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Notaguchi, M., Kurotani, K-I., Sato, Y., Tabata, R., Kawakatsu, Y., Okayasu, K., Sawai, Y., Okada, R., Asahina, M., Ichihashi, Y., Shirasu, K., Suzuki, T., Niwa, M., Higashiyama, T. (2020). Cell-cell adhesion in plant grafting is facilitated by β-1,4-glucanases. Science. 369(6504):698-702. doi: 10.1126/science.abc3710.

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Grafting being an important asexual plant propagation method, is used to combine certain attributes that do not occur naturally in a single plant such as better fruiting from the scion and disease or stress resistance from the rootstock. The major problem that exists in grafting is that it is successful only in closely related species. This research article by Notaguchi et al. 2020 describes the role of β-1,4-glucanases in the successful intra and inter family grafts of different plant species.

The scientists established that *Nicotiana benthamiana* (Nb) can be used either as stock or scion in successful interfamily grafts. It was discovered that in *Glycine max* (Gm)/*Chrysanthemum morifolium* (Cm) heterograft (Scion/Stock), grafting was unsuccessful as compared to Cm/Cm homograft and Nb/Cm heterograft (Fig. 1, A to C). A necrotic layer at the graft junction was also observed in Gm/Cm but not in Cm/Cm and Nb/Cm (Figure 1, D to F). A necrotic layer at the graft junction is an indicator of failed cell-cell adhesion which means that Nb can contribute to successful cell-cell adhesion in interfamily grafts. To further experiment on this hypothesis, seven Nicotiana species were used as stock or scion in interfamily grafting in which the other partner was from 84 species in 42 families. Successful interfamily grafting was reported in 73 species from 38 families (Figure 2, A and B).

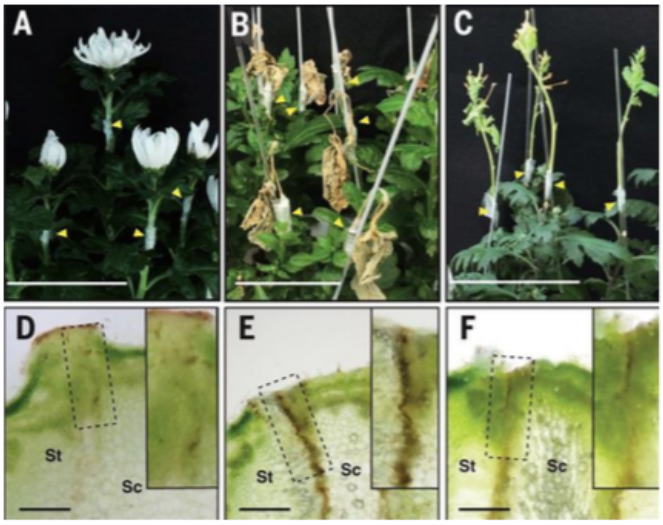

Figure 1. Success of *Nicotiana* interfamily graft marked through absence of necrotic layer. (A to C) Homografts and heterografts of *Cm*, *Gm* and *Nb* scions respectively on *Cm* stock shown after 4 weeks of grafting. Graft junctions are denoted by yellow arrowheads. Scale bars, 10cm. (D to F) Transverse section of graft junction in (A to C). Dashed rectangles show the position of insets. St- stock, Sc- scion. Scale bars, 1mm. From Notaguchi et al., 2020. Reprinted with the permission from AAAS.

To further analyze the cellular mechanism behind the Nb interfamily grafting success, transcriptomes at the graft junction of Nb/At heterograft vs intact plants were compared after 2 hours to 28 days after grafting (DAG). Upregulated genes at different time periods were involved in auxin response, wound repair and vascular development. These genes also showed a similar expression pattern in Nb/Nb homografts but with comparatively lowered expression levels. Gene ontology (GO) analysis of 189 early upregulated genes in Nb/At heterograft showed that these genes are associated with extracellular region, cell wall and apoplast that led the scientists to focus on the genes involved in the cell wall modifications. At the graft junction, cell wall reconstruction/modifying enzymes like β-glucanases, expansin and xyloglucan hydrolase were also upregulated. Laser microdissection of the graft junction confirmed the increased expression of cell wall modification enzymes. Through different dye tracer experiments, apoplastic and symplastic transport at the graft junction was observed after 3 days of grafting. Formation of xylem bridge and movement of mRNA and proteins across the graft junction were reported. This meant that within 3 days after grafting, cell-cell adhesion at the graft junction was complete and cell wall reconstruction/modifying genes played an important role during this period.

Comparative transcriptome analysis of Nb/At vs Gm/At heterografts was done and it was found that out of 189 early up-regulated genes in Nb/At, only 110 homologous genes in Gm/At were showing a similar expression pattern. The other 79 homologous genes of Gm were found through GO analysis to be related with the extracellular region and cell wall. From these 79 genes, some are involved in cell wall reconstruction at the graft junction both in intra and inter family grafts with Nicotiana. NbGH9B3, which encodes β-1,4-glucanase, was upregulated after 1DAG in Nb/At but its homolog in Gm/At showed no change. It was already known that β-1,4-glucanase is involved in cellulose digestion/cell wall relaxation during plant growth processes. To further analyze its role in grafting success, NbGH9B3-VIGS (Virus induced Gene Silencing) and NbGH9B3-KO (Knockout) lines were created and a subsequent decrease in grafting success was reported.

The role of β-1,4-glucanase in intrafamily grafting in other genera including Gm, maize (Zm), morning glory (Im) and At was also studied. One GH9 family gene was upregulated in Gm, Im and At homografts after 7 days of grafting but not in Zm. Zm being a monocot also lacks cambial activity. Gm and Im grafted on At also showed up-regulation of GH9B3 gene which indicated that GH9B3 gene expression is conserved across genera. T-DNA insertion mutants of *Arabidopsis*

CELLULASE3, a *GH9B3* clade gene were established and seedling micrografting was performed to assess the grafting success in cel vs WT plants. It was found that in Arabidopsis, GH9B3 is more important for shoot growth after grafting rather than grafting itself.

Figure 2. Nicotiana grafting compatibility with different plant species. (A) *Nicotiana benthamiana* Nb or *N. tabacum* (Nt, marked in the photo) used as scion . **Figure 2.** (B) or stock in different interfamily grafts Information regarding the other partner of interfamily grafts and the date of image taken after grafting can be found in the original article Fig. S3. Graft junctions are denoted by yellow arrowheads. Scale bars, 5cm. From Notaguchi et al., 2020. Reprinted with the permission from AAAS.

The authors concluded that Nicotiana is successful in different interfamily grafts because of a mechanism that usually occurs in homografts. To further explore the application of this research, scientists used Nb as interscion to establish interfamily grafts between species which were not successful earlier (Figure 3).

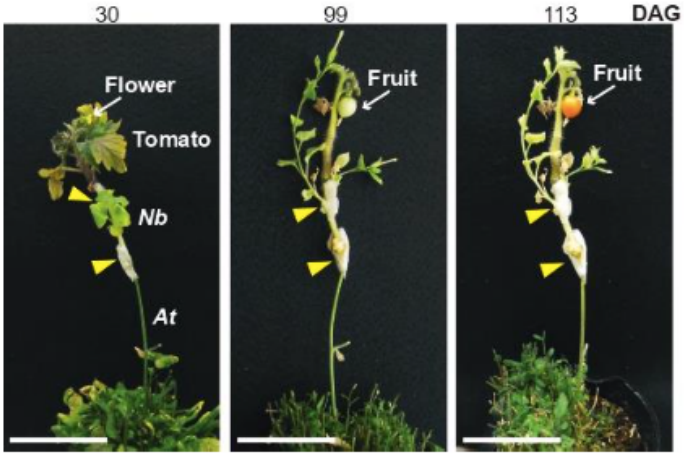

Figure 3. Successful interfamily grafts established using Nicotiana interscion. Tomato as scion established on *Arabidopsis* as stock using *Nicotiana* interscion. Days after grafting are on top of each photograph. Graft junctions are denoted by yellow arrowheads. Scale bars, 5cm. From Notaguchi et al., 2020. Reprinted with the permission from AAAS.