



The Role of Raffinose Family Oligosaccharides in Seed Membrane Stabilization

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Raffinose family oligosaccharides (RFOs) such as raffinose, stachyose, and verbascose are α -galactosyl derivatives of sucrose that accumulate to high concentrations in orthodox seeds during the late stages of maturation. Decades of physiological, biophysical, and molecular-genetic evidence led us to the conclusion that RFOs are not mere storage carbohydrates but active protectants of cellular membranes during the extreme water loss that accompanies seed desiccation. This article summarises the mechanisms by which RFOs stabilize membrane bilayers by direct interaction with phospholipid headgroups, suppression of the membrane phase-transition temperature, promotion of intracellular vitrification, and inhibition of sucrose crystallization and summarizes the genetic and comparative evidence linking RFO accumulation to desiccation tolerance, seed longevity, and vigour.

Keywords- Desiccation tolerance, α galactosyl derivatives, seed longevity, galactinol, glass transition temperature, intracellular vitrification

Introduction

Orthodox seeds of most angiosperms complete their developmental programme with a remarkable physiological feat i.e. they lose the majority of their cellular water often to below 10% moisture content and enter a quiescent, metabolically arrested state that can persist for years or decades without loss of viability. This capacity, known as desiccation tolerance (DT), depends on a coordinated suite of protective mechanisms that prevent or limit damage to macromolecules and, critically, to cellular membranes, which are the structures most vulnerable to mechanical and chemical injury as the hydration shell around lipid headgroups is stripped away. Among the protective solutes implicated in this process, soluble sugars occupy a central place. Non-reducing disaccharides such as sucrose, together with the raffinose family oligosaccharides (RFOs), accumulate co-ordinately in seed embryos during the acquisition of desiccation tolerance, and their disappearance marks the loss of tolerance in non-orthodox or immature tissues. (Koster and Leopold, 1988; Blackman et al., 1992). RFOs are synthesized from sucrose through the sequential addition of activated galactose units donated by galactinol, a reaction catalysed by galactinol synthase, raffinose synthase, and stachyose synthase. Because RFOs are structurally related to sucrose but carry additional galactosyl residues, they possess distinct physicochemical properties such as larger hydrodynamic volume, greater hydrogen-bonding capacity, and a stronger tendency to form stable, high-viscosity glasses, that make them particularly effective at protecting membranes and macromolecules during water loss.

Biosynthesis and Accumulation of RFOs in Developing Seeds

Raffinose family oligosaccharides are α -galactosyl derivatives of sucrose that are nearly ubiquitous across the plant kingdom. The synthesis starts with the reaction of myo-inositol and UDP-galactose into galactinol-by-galactinol synthase. Raffinose is then formed with the help

of raffinose synthase using sucrose and galactinol as substrates, and stachyose is subsequently formed by stachyose synthase. The enzyme α -galactosidase carries out the hydrolysis, which releases and makes available for use the stored carbon during germination, resulting in sucrose and galactose (Advances in Biotechnology review of RFO metabolism, 2024). RFOs accumulate progressively in all parts of the developing seed, including endosperm, embryo, and seed coat. While the relative contents of galactinol, raffinose, and stachyose vary among the species and tissues, their accumulation is tightly correlated in time with the emergence of desiccation tolerance during seed maturation in soybean, pea, and wheat embryos, and both the sugars disappear once desiccation intolerance becomes established.

Mechanisms of Membrane Stabilization by RFOs

• Direct Interaction with Phospholipid Headgroups

The most direct biophysical evidence for the membrane-protective role of RFOs comes from model liposome studies performed by Hinch and colleagues. Using large unilamellar vesicles of egg phosphatidylcholine subjected to controlled air-drying, they found that, raffinose and its higher homologues stabilize the membrane bilayer by inserting into the lipid headgroup region during dehydration, so as to maintain spacing between headgroups and preventing the close packing that leads to leakage and fusion (Hinch et al., 2003). Critically, the protective efficiency scaled with the degree of polymerization (DP) of the oligosaccharide: sucrose (DP2), raffinose (DP3), stachyose (DP4), and verbascose (DP5) were tested side by side, and with increasing DP the sugars were progressively more effective at restricting both leakage of entrapped aqueous contents and membrane fusion upon rehydration, with a tight linear correlation seen between the two forms of damage. This dose-dependent effect of chain length implies that the larger RFOs are mechanistically superior membrane protectants than sucrose alone, and this consistent with the observation that seeds progressively convert a portion of their sucrose pool into raffinose and stachyose as they mature and desiccation tolerance is acquired. Analogous experiments carried on oligosaccharide combinations, including a sucrose–raffinose mixture, revealed that the strength of hydrogen bonding to phospholipids and the ability to suppress transition temperature correlated closely with vitrification (glass-forming) capacity, in the order : trehalose $\approx$ sucrose–raffinose > sucrose > glucose > mannitol.

• Suppression of the Membrane Phase Transition

As seeds dry, the hydration shell that normally keeps the membrane phospholipids in the fluid, liquid-crystalline state is gradually lost. In the absence of protectants, this loss of water drives the bilayer into a rigid gel phase with an elevated transition temperature; upon rehydration, membranes passing back through this transition are subjected to transient leakage and irreversible fusion. The addition of sugars that form hydrogen bonds with the phosphate and glycerol moieties of the phospholipid headgroup can partially mimic the effects of water, replacing it, at least in part, in the hydration shell. By doing so they keep the bilayer in a fluid state, or close to it, even when almost completely desiccated, thus reducing the transition temperature. RFOs, by virtue of their multiple hydroxyl groups, are effective in this role, providing the hydrophilic interactions needed for membrane and protein stabilization under water-deficit conditions.

• Vitrification and Prevention of Sucrose Crystallization

A second, complementary mechanism operates at the level of the whole cytoplasm rather than the individual bilayer. As cellular water content falls below a critical threshold level, the concentrated mixture of sugars and other solutes in the cytosol can either crystallize or solidify into an amorphous, glass-like (vitreous) state. Only the latter is protective as intracellular glasses are extremely viscous, which slows diffusion-dependent deteriorative reactions to a negligible rate and mechanically immobilizes and supports membranes and macromolecules during dormancy. A higher proportion of RFOs relative to sucrose raises the glass transition temperature and prevents sucrose from crystallizing out of solution during drying, that favours the formation of a stable vitreous matrix. Because sucrose crystals cannot protect membranes and their formation locally depletes the protective sugar pool, the oligosaccharides which resist

crystallization far more strongly than sucrose alone and act as a molecular "antifreeze" for the drying cytoplasm, a role first proposed by Caffrey and colleagues, who demonstrated that oligosaccharides can protect membranes by preventing the crystallization of sucrose during drying (Caffrey et al., 1988).

• **Osmotic Adjustment and Protein/Organelle Protection**

Beyond their direct membrane interactions, RFOs contribute to osmotic adjustment, helping seed and vegetative cells maintain turgor and stabilize proteins under drought and desiccation stress conditions by acting as compatible osmolytes. In resurrection plant species, elevated RFO levels during desiccation likewise help stabilize membrane phospholipids in vegetative tissue, extending the same protective logic seen in seeds to whole desiccation-tolerant plants. Related galactosyl cyclitols, which co-occur with RFOs in some seeds, have additionally been proposed to contribute to the stability of organelles, membranes, enzymes, and other macromolecules, and to the glassy state itself.

RFOs, Seed Storability, Vigor, and Longevity

Seed longevity is the length of time a seed remains viable in storage depends on the same protective infrastructure that confers initial desiccation tolerance, since progressive deterioration during storage involves membrane damage, loss of DNA integrity, mitochondrial dysfunction, protein damage, and disruption of antioxidative defences. Because RFOs help to maintain membrane integrity through both the initial drying event and the subsequent dry, quiescent storage period, RFO content has emerged as an crucial biochemical correlate of, and candidate tool for improving, seed vigour and longevity in gene-bank. Comparative work across legume and cereal seeds shows that a reduction in RFO content below a threshold level is related to reduced desiccation tolerance and poorer storability, even though very high RFO levels are nutritionally undesirable because of their fermentation by gut microbiota after ingestion.

Taken together, the evidence from model membranes, whole seeds, and genetically modified plants supports a coherent model in which RFOs contribute to seed membrane integrity through at least four, non-mutually-exclusive routes:

- Direct hydrogen-bonding insertion into the phospholipid headgroup region, spacing headgroups apart and preventing close packing and fusion during drying.
- Depression of the membrane gel-to-liquid-crystalline phase transition temperature, keeping the bilayer closer to its native fluid state in the dry state.
- Promotion of a stable intracellular glassy state and suppression of damaging sucrose crystallization, immobilizing membranes and macromolecules during quiescence.
- Contribution to osmotic adjustment and stabilization of associated proteins and organelles under water-deficit conditions.

Conclusion

The weight of biochemical, biophysical, and genetic evidence establishes raffinose family oligosaccharides as active, mechanistically well-characterized protectants of seed membranes rather than incidental storage carbohydrates. Liposome studies show that raffinose, stachyose, and verbascose insert into phospholipid headgroups and suppress the membrane phase-transition temperature in a manner that scales with their degree of polymerization, while whole-seed physiological and genetic studies confirm that RFO accumulation is temporally and causally linked to the acquisition of desiccation tolerance, and that manipulating RFO biosynthesis genes correspondingly delays or enhances tolerance. Complementary evidence indicates that RFOs also support intracellular vitrification and prevent the crystallization of sucrose, extending their protective role from the level of the individual membrane to the entire desiccated cytoplasm. Together, these mechanisms explain why RFO content is now regarded as a functional biomarker and a potential breeding or seed-treatment mechanism for improving seed storability, vigour, and longevity in crop germplasm conservation. Future work combining structural biology of sugar-lipid interactions with field-level assessment of storability across

diverse germplasm will be valuable for translating this mechanistic understanding into practical seed-quality improvement.

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