *AiDR6* is specifically localized to chloroplasts of stomata. Furthermore, compared to the wild type, *N. benthamiana* overexpressing *AiDR6* exhibited a very distinct stomatal closure phenotype. These results suggest that *AiDR6* plays a critical role in regulating the *Epichloë* endophyte mediated *A. inebrians* drought tolerance. The functional analysis of *AiDR6*, along with further elucidation of its regulatory mechanisms, will advance the understanding of drought tolerance mechanisms in *Epichloë*-grass symbionts.