Neotyphodium lolii endophyte improves drought tolerance in perennial ryegrass (*Lolium perenne*. L) through broadly adjusting its metabolism

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Abstract

Perennial ryegrass (*Lolium perenne*) is a widely used pasture grass that is frequently infected by *Neotyphodium lolii* endophyte. The presence of *N. lolii* enhances grass resistance to several biotic and abiotic stresses such as insect, herbivory and drought. Recent studies suggest the effect of *N. lolii* on ryegrass drought tolerance varies between grass genotypes. However, little is known about the molecular basis of how endophytes improve grass drought tolerance, why this effect varies among grass genotypes, or how the endophytes themselves respond to drought stress. This knowledge will not only increase our knowledge of beneficial plant-microbe interactions, but will also guide better use of endophytes, such as selection of specific endophyte - cultivar combinations for growth in arid areas.

In this study, a real time PCR method that can accurately quantify *N. lolii* DNA concentration in grass tissue was developed for monitoring endophyte growth under drought. The effect of *N. lolii* on growth of 16 perennial ryegrass cultivars under drought was assessed, and a pair of endophyte-infected grasses showing distinct survival ability and performance under severe drought stress was selected. The transcriptome profiles of these two endophyte-infected grasses, as well as their clonal endophyte-free grasses, were analyzed using high-throughput RNA sequencing. The expression of endophyte and grass genes responsive to drought was analyzed simultaneously using different bioinformatic tools.

The results demonstrated that *N. lolii* enhanced the growth of perennial ryegrass under drought, but the effect varied between cultivars. On the molecular level, endophytes living in both drought-tolerant and drought-sensitive grasses responded to drought in similar ways, including increased expression of endophyte genes related to scavenging of reactive oxygen species (ROS), DNA replication and the cell cycle, and also reduced expression of genes involved in alkaloids biosynthesis. The presence of endophytes also led to enhanced grass tolerance that was associated with broad adjustments in the plant metabolism. This included up regulation of grass genes

involved in chloroplast maintenance and protection, osmotic adjustment and ROS scavenging capability. The extent of these endophyte-associated effects was greater in the drought tolerant grass genotype than in the drought sensitive genotype.

This work highlights the role of fungal endophytes in grass drought stress tolerance and provides a comprehensive insight into the mechanisms involved.

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