ABSTRACT

The oomycetal elicitor *β*-cryptogein has been known to induce phenolic metabolites accumulation and pathogen/disease resistance in tobacco, however, information regarding its effect on other than enhanced accumulation of phenolic metabolites is scant and not much attention has been given to oxidative status in cryptogein-transformed plant. Moreover, there were no reports on primary metabolites changes in response to *β*-cryptogein. Therefore, this study was carried out with the aim to assess metabolite purturbations, antioxidative status and pathogen resistance in response to constitutive expression of *β-cryptogein* gene. Tobacco (*Nicotiana tabacum* L.) hairy roots and plantlets (spontaneously regenerated from hairy roots) expressing *β-cryptogein* gene under *CaMV 35S* promoter were established. Hairy roots were used primarily to analyse metabolite perturbations in order to gain a better understanding of *β*-cryptogein induced secondary metabolite accumulation and its connection with changes in primary metabolites, whereas, experimental system with plantlets was explored to evaluate the antioxidative status in response to constitutive expression of *β-cryptogein* gene. Metabolite analyses in hairy root suggested increment of phenolic compounds, fatty acid derivatives, alkaloids (nicotine and nornicotine), benzenoids, sugars (sucrose, fructose, galactose and lactose) and few metabolites originated from glycolysis pathway intermediates, in response to *β-cryptogein* expression, However, suppression of terpenoids and many amino acids (except proline) synthesis was also noticed in *β-cryptogein* -transformed hairy roots. Plantlets expressing *β-cryptogein* also showed accumulation of phenolics (wall-bound phenolics, total soluble phenolics and chlorogenic acid). Increased gene expression of phenylalanine ammonia lyase (*PAL*), 4-coumaryl CoA ligase (*4CL*), coniferyl alcohol dehydrogenase *CAD*), genes along with enhanced enzyme activities of PAL, 4CL and CAD were noticed in *β-cryptogein* expressing hairy roots and plantlets as compared to their respective controls. These findings supported the phenolics increment in both hairy roots and plantlets expressing *β-cryptogein* gene compared to control. Decreased expressions of *3-hydroxy-3-methylglutaryl-CoA reductase* (*HMGR*) and *1-deoxy-D-xylulose-5-phosphate synthase* (*DXS*) in Ri-crypt-transformed hairy roots and Ri-crypt-transformed plantlets as compared to their respective controls were observed which supported the terpenoid metabolite decrement in the same. Enhanced accumulation of total lignin content was noticed in Ri-crypt-transformed plantlets as compared to Ri-transformed plantlets, which were in agreement with CAD expression and enzyme activity results. Antioxidative status was uplifted in both hairy roots and plantlets upon constitutive expression of *β-cryptogein* as total flavonoid content, total phenolics and activities of antioxidative enzymes (peroxidase, catalase, ascorbate peroxidase and superoxide dismutase) were enhanced. Enhanced antioxidative status resulted in an apparent reduction in oxidative stress indicators such as H_2O_2, superoxide radicals and malondialdehyde content. Enhanced accumulation of wall-bound phenolics and total lignin content strengthened the cell wall in *β-cryptogein* expressing plantlets. The *β-cryptogein*-expressed leaves showed delayed degradation upon infiltration of fungal pathogen (*Alternaria alternata*) might be due to the enhanced antioxidative status and strengthen-cell wall. The expression study of defense gene *PR1a* showed higher expression in *β-cryptogein* expressing hairy roots and plantlets expressing *β-cryptogein*.

Keywords: *Nicotiana tabacum*, *β*-cryptogein, antioxidative status, hairy roots, plantlets, metabolites perturbation