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Content

DRYING WITHOUT DYING: REVEALING THE ROLE OF LATE EMBRYOGENESIS ABUNDANT PROTEINS DURING DESICCATION IN *RAMONDA SERBICA*

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Introduction: Resurrection plants (such as *Ramonda serbica*) can survive a long desiccation period and fully resume their metabolism upon watering. The hallmark of desiccation tolerance (DT) is the accumulation of protective, intrinsically disordered proteins (IDPs), called late embryogenesis abundant proteins (LEAPs). Although their high structural plasticity allows them to interact with various partners, no specific cellular targets of LEAPs have been identified so far.

Methods: To identify LEAPs involved in DT, differential transcriptome and proteome analyses of hydrated and desiccated *R. serbica* leaves were performed. The identified LEAPs were structurally characterised and classified. To evaluate their structural properties *in vitro* and their potential functions *in vivo*, the representative RsLEA proteins, were produced in *Escherichia coli* using recombinant DNA technology.

Results: Members of the LEA4 protein family represent the majority of desiccation-inducible LEAPs. Even 17 proteins belonging to the LEA4 protein family group were induced by desiccation. They show high disorder propensity (82 %), and at the same time, a high tendency to form α-helices (>80%). Although recombinant DNA technology has traditionally been used to overexpress and purify various globular proteins, the production of IDPs is challenging due to their high susceptibility to proteolytic cleavage and aggregation. Nevertheless, the representative LEAPs containing hexa-His tags immunoglobulin G-binding protein and a proteolytic TEV site were produced, purified and cleaved by TEV protease.

Conclusion: The combination of *in silico* and *in vitro* results will be crucial for the identification of endogenous partners of LEAPs, providing further insight into their role in DT.

Key words: late embryogenesis abundant proteins; desiccation tolerance; recombinant DNA technology; intrinsically disordered proteins

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