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MELATONIN-INDUCED METABOLIC ALTERATIONS IN LOTUS CORNICULATUS CALLI: AN UNTARGETED METABOLOMIC PROFILING

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A plethora of studies has highlighted the pleiotropic role of melatonin and its interaction with other hormones in various plant physiological processes. The exogenous administration of melatonin regulates aerial and root development, seed germination and photosynthesis. This molecule lowers the levels of reactive oxygen and nitrogen species and promotes plant resistance to stresses. The effects of melatonin in triggering the biosynthesis of secondary metabolites of relevance for human health appear to be concentration, species and organ dependent.

Lotus corniculatus is a forage legume able to synthesize a wide range of flavonoids in its organs and tissues. A preliminary investigation has shown that the ROS scavenging and anti-apoptotic activity of its leaf methanolic extracts seems to be dependent on the levels of flavonoids. Thus, in the present study we aimed at verifying whether melatonin administration could induce the accumulation of useful metabolites in in vitro grown L. corniculatus cells. To test this hypothesis, calli of a diploid genotype of L. corniculatus has been treated with 100 µM of melatonin for 24, 48, 72 h or 7 days, and their metabolic profiles were examined using untargetedLC/MS QTOF analysis followed by statistical analyses performed withMetaboAnalyst 5.0 platform. In keeping with what found in literature, manypathways were significantly influenced by the exogenous application ofmelatonin.

Purine, pyrimidine, amino acid, lipid, and carbohydrate biosynthesis and degradation were among the pathways of primary metabolism to be influenced. Among secondary metabolites, both terpene and phenolic compounds were affected. For the former class, a significant increase in the content of presqualene diphosphate and farnesil diphosphate was found up to at 72 h. Likewise, the increase in the significance of the phenylpropanoid pathway was proportional to the exposure time up to 3 days. This was likely due to the increase in the levels of trans cinnamate, the entry metabolite of this pathway. The same compound was no longer detected after a week of treatment. This could suggest a transient increase in the activity of the PAL enzyme in the first days of treatment to give off a substrate which in turn serves to boost the biosynthesis of the downstream pathways like flavonoids and, among them, anthocyanins. Notably, an anthocyanidin increased significantly only at 72h, whereas a re-direction of the pathway between di-hydroflavonols and flavonols was assessed after 7 days of treatment, when a significant increase in dihydrokaempferol was accompanied by a significant reduction of quercetin.

Overall, melatonin seems to control the biosynthesis of several compounds of pharmacological interest in Lotus calli. However, the timing of their biosynthesis and degradation remains to be investigated in order to be able to exploit Lotus calli as a bioreactor for the controlled production of compounds with nutraceutical properties.