Orientin Production

Genes and production system for efficient orientin and isoorientin flavonoid production in duckweed

Overexpression of LtP1-L to increase orientin and isorientin production

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Tech ID: 23.702

Patent Literature

Publication: WO2024/110539 A1

Dr Shengjun Li and researchers at *Qingdao Institute* of Bioenergy and Bioprocess Technology, China have identified novel P1-L genes from the duckweed stains *Lemna turionifera* and *Landoltia punctata* and created transgenic *Lemna turionifera* duckweed stains that through over expression of LtP1-L and/or LeP1-1 produce high levels of orientin and isoorientin flavonoids, released in aqueous solution. LtP1L is key in accumulating CFGs, opens up the potential for further metabolic engineering approaches in duckweed for high flavone accumulation.

Orientin and isoorientin are C-glycosylated flavones, like other flavonoids, C-glycosylated flavones have been used as valuable nutraceuticals with antioxidant, anti-inflammatory, anti-proliferative and cardioprotective activities reported. Orientin and isoorientin are mainly extracted from plants through various methods such as traditional extraction, enzymatic extraction or synthesis by chemical means, resulting in high production costs. Moreover, the method of extracting flavonoids by collecting plant resources from nature is no longer sufficient to meet the growing market demand. Therefore, methods that are easy to scale and automate for the production of orientin and isoorientin are required. In addition, isoorientin was reported to improve the stability of silver nanoparticles, reduce their toxicity, and enhance the inhibitory effect on α-glucosidase and pancreatic lipase, exhibiting the further development of highly stable and lowly cytotoxic AgNPs-Iso on Type II diabetes and obesity. Isoorientin exhibited high photoprotective activity against UV-B (290–320 nm) radiations, revealing its possibility as an antioxidant and sunscreen agent in cosmetic formulations.

Duckweed is a floating higher aquatic angiosperm. It has been used as a medical plant for many years. Duckweed has the advantages of fast growth, easy reproduction, low production cost. Duckweed contains flavonoids such as orientin and isoorientin, with a typical content of less than 0.5 mg/g dry weight. Through a transformation system developed in the laboratory of Dr Shengjun Li, the productivity of orientin and isoorientin can be increased up to 40 times in plant tissues. **The total yield of orientin reaches 9.39 mg/g dry sample, while the total yield of isoorientin reaches 16.99 mg/g dry sample.** Most importantly, **in the liquid solution of duckweed cultivation, both orientin and isoorientin were detected with a very high concentration (orientin ~104.21 mg/L, isoorientin ~191.44 mg/L).** Therefore, genetic engineered duckweed has the potential to become a stable, high-yielding, and new source of orientin and isoorientin with the very low cost. LtP1L is key in accumulating CFGs, opens up the potential for further metabolic engineering approaches in duckweed for high flavone accumulation.

Duckweed is a non-food aquatic plant and has the advantages of fast growth, easy reproduction, low production cost and can achieve rapid production, overcoming inefficiencies of current natural plant-based methods for extraction or synthesis by chemical means. Broad application prospects with potential high economic value in industrial processes such as the food industry, health products, cosmetics and feed production.

References:

Wang S, He G, Liu Y, Wang Y, Ma Y, Fu C, Xu H, Hu R, Li S (2024). A P1-like MYB transcription factor boosts biosynthesis and transport of C-glycosylated flavones in duckweed. *International Journal of Biological Macromolecules*; 277(2): 134138. https://doi.org/10.1016/j.ijbiomac.2024.134138

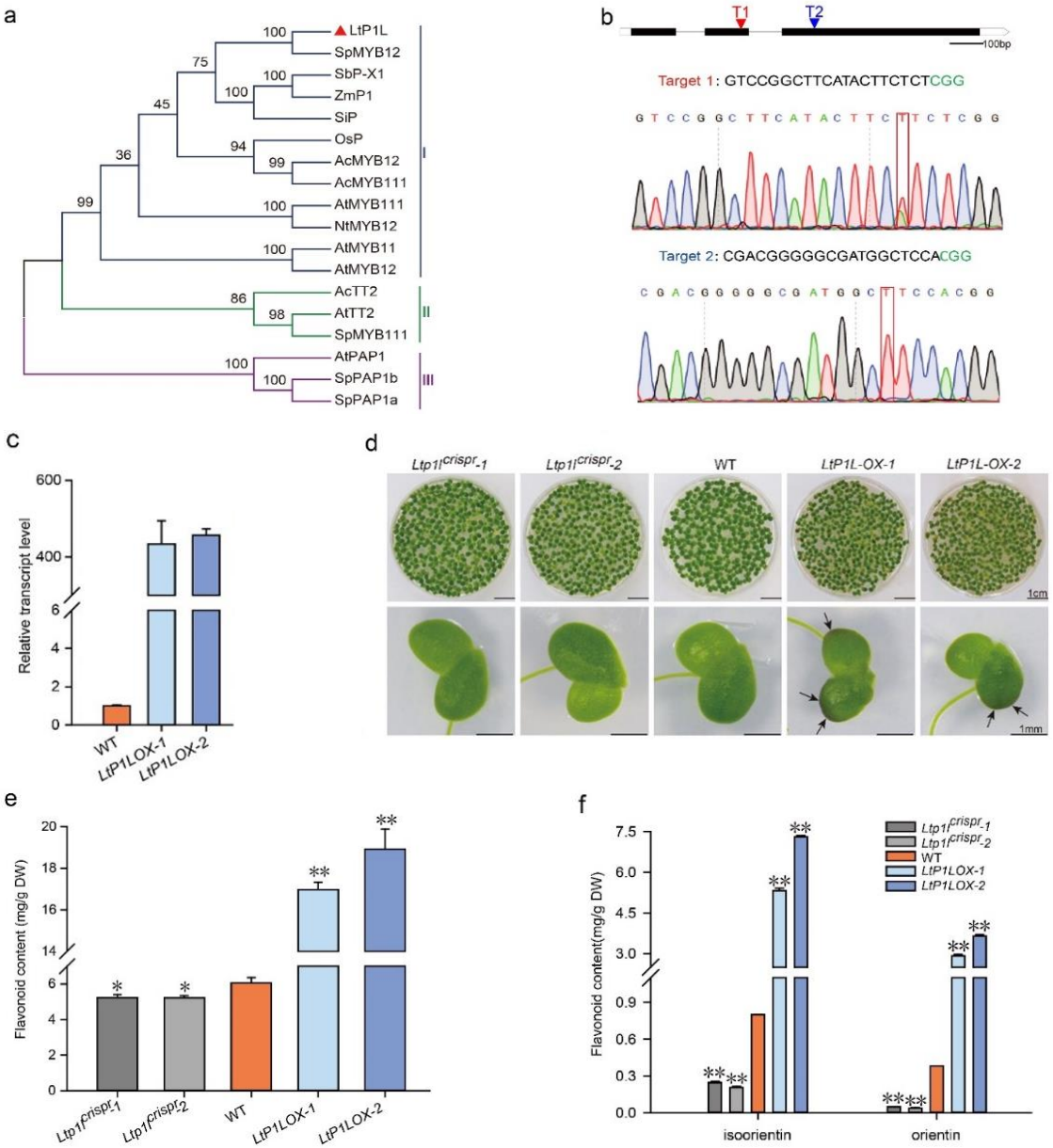

Figure 1. Functional characterization of LtP1L. **a** Phylogenetic tree was constructed using full-length protein sequences of LtP1L and its homologues from eight plant species (**Table S1**). Three groups are shown in different colours. **b** Loss-of-function mutants of *LtP1L* generated by CRISPR/Cas9. Selected target 1 (T1) and target 2 (T2) are located in the second and third exons of *LtP1L*, respectively. Sequencing analysis revealed a T insertion at T1 and a T insertion at T2 in the mutants, both of which resulted in a frameshift and premature termination of protein translation. **c** qRT-PCR analysis of *LtP1L* transcript abundance in the wild type (WT) and *LtP1L* overexpression lines. **d** Phenotype of the WT and transgenic duckweed in the SH medium for two weeks. The bottom lane showed a representative frond for each genotype. **e** Quantification of total flavonoids in the WT and transgenic duckweed. **f** Quantification of orientin and isoorientin in the WT and transgenic duckweed. DW, dry weight. Bars represent standard deviation (SD) from three biological replicates. Asterisks denote significant difference between the transgenic duckweed and WT (*, $p < 0.05$; **, $p < 0.01$).

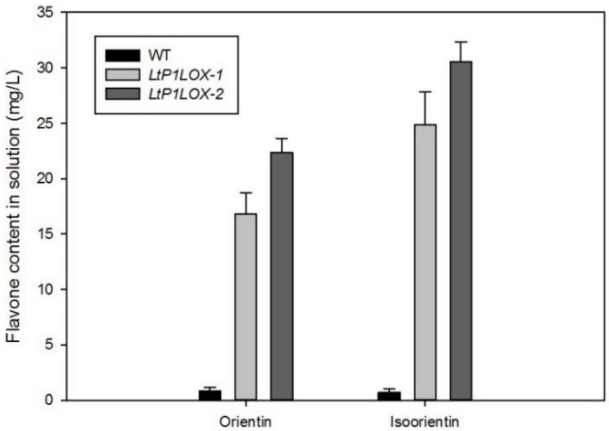

Figure 2. The content of orientin and isoorientin in duckweed cultivation solution.