Summary

Agrobacterium tumefaciens-mediated transformation is a key driver of functional genomics and genetic engineering in many economically and industrially important crops. However, achieving desirable transformation outcomes in recalcitrant plants such as *Hypericum perforatum* L., commonly known as St. John's wort remains challenging due to incompatible plant-bacterium interactions. Here, we report for the first time the proteomic and physiological changes in the pharmaceutically important and non-transformable medicinal plant *H. perforatum* in response to *A. tumefaciens*, using a comprehensive proteomic approach and physiological assessments. Initially, the viability of *A. tumefaciens* in the presence of *H. perforatum* cell suspension cultures was tested via antibacterial assays by assessing colony growth. Our results show that the *H. perforatum* cell suspension reduced the viability of *A. tumefaciens*, reaching 90% mortality within 24 h. Proteome analysis of *H. perforatum* cell suspension cultures challenged with *A. tumefaciens* using liquid chromatography–tandem mass spectrometry revealed 1,200 differentially expressed proteins (DEPs) (613 up-regulated and 587 down-regulated) after 12 h and 390 DEPs (174 up-regulated and 216 down-regulated) after 24 h of co-cultivation. Functional analysis identified various activated proteins in *H. perforatum* involved in mounting a defence immune response to *A. tumefaciens*. The proteomic dataset highlights the activation of the defence-related pathogenesis-related-10 protein and the accumulation of oxidative stress-related proteins, peroxiredoxin and L-ascorbate peroxidase. The induction of cell wall-modifying proteins, such as UDP-arabinopyranose mutase and pectin acetylesterases, as well as the accumulation of proteins related to secondary metabolic defence mechanisms, including phenylalanine ammonia lyase, 1,3,7-trihydroxyxanthone synthase, and benzophenone synthase, indicates a coordinated plant defence response to restrict pathogen invasion. Increased levels of malate dehydrogenase and sucrose synthase suggest a higher metabolic energy demand. In addition,

physiological analysis revealed significant stress responses in *H. perforatum*, including a progressive decrease in photosynthetic rate, along with an initial increase followed by a decrease in stomatal conductance, intracellular CO_2 concentration, and transpiration rate. Taken together, the proteomic changes and altered physiological responses of *H. perforatum* enhance our understanding of the factors underlying the recalcitrance of plants to *Agrobacterium*-mediated transformation.