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Extracellular vesicles of the apoplast of germinating lupin seeds: proteomic analysis and mechanism of release from the cells

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Extracellular vesicles (EVs) are formed within the endosomal network and released upon fusion of multi-vesicular bodies with the plasma membrane into the surrounding environment. EVs play an important role in intercellular communication by transporting proteins, nucleic acids and organic compounds. It has been shown that during seed germination plant EVs mediate the transport of proteins to the extracellular compartment (apoplast) participating in defense responses. Previous preliminary works indicate that, after 16 hours from the germination onset, γ-conglutin (γC) is the most represented protein in seed apoplast and that it is confined in vesicular material. γC from the leguminous plant *Lupinus albus* has been considered for a long time a storage protein, but recent studies definitely ruled out this function evidencing a multifaceted involvement in plant defence mechanisms against pathogens attacks. γC is structurally related to GH12 xyloglucan-specific endo-glucanase inhibitor proteins (XEGIPs), but shows its inhibitory activity only against fungal GH2 β-mannosidase.
Beside its physiological role in plant, γC set off a remarkable pharmacological interest, too. It was proved to significantly decrease glycaemia in humans and animals when orally administered. The applicative fall backs of these findings are relevant, being γC a natural dietary component that could complement pharmacotherapy in the management of diabetes.
In developing seeds, γC is deposited inside protein bodies through the secretory pathway. During germination, γC is localized in the apoplasts of germinating seeds while the storage vacuoles appeared void of the protein. How γ-conglutin reaches the extracellular regions is still matter of debate.
The present communication describes the first proteomic characterization of the EVs released outside the cotyledonary cells of lupin seeds during the very first moments of germination, and investigates some aspects of their release mechanisms. The results have been obtained by making use of a combination of methodologies such as vacuum infiltration, ultracentrifugation fractionation, 1D- and 2D-gel electrophoresis, mass spectrometry, and immunoblotting techniques.
The hypothesis that a specific set of seed-stored proteins are released outside the cells through unconventional secretory vesicles-based pathways is suggestive.
Taken together, the results here reported contribute to deep new aspects of the role of seed-originated proteins, and of the mechanisms of action and secretion of γC.

Keywords: legume proteomics, plant defence, seed germination, lupin γ-conglutin.