Tracheary element remodeling at the grafting interface of *Arabidopsis*/*Nicotiana* interfamilial graft

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Background

Interfamilial scion-rootstock interaction often results in graft incompatibility and the underlying mechanisms are largely unknown. Since vascular re-connection is a critical step towards functional assemblage of grafting partners, this study aims to establish an *At*/*Nb* interfamilial graft and further to examine the tracheid behavior at the grafting interface.

Methods

Micrografting of *At* scion on the *Nb* rootstock was performed. Three types of grafts were categorized. The symplastic and apoplastic flow was assessed in three groups of grafts. Direct SEM imaging, histology and confocal microscopy was undertaken at the grafting interface to reveal the tracheary element (TE) behavior.

Results (up to 4 figures and tables can be included)

We revealed for the first time that TE segmentation and deformation, rather *than de novo* formation from callus, took place at the early stage of graft union development (Figure 1). Following cellular deposits, the short TEs including segmented and/or newly re-differentiated ones from both partners were overlapping with each other dependent of the homogeneity of contacting TE (Figure 2). Without overlapping, the TEs at the interface would grow laterally and the TEs above and below the interface would undergo self-fusion to form insulating spiraling bundles (Figure 3). Finally, the overlapping TEs constituted a continuous network through alignment.

Conclusion

Our results provide a definitive framework for the critical process of tracheid behavior in the *At*/*Nb* distant grafts, including: (1) fragmentation and/or deformation, (2) matching, overlapping and membranation, (3) aligning or spiraling. These insights might guide us in the future in constructing more compatible distant grafts from the perspective of tracheid homogeneity.

Funding

This work was partially supported by National Natural Science Foundation of China (31671257).

References (No more than 15 references)

Deng Z., Wu H., Jin T., Cai T., Jiang M., Wang M., Liang D. (2021) A sequential three-phase pathway constitutes tracheary element connection in the Arabidopsis/Nicotiana interfamilial grafts. Frontiers in Plant Science DOI:

10.3389/fpls.2021.664342 (in press)

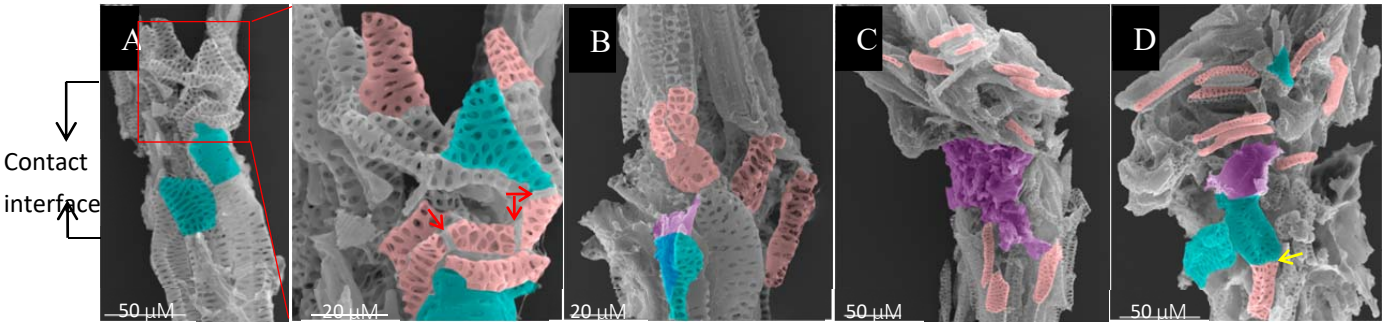

Figure 1. Local TE segmentation and cellular deposits on the TEs during graft union formation. (A) At/Nb graft union at 7 DAG. The shape of some segmented TEs became irregular. Noted that the TEs from At became widened and the end became attenuated. The red arrows indicated the cracks from a single TE. (B) At/Nb graft union at 9 DAG. Noticed that the deposit of membrane-like patches on the TEs occurred at this stage. (C) At/Nb graft union at 14 DAG. More small, short TEs accumulated at the interface and larger membrane-like patches covered the interface. (D) At/Nb graft union at 21 DAG. Short TEs further accumulated at the interface and some of them began to fuse indicated by the yellow arrows.

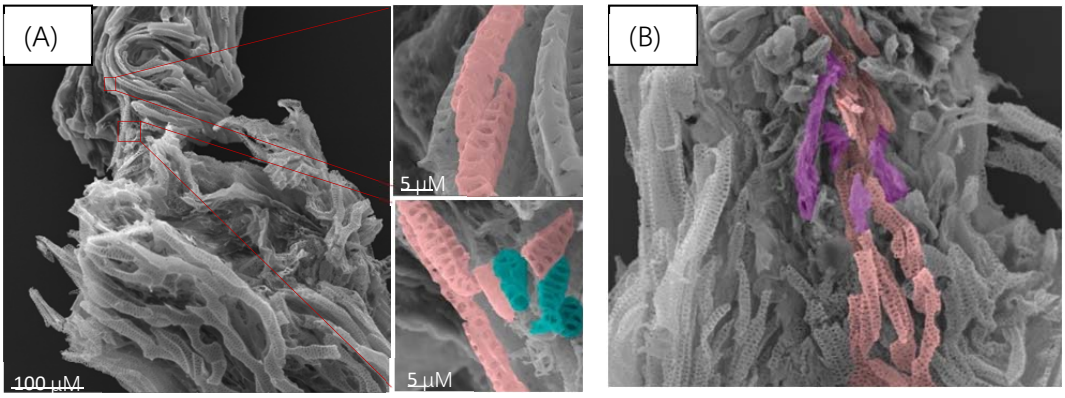

Figure 2. TE overlapping and alignment in At/Nb grafts. (A) The grafting interface in partial compatible grafts. Right top: overlapping of the top end of a Nb TE with an At TE. Right bottom: overlapping of the bottom end of the same Nb TE with other Nb TEs. (B) The reticulate TEs of At and Nb were overlapping and aligned.

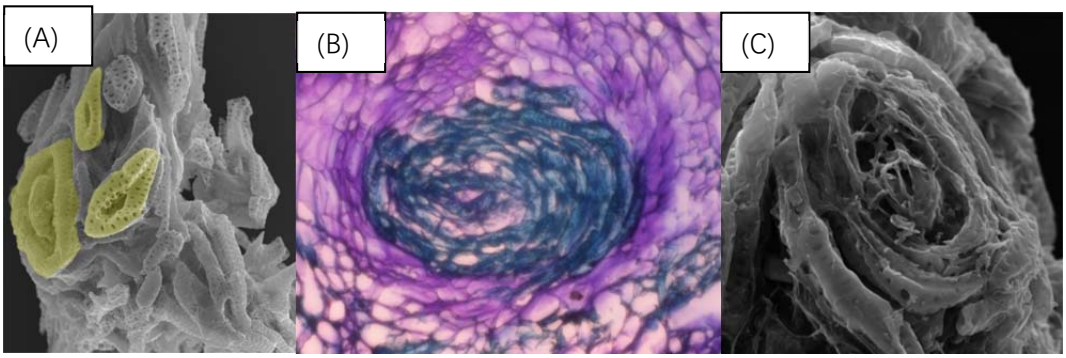

Figure 3. Formation of spiraling TE bundles in incompatible At/Nb group. (A) The circled TEs formed above the grafting interface of incompatible grafts due to self-fusion. (B) A section showing the spiraling TE bundle in the incompatible grafts. (C) The vertically spiraling TEs in the tangential face of the xylem bundles from the incompatible grafts.