

# Extracellular vesicles released in the apoplast of germinating seeds contain almost exclusively a fungal glucanase inhibitor

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Extracellular vesicles (EVs) are small, membrane-enclosed structures released from a cell into the surrounding environment. EVs play an important role in intercellular communication by transporting proteins and RNA. Plant EVs seem to mediate the transport of proteins to the extracellular compartment (apoplast) and could account for the so-called unconventional secretion pathway, where proteins lacking a signal peptide secreted in extracellular fluids. Thus, EVs participate in defense responses, acting as carriers of active proteins through the apoplast (Regente et al., 2017).

$\gamma$ -Conglutin 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 triggered by pathogens attacks (Scarafoni et al., 2010).  $\gamma$ -Conglutin shares structural similarities with members of a large family of glucanase inhibitors widely distributed across the plant kingdom, such as the xyloglucan-specific endo- $\beta$ -1,4-glucanase inhibitors (XEGIPs) (Qin et al., 2003, Scarafoni et al., 2016).  $\gamma$ -Conglutin was localized in the apoplasts of germinating seeds while the storage vacuoles appeared void of the protein. How  $\gamma$ -conglutin reaches the extracellular regions is still matter of debate.

The present work aimed to investigate if  $\gamma$ -conglutin is secreted outside the cotyledonary cells during the very first moments of seed germination is conveyed by EVs. The results obtained by a combination of vacuum infiltration, 1D- and 2D-gel electrophoresis, ultracentrifugation fractionation and immunoblotting techniques, indicate that after 16 hours from the germination onset  $\gamma$ -conglutin is the most represented protein in apoplast and that it is confined in vesicular material.

The hypothesis that the seed-stored protein is released outside the cells through an unconventional secretory vesicles-based pathway, as it occurs for sunflower seed agglutinin 1 and other seed proteins, is suggestive and needs further focused investigations.

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

## References

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