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PLANT ROOTS RELEASE SMALL EXTRACELLULAR VESICLES CARRYING A MOLECULAR TOOLKIT FOR DEFENCE AGAINST PATHOGENS

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Cells release extracellular vesicles (EVs) naturally conceived to work as smart bioshuttles with a prominent role in cell-cell communication. While the research in animal biology has shown the great potential of EVs in diagnostics and therapeutics, EV knowledge still lags behind in plant science.

Recent evidences have shown that plants produce round-shaped nano and microvesicles outlined by a phospholipid bilayer delivering different biomolecules such as lipids, proteins, nucleic acids (microRNA, long non-coding RNAs) and other metabolites to recipient cells, influencing their biological processes and driving cell-cell communication, even between different organisms and species. In particular, proteomic studies have demonstrated the important presence of proteins with antifungal and hydrolytic activities, suggesting that EV may have specialised functions in plant defence.

Here we report the purification of round-shaped small vesicles (EVs) by differential ultracentrifugation of a sampling solution containing root exudates collected from hydroponically grown tomato plants. Dimensional analyses shown that the size of root-released EVs ranges in the nanometric scale (50–100 nm). EV morphological characterization by Scanning Electron Microscopy confirmed the presence of intact EVs in close vicinity to the expected size range.

Shot-gun proteomic analysis of root-derived EVs revealed the presence of numerous proteins known to be involved in plant-microbe interactions such as endochitinases and glucan-endo-1,3 beta- glucosidase B precursors and putative late blight resistance protein homologs. Furthermore, root-released EVs were shown to inhibit in vitro the spore germination and the development of germination tube of the plant pathogens *Fusarium oxysporum*, *Botrytis cinerea*, and *Alternaria alternata*.

To summarise, here we demonstrate for the first time that plants release EVs in the surrounding environment. The data corroborate the hypothesis that EVs are novel components of the innate immune system in plants. From a biotechnological perspective, these results pave the way for the use of plants as

biofactories of extracellular vesicles with great potential in plant protection.