

Cell wall removal triggers global epigenetic erasure and lineage competition to govern plant regeneration

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Single differentiated plant cells, such as leaf mesophyll cells, can regenerate into whole plants after cell wall removal (protoplasting). Distinct from standard explant regeneration, this process provides a direct demonstration of cellular totipotency and is widely used in plant biotechnology, but its molecular basis is poorly understood. Here we show that cell wall removal triggers global nucleosome disassembly and epigenetic erasure, accompanied by widespread transcriptional pausing. Using single-cell and spatial transcriptomics, we find that protoplast-derived callus cells enter a fate-labile “limbo” state with mixed identities and no spatial patterning. Over time, these cells gradually resolve into vascular, shoot, and root lineages. The competitive balance among these fates determines whether regeneration succeeds; shifting this balance away from vascular fate promotes shoot regeneration. Our findings suggest that the cell wall acts as a structural guardian of epigenetic identity, providing a mechanistic basis for protoplast totipotency, and informing strategies to improve crop transformation.

Date : September 9 (Wed), 2026

16:00 ~ 17:30

Place : SA329 Seminar Room

(Science and Agricultural Build.)



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