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Transgenic *Spirodela polyrhiza* and *Lemna minor* for Oral Delivery of Highly Glycosylated Human Interferon Alpha-2b

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Duckweeds are a group of aquatic plants that represent an attractive platform for molecular farming due to their rapid vegetative propagation, genetic stability, and low cultivation costs. In this study, we investigated the production of recombinant human interferon alpha-2b (HsIFN α 2b), a cytokine widely used for antiviral and anti-proliferative therapies, in duckweed, with potential to reduce costs associated with downstream processing, cold-chain storage, and parenteral administration.

Respiratory viral infections remain a major global health burden, causing significant morbidity, mortality, and economic losses, while access to prevention and treatment remains limited in many low-income countries. Oral delivery of HsIFN α 2b has been shown to produce a systemic immune response at doses up to 104-fold lower than required for injections, potentially reducing its adverse effects. Moreover, in edible duckweeds, the plant cell wall may provide bioencapsulation, protecting recombinant protein from digestion via gastrointestinal enzymes.

To improve the protein stability and biological activity of HsIFN α 2b, four artificial N-glycosylation sites were introduced by site-directed amino acid substitution. Transgenic lines of *Spirodela polyrhiza* 162 and *Lemna minor* 8623 expressing 4N-HsIFN α 2b were generated via *Agrobacterium*-mediated transformation and indirect organogenesis. LC-MS glycan analysis of plant 4N-HsIFN α 2b identified complex plant-type glycans, indicating Golgi-mediated processing and targeting to the secretory pathway.

Biological activity was assessed using human A375 melanoma cells carrying an ISRE-mCherry reporter. Plant-produced 4N-HsIFN α 2b induced activation of the IFNAR signalling pathway, resulting in an approximately threefold increase in reporter activity at 70 ng ml⁻¹ compared with the buffer control, demonstrating functional activity in human cells.

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References

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