Metabolic profiling of endophyte-infected and endophyte-free ryegrass grown under sufficient water supply and drought

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Metabolic profiling using gas-chromatography mass spectrometry was performed for endophyte-infected (E+) and corresponding endophyte-free (E-) clones of two ryegrass genotypes cultivated under sufficient water supply and drought stress. In total, 243 metabolites representing both known and unknown compounds were analysed for samples taken at the end of the drought stress period and after rewatering (n=10 replications per level of genotype, endophyte infection, and water supply).

Before statistical analysis the data were normalised using vector normalisation. For each metabolite, comparisons between control and drought treatment as well as between E+ and E- were carried out by the calculation of t-values ($P \leq 0.01$ with Bonferroni correction). Most metabolites showed significantly higher levels in the control as compared to the drought treatment. However, some compounds, including proline, which is a well-known indicator for drought stress, but also histidine, indole derivates, phenylalanine, sucrose, and tryptophane were more abundant under drought stress. On the other side, only two metabolites (mannitol and an unknown compound) differed significantly between E+ and E- variants. Mannitol is known as a fungal metabolite contributing to osmotic adjustment of E+ plants. For this substance, ANOVA elucidated a significant interaction between endophyte and water supply. Mannitol was more abundant in E+ than in E- and this effect was more pronounced under sufficient water supply than under drought stress.

To investigate entire metabolite profiles discriminating between drought/control and E+/E-, we carried out a principal component analysis (PCA). The first three principal components accounted for 56% of the total variance. As expected, PCA allowed clear clustering between control and drought treatment. This separation is particularly reflected by the first two components. Component 1 features positive values for those substances, which were found to be increased in the drought condition (e.g. proline, histidine, indole derivates, sucrose, tryptophane), and negative values for most other substances. Component 2 contains positive peaks for (among many unidentified substances) ferulic acid, fumaric acid, hexose, oxalate, and xylose, and negative peaks for example for citric acid, succinic acid, and gluconic acid. Component 3 shows positive peaks for example for citric acid, hexose and trehalose, while negative peaks are observed for pipecolic acid, quinic acid and xylose. Furthermore, both genotypes formed separate clusters indicating genotype-specific metabolic profiles. The differences between the two genotypes were more pronounced under control and re-watering conditions than under the influence of drought. The shift vectors between drought and control conditions were very similar for all endophyte × genotype combinations, although the genotypes clearly separate with respect to component 3. At the end of the re-watering phase the difference between the two treatment groups (drought and control) was qualitatively still the same, although greatly diminished in magnitude. The shift vectors between E+ and E- status were less consistent. The effect of endophyte infection strongly depended on the grass genotype and water supply.

In conclusion, metabolic profiling showed that drought caused gross changes in plant metabolism involving the majority of measured compounds. While the production of most metabolites reduced under drought stress, some substances, including proline increased. Re-watering induced “regeneration” of the metabolic profiles resulting in only small residual differences between treatment groups after 10 days. Endophyte infection clearly yielded differences in metabolic profiles, which were also strongly dependent upon plant genotype and environmental conditions. Only a few substances, like the fungal metabolite mannitol, were generally increased in infected plants.